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MARCUS VINICIUS SILVA SANTOS

GEOQUÍMICA E MICROBIOMA DE SEDIMENTOS DE MANGUEZAL DA BAÍA DE TODOS OS SANTOS IMPACTADO POR METAIS EM ÁREA DE REFINO DE PETRÓLEO

Salvador
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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Geoquímica: Petróleo e Meio Ambiente – Pospetro, Instituto de Geociências da Universidade Federal da Bahia, como requisito para obtenção do título de Doutor em Geoquímica do Petróleo e Ambiental.

Orientadora: Profa. Dra. Olívia Maria Cordeiro de Oliveira
Corientadora: Profa. Dra Vânia Maria Maciel Melo

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Banca examinadora

Olívia Maria Cordeiro de Oliveira – Orientadora______________________________
Doutora em Geologia pela Universidade Federal da Bahia
Universidade Federal da Bahia

Fabio Alexandre Chinalia ____
Doutor em Environmental Microbiology pela University of Aberdeen
Universidade Federal da Bahia

Danúsia Ferreira Lima ____
Doutora em Geologia pela Universidade Federal da Bahia
Universidade Federal da Bahia

Icaro Thiago Andrade Moreira ____
Doutor em Geologia pela Universidade Federal da Bahia
Universidade Federal da Bahia

Carlos Eduardo Veiga de Carvalho _______________________________________
Doutor em Geociências (Geoquímica) pela Universidade Federal Fluminense
Universidade Estadual do Norte Fluminense Darcy Ribeiro

Tarcísio José Domingos Coutinho ____
Doutor em Bioinformática pela Universidade Federal de Minas Ferais
Instituto Federal de Educação, Ciência e Tecnologia do Ceará

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OLIVIA MARIA CORDEIRO DE OLIVEIRA
DIRETOR
2322888

(Assinado eletronicamente em 30/03/2021 14:16)
ICARO THIAGO ANDRADE MOREIRA
PROFESSOR DO MAGISTERIO SUPERIOR
3897782

(Assinado eletronicamente em 14/04/2021 16:34)
FABIO ALEXANDRE CHINALIA
PROFESSOR DO MAGISTERIO SUPERIOR
1919894

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Mário Lago

RESUMO

A Baía de Todos os Santos (BTS) é pioneira na exploração do petróleo no Brasil, onde atualmente está instalada na porção nordeste a Refinaria Landulpho Alves Mataripe – RLAM, o porto de Aratu e o polo Petroquímico de Camaçari. Pela presença de manguezal no entorno da refinaria e pelos derramamentos de óleo registrados nos últimos anos, diversas pesquisadores têm demonstrado interesse em compreender os impactos desses eventos ao meio ambiente através de biomonitoramento de fauna, flora e microbiota. Por serem importantes no funcionamento dos manguezais e pela dificuldade de cultivo com técnicas tradicionais, os microrganismos têm sido cada vez mais estudados através de técnicas moleculares com auxílio da bioinformática, o que tem possibilitado a descoberta de novos táxons e elucidação de rotas metabólicas. Nesse contexto, o objetivo deste trabalho foi descrever a diversidade taxonômica e funcional do microbioma de manguezal sob influência de metais em área de exploração de petróleo na Baía de Todos os Santos, além de determinar a qualidade dos sedimentos através de índices geoquímicos e valores guia de qualidade internacionalmente conhecidos. Para tal, 30 amostras de sedimentos superficiais foram coletas em duas áreas, sendo 15 provenientes do Rio São Paulo, conhecidamente poluído, e 15 da Praia do Caboto, área que já serviu como controle para metais em estudos anteriores. As amostras foram acondicionadas em caixas térmicas e alíquotas foram destinadas à análise granulométrica e quantificação de carbono orgânico total, fósforo disponível, enxofre e nitrogênio totais e os elementos Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti, V e Zn. Outra parcela foi submetida à extração de DNA e posterior PCR para sequênciamento da região V4 do 16S rRNA e preparação da biblioteca metagenômica através do Illumina MiSeq. Ferramentas de Bioinformática foram utilizadas para classificação taxonômica e genes foram preditos através do PICRUSt2. Os resultados mostraram que ambas as áreas estão poluídas por metais, mas a Praia do Caboto apresentou concentrações mais elevadas para a maioria destes elementos, o que pode estar relacionado ao sedimento de granulometria mais fina dessa área em relação às amostras do Rio São Paulo. Concentrações de As e Cd estiveram abaixo do limite de quantificação em todas as amostras. A análise de redundância mostrou que Cu, enxofre total e nitrogênio total foram positivamente relacionados às amostras da Praia do Caboto. Quanto aos microrganismos, as amostras da Praia do Caboto apresentaram maior riqueza e diversidade, contudo, em ambas as áreas houve predominância dos filos Proteobacteria, Firmicutes e Bacteroidetes. Os dados sugerem a influência de poluentes orgânicos oriundos do petróleo na distribuição de alguns gêneros, tais como *Exiguobacterium*, *Bacilli* e *Brassicibacter*, predominantes nas amostras do Rio São Paulo. Não foi encontrada diferença funcional significativa entre as áreas, entretanto, as rotas metabólicas foram associadas a diferentes organismos. Na Praia do Caboto os genes de resistência a metais e HPAs foram associados principalmente ao gênero *Desulfosarcina* e no Rio São Paulo ao gênero *Mycobacterium*, confirmando a redundância funcional comum aos microrganismos.

PALAVRAS-CHAVE: Baía de Todos os Santos. Manguezal. Sequênciamento em larga escala. Petróleo.

ABSTRACT

Todos os Santos Bay (BTS) is a pioneer in oil exploration in Brazil, where the Landulpho Alves Mataripe Refinery – RLAM, the port of Aratu and Petrochemical Complex of Camaçari are current installed in the northeastern portion. Due to the presence of mangroves in the vicinity of the refinery and the oil spills recorded in recent years, several researchers have shown interest in understanding the impacts of these events on the environment through the biomonitoring of fauna, flora and microbiota. For playing an important role in mangroves and the difficulty of cultivation with traditional techniques, the mircrorganisms have been increasingly studied through molecular techniques with the help of bioinformatics, which has the potential discovery of new taxa and elucidation of metabolic pathways. In this context, the main goal of this study was to describe the taxonomic and functional diversity of the mangrove microbiome under the influence of metals in an oil exploration area in the Todos os Santos Bay, in addition to determining the quality of sediments through geochemical indexes and internationally known references values. For this purpose, 30 samples of superficial sediments were collected in two areas, of which 15 from the São Paulo River, known to be polluted, and 15 from Caboto Beach, an area that has served as a control point for metals pollution in previous studies. The samples were stored in thermal boxes and aliquots were used to granulometric analysis and quantification of total organic carbon, available phosphorus, total sulfur, total nitrogen and the elements As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti V and Zn. Another portion was subjected to DNA extraction and subsequent PCR for sequencing the V4 region of the 16S rRNA and preparation of the metagenomic library through the Illumina MiSeq. Bioinformatics tools used for taxonomic classification and the genes were predicted throught PICRUSt2. The results showed that both areas are polluted by metals, but Caboto Beach had the highest concentrations for most of these elements, which may be related to the finer granulometry sediment in this area compared to samples from the São Paulo River. As and Cd were below the limit of quantification in all samples. The redundance analysis showed that Cu, total sulfur and total nitrogen were positively related to samples from Caboto Beach. For microorganisms, samples from Caboto Beach had greater richness and diversity, however, in both areas there was a predominance of the Proteobacteria, Firmicutes, and Bacteroidetes phyla. The data suggest the influence of organic polluants from petroleum on the distribuition of some genus, such *Exiguobacterium*, *Bacilli* and *Brassicibacter*, prevalent in samples from the São Paulo River. No significant functional difference was found beetwen the areas, however, the metabolic routes were associated with differents organisms. In samples from Caboto Beach the metals and PAHs resistance genes were associated mainly with the genus *Desulfosarcina* and in the samples from São Paulo River with the genus *Mycobacterium*, confirming the functional redundancy tipical to microorganisms

KEYWORS: Todos os Santos Bay. Mangrove. High throughput Sequencing. Oil.

SUMÁRIO

1 INTRODUÇÃO

A Baía de Todos os Santos (BTS) é considerada palco das ações pioneiras dos anos 50 da exploração do petróleo no Brasil, principalmente nos municípios de São Francisco do Conde, Candeias e Madre de Deus (FIORANTI, 2013). Até meados dos anos 80, a BTS foi o único local no Brasil a produzir petróleo (OLIVEIRA, 2013), atividade que modificou a organização social e econômica do estado da Bahia especialmente a partir das décadas de 60 e 70. Nesse contexto, esse desenvolvimento fomentou a criação do complexo petroquímico de Camaçari, do centro industrial de Aratu e de outras indústrias dispostas pelo recôncavo baiano. Em contrapartida, essas atividades têm contribuído para geração de impactos ao meio ambiente, afetando principalmente o manguezal e seus compartimentos ambientais a partir da liberação de metais e componentes de petróleo, representando riscos à biota especialmente pela possibilidade de biomagnificação na cadeia alimentar (MOREIRA, 2011) que muitos desses poluentes possuem.

As atividades de exploração, refino e transporte de petróleo requerem atenção especial quanto aos riscos de vazamentos ao mar, os quais geram impactos por vezes incalculáveis em toda a sua real dimensão. Nesse sentido, diferentes grupos de pesquisadores têm buscado se aprofundar nessa questão, avaliando as alterações à biota e ao ambiente e formas de mitigação e recuperação ao longo de tempo. Projetos de monitoramento buscam manter atualizados os dados de concentração de subprodutos de petróleo, comparando com valores de referência quando disponíveis, para as matrizes água, sedimento e biota; em outra linha de estudo, busca-se medidas que fomentem a biorremediação de ambientes impactados por atividades petrolíferas; não menos importante, procura-se ainda a compreensão de como os organismos vivos e as comunidades bacterianas relacionam-se à contaminação do petróleo, já que essas são fundamentais para o funcionamento do ecossistema.

Os microrganismos representam o maior reservatório de biodiversidade em florestas naturais, sendo imprescindíveis para o funcionamento dos ecossistemas (RODRIGUES et al., 2013), contribuindo para a transformação dos elementos presentes na ciclagem de nutrientes (De MANDAL, 2015), degradação de xenobióticos, produção de gases do efeito estufa, fixação do nitrogênio e outros processos ainda não bem esclarecidos (HUGHES et al., 2001). São os organismos mais abundantes no planeta, apresentando adaptações a praticamente todo tipo de ambiente, exibindo alta diversidade metabólica, sendo capazes de decompor uma elevada variedade de contaminantes ambientais (OVREAS, 2000). Por esse motivo, estuda-se a empregabilidade desses organismos na biorremediação de ambientes impactados por atividades petrolíferas (VIDALI, 2001; COELHO, 2005).

Estima-se que apenas 1 g de solo contenha mais de dez bilhões de microrganismos distribuídos em aproximadamente 4 mil espécies (TORVISK; OVREAS, 2002), entretanto, aproximadamente 99% destes não podem ser cultivados por técnicas convencionais, o que dificulta a compreensão da diversidade genética e estrutura populacional (AMANN et al., 1995; HUGENHOLTZ et al., 1998). Técnicas moleculares desenvolvidas a partir dos anos 90 possibilitaram avanços nos estudos de ecologia microbiana sem a necessidade de cultivo (LECKIE, 2005) utilizando regiões específicas do material genético (DNA ou RNA) diretamente das amostras ambientais (RANJARD et al., 2000). Anteriormente, os métodos tradicionais possibilitavam identificar grupos funcionais, a abundância relativa e a estrutura da comunidade (AMMAN et al., 1995), mas a identificação se dava a partir das necessidades nutricionais e da observação morfológica direta (RITCHIE, 2000), subestimando os valores reais encontrados na natureza (KIRK et al., 2005). Dessa forma, o sequênciamento molecular em larga escala aliada a metagenômica consiste em uma técnica que possibilita identificar a partir de amostras ambientais uma grande variedade taxonômica não cultivável por métodos clássicos, bem como genes funcionais e rotas metabólicas (PAES, 2008; PIZA, 2004; SILVA, 2003), aferindo também o efeito da presença de contaminantes na dinâmica populacional dos microrganismos (OGRAM, 2000).

Os manguezais compreendem um ecossistema complexo e peculiar, abrangendo organismos adapatados a variações de salinidade, a sedimento predominantemente anóxido e rico em matéria orgânica em decomposição. Distribuindo-se na faixa litorânea de regiões tropicais e subtropicais, podem ser considerados hospot de diversidade e sensíveis à poluição devido a predominância de sedimento lamoso de granulometria fina, o qual facilmente retém metais e poluentes orgânicos. Se tratando de metais, a porção norte/nordeste da BTS é conhecida pela contaminação por Pb e Cd proveniente de usina de fundição abandonada no município de Santo Amaro – BA (SANTOS et al., 2016). Outros metais de origem antrópica incluem Zn, Cr, Cd e V que tem potencial de provocar alterações severas na comunidade microbiológica (KACI et al., 2016) e podem alterar o equilíbrio do ecossistema, uma vez que esses organismos participam da decomposição da matéria orgânica, transformação de poluentes (BENOIT, 2003), além de influenciar na disponibilidade e associação de elementos químicos com outros ecossistemas (STOLZ, 1999). Dessa forma, este estudo se propõe a ampliar os conhecimentos acerca da diversidade miocrobiana em sedimentos de manguezal da Baía de Todos os Santos em função dos impactos por metais.

2 OBJETIVO

Avaliar a diversidade taxonômica e funcional do microbioma de manguezal impactado por metais em área próxima a refinaria de petróleo na Baía de Todos os Santos através de sequênciamento genético em plataforma de nova geração.

2.1 OBJETIVOS ESPECÍFICOS

- Avaliar a qualidade dos sedimentos de manguezal na BTS e os riscos oferecidos à biota.
- Determinar as características edáficas e os teores de metais em sedimentos de manguezais sob influência de atividades da cadeia petrolífera e de áreas não contaminadas da BTS;
- Isolar DNAs metagenômicos de sedimentos de manguezais de áreas contaminadas e não contaminadas da BTS;
- Construir bibliotecas de RNAr 16S a partir dos DNAs metagenômicos e sequênciar em plataforma de nova geração;
- Descrever e comparar a diversidade taxonômica e funcional das bibliotecas de amplicons com o auxílio de ferramentas de bioinformática;
- Realizar análises multivariadas cruzando dados bióticos e abióticos.
- Identificar possíveis bioindicadores de contaminação por petróleo e metais

3 MATERIAIS E MÉTODOS

Para cumprir os objetivos propostos nesse trabalho, foram realizadas atividades de campo, de laboratório, bem como pesquisa bibliográfica durante os quatro anos de duração do doutorado do presente autor. Dessa forma, os dados obtidos foram compilados, tabulados, tratados, discutidos e expressos na forma de dois artigos destinados a revistas científicas. Para facilitar a compreensão das etapas de campo e laboratório, um fluxograma das mesmas é apresentado na Figura 1.

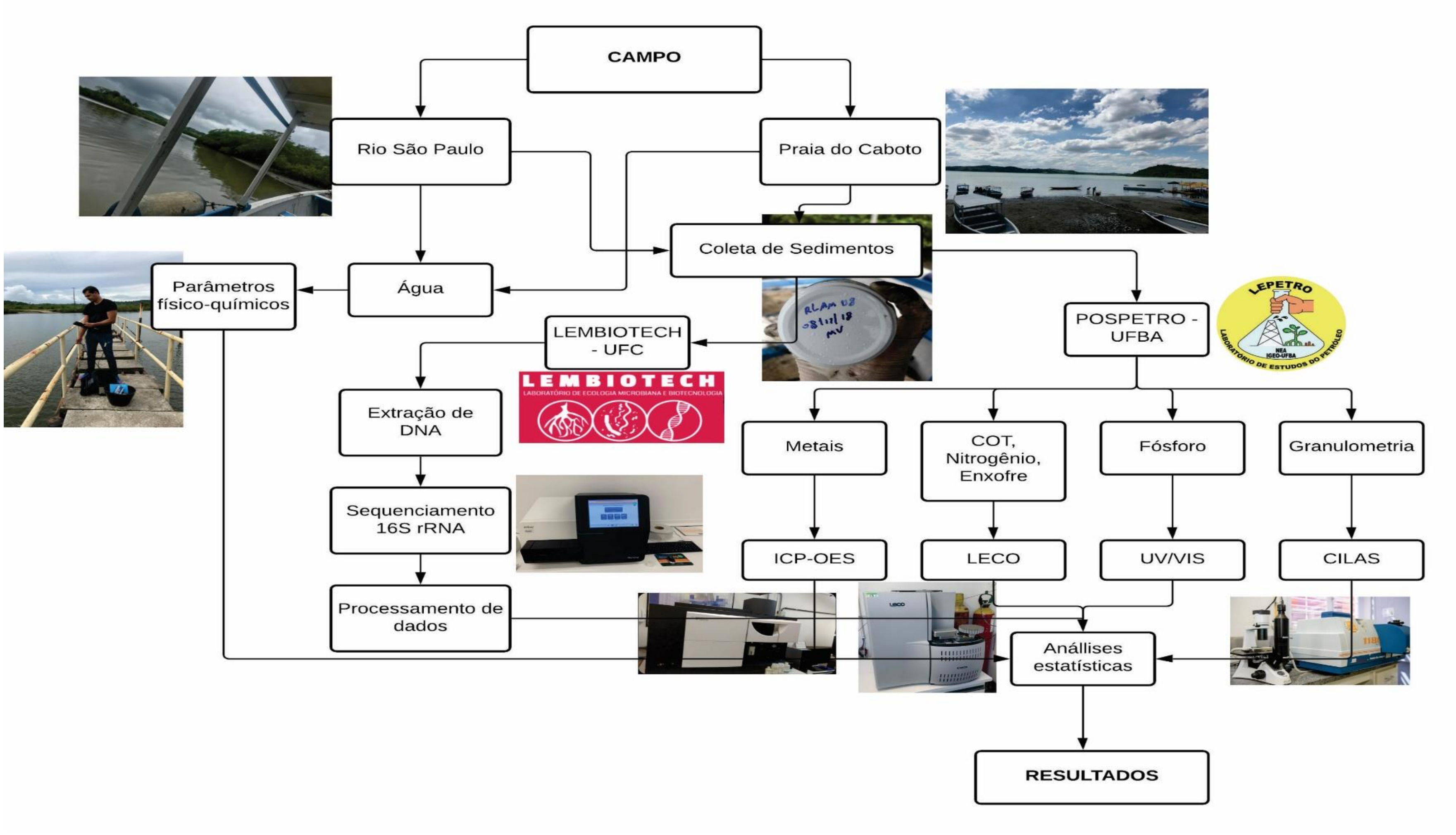
3.1 CARACTERIZAÇÃO DA ÁREA DE ESTUDO

A Baía de Todos os Santos (Figura 2) compreende a segunda maior baía do Brasil, com 184 Km de costa e área total de 1.233 Km^2 disposta entre as coordenadas 08º30’ e 18º30’ S e 37º30’ e 46º30’ W, sendo reconhecida mundialmente pelas belezas naturais e pela exploração industrial. Em seu entorno há extensa área urbana com população de cerca de 3 mihões de habitantes, forte zona industrial, destancando-se a Base Naval de Aratu, o Polo Petroquímico de Camaçari e zona de exploração e refino de óleo e gás entre os municípios de São Francisco do Conde e Candeias (CRA, 2004).

3.2 PROCEDIMENTOS DE COLETA E PREPARO DAS AMOSTRAS

As amostras de sedimentos foram coletadas em duas áreas de manguezal na porção noroeste da BTS, local de conhecida atividade de exploração de petróleo há mais de 60 anos. A primeira área, o rio São Paulo, localiza-se ao lado da RLAM, cuja área de drenagem compreende 37 Km^2 e onde estão instalados diversos poços de petróleo. A segunda compreende a praia do Caboto, mucipio de Candeias, onde há um pequeno distrito com cerca de 2000 habitantes e que já foi considerado ponto de controle para poluição por metais. A partir de visita prévia, 30 pontos foram determinados e devidamente georreferenciados através de aparelho GPS modelo GPSMAP 64s da Garmin (coordenadas disponíveis nos anexos dessa tese). Os pontos foram denominados de SPR1 à SPR15 e de CAB1 à CAB15 no Rio São Paulo e Praia do Caboto, respectivamente (Figura 3).

Figura 1 – Fluxograma da metodologia de laboratório e campo utilizada nesse trabalho



Fonte: Elaborado pelo autor

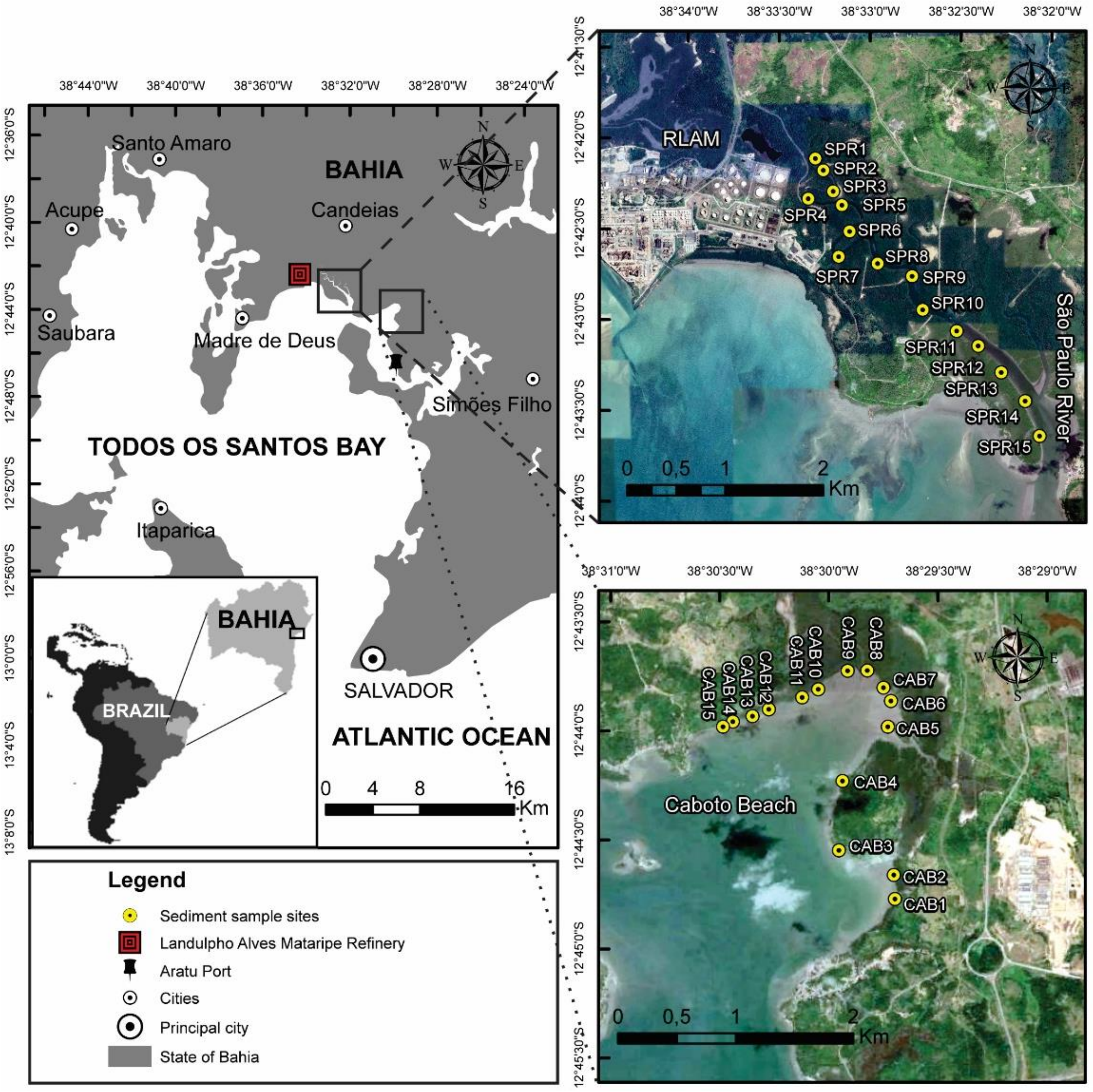
Figura 2 – Vista aérea da Baía de Todos os Santos

Fonte: Guia Geográfico Bahia – Baía de Todos os Santos

As amostras de sedimentos foram coletadas da camada superficial (0-15 cm) da região intertidal com auxílio de pá plástica previamente ambientada, sendo transferidas para frascos de vidro descontaminados e estocados em caixa térmica com gelo para transporte ao Laboratório de Estudos do Petróleo – LEPETRO do Instituto de Geociências da Universidade Federal da Bahia. Em campo, foram realizadas aferições dos parâmetros físico-químicos temperatura pH, Eh, condutividade elétrica, salinidade, turbidez, oxigênio dissolvido, sólidos totais dissolvidos introduzindo os eletrodos da sonda multiparâmetro Horiba D-54 na água marinha ou estuarina mais próxima aos pontos de coleta dos sedimentos, que se encontravam submersos no momento da coleta.

Em laboratório, alíquotas das amostras destinada à determinação de nutrientes, carbono orgânico total e metais foram secas em liofilizador (Liotop L101), peneiradas a 2 mm e posteriormente homogeneizadas para análises químicas

Figura 3 – Mapa de localização e pontos de coleta de sedimentos no Rio São Paulo e Praia do Caboto



Fonte: Adaptado pelo autor a partir de imagens do Google Earth

3.3 CARACTERIZAÇÃO GRANULOMÉTRICA

Iniciamente foi realizada a desagregação dos torrões e peneiramento em malha de 10 mesh (2 mm). Após, parcelas de 3 g de amostras foram transferidas para cadinho de porcelana e introdudizas na mufla para calcinação da matéria orgânica, permanecendo por 3 horas à 450 ºC. Em seguida, após o resfriamento, as amostras foram peneiradas em malha de 35 mesh (500 um), alocadas em tubos Falcon de 50 mL e adicionados 10 mL do dispersante hexametafosfato de sódio a 0,1 $mol.L^{-1}$ para posterior agitação por 4 horas, evitando assim a floculação. A determinação se deu através do analisador de partículas com difração a laser modelo Cilas 1064 seguindo a classificação de Folk e Wark (1957). Os resultados são apresentados através do diagrama de Shepard gerados no software SysGran 3.0.

3.4 DETERMINAÇÃO DOS METAIS, AS, NUTRIENTES E CARBONO ORGÂNICO TOTAL

Para quantificação dos metais, foi realizada digestão ácida parcial das amostras conforme protocolo descrito por USEPA method 3051A. Dessa forma, em alíquotas de 1 g de amostra foram adicionados 10 mL de HNO_3 ultrapuro (Merck-Darmstadt, Germany) e 10 mL de água ultrapura em tubos teflon e levados ao forno microondas modelo Provecto DGT 100 para digestão a 160 ºC e tempo de espera (hold) 20 minutos. As amostras foram transferidas para balões de 50 mL, sendo filtradas em papel Whattman, e posteriormente avolumadas com água ultrapura. A quantificação dos elementos As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Ti, Pb, V e Zn foi realizada por Espectrofotometria de Emissão Óptica com Plasma Indutivamente Acoplado (ICP-OES – Agilent Technologies 700 series model). Brancos analíticos foram realizados para corrigir possíveis interferências relativas ao método. Todos os materiais utilizados haviam sido descontaminados previamente em solução de HNO_3 por 24 horas e rinçados em água ultrapura. Para garantir o controle do método, realizou-se certificação através de material de referência – Certified Reference Material STSD-4 (esturine sediment).

Dentre os nutrientes, foi realizada a quantificação de fósforo assimilável, nitrogênio total, carbono orgânico total e enxofre total. Para determinação do fósforo, 0,4 g da amostra foi transferida para tudo de ensaio graduado 50 mL, adicionado 10 m de HCl 1 mol.L^{-1} e posteriormente submetido à agitação por 16 horas em mesa agitadora. Após, os produtos foram transferidos para tubos de ensaio para centrifugação a 3000 RPM por 3 minutos. A cada 1 mL de amostra foi adicionada 10 mL de água deionizada, 0,8 mL de solução ácida de molibdato + tartarato e 0,2 mL de ácido ascórbico 0,1 g.mL^{-1}. Após misturado e colocado em repouso, o fósforo foi quantificado no espectrofotômetro (Cary 60) em 880 nm. O nitrogênio total, carbono orgânico total e o enxofre total foram determinados por combustão (950 ºC) a partir de 1 g de amostras previamente descarbonatadas pelo Analisador Elementar LECO CN 628 e CN 628S com precisão < 1 ppm e LDM de 0,10%.

3.5 EXTRAÇÃO DE DNA E PREPARAÇÃO DAS BIBLIOTECAS METAGENÔMICAS

A extração de DNA se deu a partir de 0,5 g (peso úmido) de sedimento através do PowerLyzer PowerSoil DNA Isolation Kit (MoBIO laboratories, Carlsbad, CA, USA), seguindo protocolo do fabricante. As extrações foram realizadas em triplicatas e a quantidade e concentração do DNA extraído foi estimado utilizando Nanodrop 1000 (Thermo Scientific, Waltham, MA, USA). A biblioteca de amplicons foi preparada a partir da região V4 de gene 16S rRNA conforme fabricante (ILLUMINA, 2013) usando primers específicos para essa região (515F/806R) (CAPORASO et al., 2011). Na primeira etapa de amplificação foram usadas 25 uL de seis compostos distintos, dos quais 14,8 uL foram de nucleasse-free water (Certified Nuclease-free, Promega, Madison, WI, USA), 2,5 uL de 10x High Fidelity PCR Buffer (Invitrogen, Carlsbad, CA, USA), 1,0 uL de 50 mM $MgSO_4$, 0,5 uL de cada primer (10uM concentration, 200 pM final concentration), 1,0 unit of Platinum Taq polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), e 4,0 uL de template DNA (10 ng). Para a realização da PCR, o DNA foi desnaturado a 94 ºC por 4 minutos com 25 ciclos a 94 ºC por 45 s, então 60 ºC por 60 s e 72 ºC por 2 minutos, com extensão final de 10 minutos a 72 ºC. Na etapa seguinte, foi adicionado em ambas extremidades do produto amplificado a unique pair of Illumina Nextera XT indexes (Illumina, San Diego, CA). Cada 50 uL do conteúdo da reação continha 23,5 uL de nuclease-free water (Certified Nuclease-free, Promega, Madison, WI, USA), 5,0 uL de 10x High Fidelity (Invitrogen, Carlsbad, CA, USA), 1,0 unit of Platinum Taq polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), e 5,0 uL dos produtos obtidos na PCR anterior. Para essa segunda PCR, a desnaturação inicial do DNA se deu a 95 ºC por 3 minutos, realizando-se 8 ciclos a 95 ºC por 30 s, 55 ºC por 30 s, e 72 ºC por 30 minutos, com extensão final a 72 ºC por 5 minutos.

Após, os produtos da PCR foram purificados com Agencourt AMPure XP-PCR purification beads (Beckman Coulter, Brea, CA, USA) seguindo o protocolo estabelecido pelo fabricante. A quantificação foi realizada com dsDNA BR assay Kit (Invitrogen, Carlsbad, CA, USA) no fluorômetro Qubit 2.0. Posteriormente, foi realizado pool com diferentes volumes de cada biblioteca em um tubo em que cada amostra foi representada equitativamente. Após a quantificação, a molaridade do pool foi averiguada e diluída para 2 nM, desnaturado e diluído para uma concentração final de 8,0 pM com 20% PhiX (Illumina, San Diego, CA, USA) spike para sequênciamento no Illumina MiSeq (Illumina, San Diego, CA, USA).

3.6 PROCESSAMENTO DE DADOS

Os dados obtidos no sequênciamento foram analisados usando as ferramentas de bioinformática descritas a seguir: os adaptadores foram trimados a partir de arquivos raw fastq demultiplexado usando Cutadapt v1.8 (MARTIN, 2011) no modo paired-end, sendo a qualidade das reads avaliadas usando FastQC v.0.1.1.8 (ANDREWS, 2012) e and vsearch v2.10.4 (ROGNES, 2016). Análises subsequentes foram realizadas no V usando o pacote DADA2 v. 1.11.1 (CALLAHAN, 2016) conforme pipeline segeridos pelos autores e ajustados para o presente trabalho. O produto foi uma tabela não quimérica de sequências variantes de amplicons (ASVs) (CALLAHAN, 2017) que registra a quantidade de vezes que cada ASV foi observada em cada amostra. A ferramenta DADA2 identifica mais variações reais e produz menos sequências espúrias do que os tradicionais métodos de agrupamento usando sequências taxonômicas operacionais (OTUs) (CALLAHAN, 2016). A classificação taxonômica e remoção de sequências de outros grupos não bactéria ou arqueias foram reaizadas a partir de banco de dados de referência SILVA nr v.132 (YILMAZ et al., 2014). A análise de genes funcionais foi realizada através do softawe PICRUSt2 (versão 2.3.0) com configuração padrão.

3.7 ANÁLISES ESTATÍSTICAS

Foi realizado o cálculo estimador de alfa e betadiversidade e testatos quanto a normalidade dos dados pelo teste de Shapiro-Wilk. Os índices de Shannon, Obseved ASVs e Chao 1 foram paramétricos ($p > 0,05$), então realizou-se análise de variância unilateral e teste post-hoc de diferença para sugnificância de Tukey para comparações múltiplas de médias com intervalo de 95% de confiança. Para o índice de Simpson, foi utilizado o teste não paramétrico de Kruskal-Wallis. Para estimar o quão representativa as amostras eram em relação a comunidade bacteriana, o estimador de cobertura de Good foi calculado para todas as amostras, assim como as curvas de rarefação. As amostras foram agrupadas pelo método de pares não ponderados com média aritmética (UPGMA), um método baseado na média aritmética para determinar padrões de agrupamento entre as host espécies. UPGMA baseado em dissimilaridade Bray Curtis para abundâncias relativas de ASVs transformadas de médias Hellinger a nível de gênero foi produzido no software estatístico R (R CORE TEAM, 2014) utilizando o pacote Vegan (OKSANEN et al., 2015). Todos os gráficos foram gerados usando o ambiente R v.3.5.3 (R CORE TEAM, 2016).

As análises estatísticas para variáveis geoquímicas foram realizadas no software livre Past, utilizando o teste não paramétrico de Mann-Whitney ($p < 0,05$).

4 EVALUATION OF METAL CONTAMINATION IN MANGROVE ECOSYSTEMS NEAR OIL REFINING AREAS USING CHEMOMETRIC TOOLS AND GEOCHEMICAL INDEXES

ABSTRACT

The northern and northeastern portion of the Todos os Santos Bay (TSB) is known for the presence of an oil refinery in addition of other activities with significant potential for impact on the environment. 30 samples of superficial mangrove sediment were collected in two diferente locations: on the banks of the São Paulo River near the Landulpho Alves Mataripe Refinery (RLAM) and at Caboto Beach, a place that was once a control point in studies of metal pollution. After the determination of potentially toxic elements (Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti, V, Zn), the quality of the sediment was evaluated using the concentrations of these elements associated with geochemical parameters (TOC, P, S, and granulometry). In this way, the pollution indexes (EF, Igeo, PN) were calculated in addition to the comparison with the guide values for the sediment quality (TEL, PEL, ERL, ERM). Among the elements analyzed, Cu also showed levels (92.71–97.54 mg kg^{-1}) very close to PEL (108 mg kg^{-1}). At 13 sampling points, Cr concentrations were higher (56.16–66.01 mg kg^{-1}) than TEL (52.3 mg kg^{-1}). Ba showed significant concentrations in 6 samples collected on the S˜ao Paulo River, a region close to the oil refining area. The enrichment factor (EF) showed that most elements did not show enrichment, except for Zn. Through Igeo there was a tendency towards serious pollution of Ba, Cu, and Zn; moderately polluted by Cr. Principal component analysis (PCA) and Spearman's classification showed a correlation greater than 70% between the variables. According to Nemerow Synthetic Pollution (PN), both areas are polluted by Al, Ba, Cr, Cu, Fe, Mn, Ni, Ti, V, and Zn.

Keywords: Geochemistry Inorganic, Oil Refining, Mangrove, Multivariate Analysis, Environmental Assessment

4.1 INTRODUCTION

Mangroves are an important habitat for organisms that live in intertidal coasts zones of tropical and subtropical estuaries, being a complex and high productivity ecosystem that maintains marine biodiversity (SHI et al., 2019; Da SILVA JUNIOR et al., 2020). It provides balance to the environment and supports many families who live in surroundings and fish mainly crabs and other crustaceans (REZENDE et al., 2015). On the other hand, mangroves have suffered impacts in various parts of the world, attributed to human activities associated with population growth and the development of industries in coastal regions (WANG; ZIN, 2013; REIS-FILHO et al., 2019; ALMEIDA et al., 2020). Worldwide, mangroves cover an area between 150,000 to 188,000 km^2

in the estuary of 124 countries, which Bahia has the largest mangrove area in northeastern Brazil.

Crude oil is a mixture with a predominance of aliphatic and aromatic hydrocarbons, polar compounds in addition to metals such as Ni, V, Na, Cu, and U (NICOLAUS et al., 2017; PINHEIRO et al., 2020), which have toxic and mutagenic properties for a biota. For metals, acute toxicity depends on the concentration and physical-chemical parameters of water bodies (LEUNG et al., 2005; OLDHAM et al., 2014; BRITO et al., 2020). When incorporated into marine sediments, they become this important tool for environmental diagnostic studies (OLIVEIRA et al., 2017).

The Todos os Santos Bay (TSB) is recognized for its pioneering role in oil exploration in Brazil, an activity that has been carried out since the 1950s mainly in the cities of São Francisco de Conde, Madre de Deus, and Candeias (De ALMEIDA et al., 2018). Thus, the mangroves near these industries have been impacted over time by the accumulation of metals and compounds derived from petroleum, which causes a risk of biomagnification in the trophic chains (WAGENER et al., 2010; NASCIMENTO et al., 2017). From this, the distribution and bioavailability of these pollutants for biota have been monitored (DA SILVA JÚNIOR et al., 2020).

The main metals present in Todos os Santos Bay include Zn, Cr, Cd, V e Pb from the Subaé River (HATJE et al., 2006). In particular, Pb comes from a deactivated foundry that produced 32 x 10^6 kg of this metal in the municipality of Santo Amaro da Purificação, just 12 km from the mouth of the Subaé River (Da SILVA JUNIOR et al., 2016; MOTTA et al., 2018; Da SILVA JÚNIOR et al., 2020). The TSB provides the highest GDP in Bahia due to the Camaçari petrochemical complex created in the 1970s (CELINO et al., 2008). On the other hand, it recorded accidents by oil spills over the years, such as those that occurred in the years 2000, 2001, 2002, 2009, 2010, and 2012 (DO Ó MARTINS et al., 2019).

Due to the proximity of the TSB mangrove to the Landulpho Alves Mataripe refinery, the state government created in 1999 the Environmental Protection Area (EPA) of the Todos os Santos Bay, which covers 800 km^2 of extension including waters and islands (CELINO et al., 2008).

In this context, Petróleo Brasileiro S.A. (PETROBRAS) has supported since 2004 studies that seek to quantify and mitigate the environmental impacts caused by it. Thus, this paper aimed to analyze the concentrations of the elements Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti, V, and Zn in superficial sediments in two mangroves forest areas near to oil activity in Todos os Santos Bay and assess the quality of sediments against potential risks to biota through reference values established by international agencies.

4.2 MATERIAL AND METHODS

4.2.1 Study area

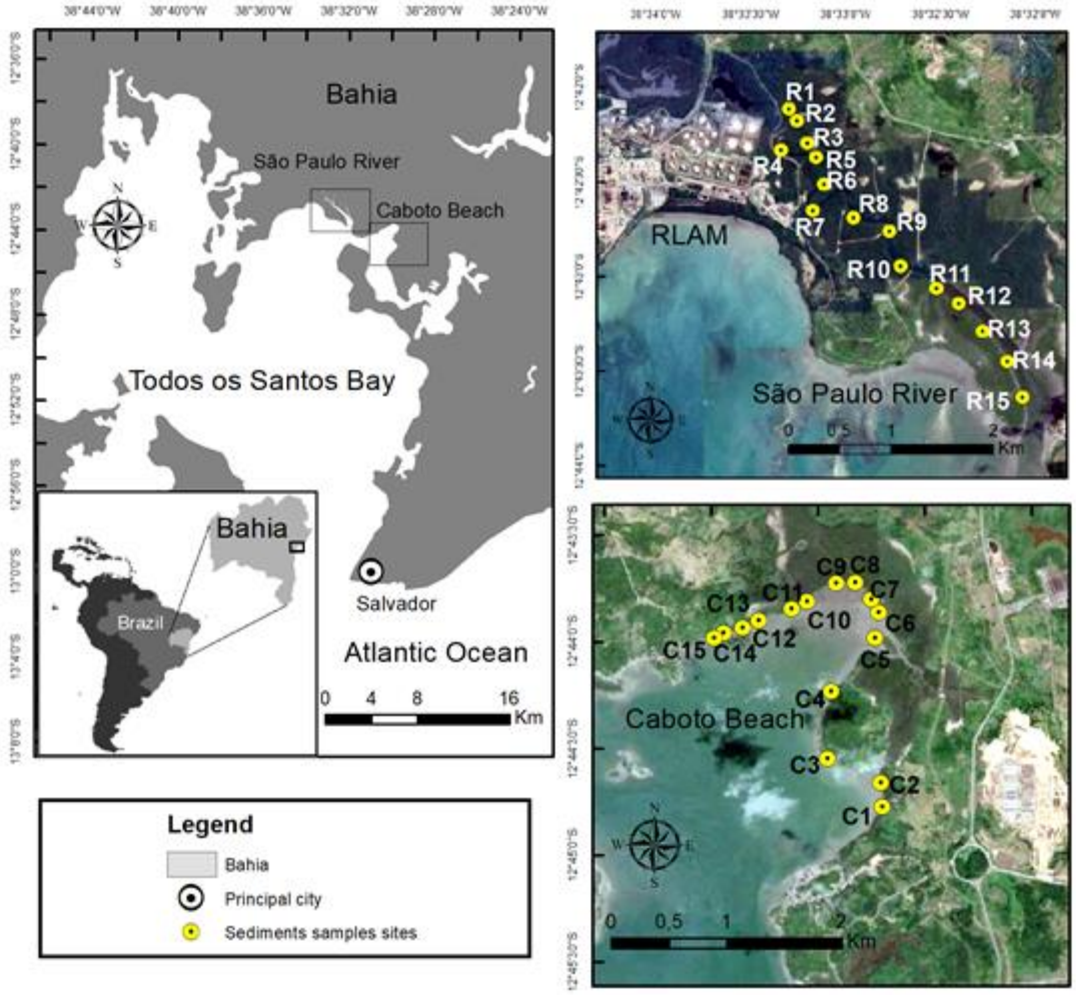
The collections were carried out in two areas between the coordinates 12°35’30” S – 13°07’30” S and 38°29’00” W – 38°48’00” W. One of them comprises the São Paulo River – SPR where Landulpho Alves Mataripe Refinery (RLAM) and another comprises the Caboto Beach in the municipally of Candeias (Figure 4). In each area, 15 samples of the surfaces layer (0-15 cm) were collected employing a previously decontaminated polyethylene shovel and transferred to hermetically sealed packages to transport in a thermal box at 4 °C to the laboratory. The samples were lyophilized for 24 hours and ground in a ball mill.

4.2.2 Physico-chemical parameters, granulometric determination and nutrients

The pH and potencial redox (Eh) of the water were measured in loco using the previously calibrated multiparameter probe (Horiba D-54, Horiba). The temperate was obtained by means of a portable codutivimeter (Handylab1, Schott) inserting the electrodes in the interstitial water.

For the grain size analysis, aliquots of 1.5 g of dry sediment sieved in a 500 µm (35 mesh) were transferred to 50 mL Falcon tubes, which 10 mL of 0.1 mol L^{-1} sodium hexametaphosphate were added and transferred to a shaker for 4 h. The grain size determination was performed by means of a lases diffraction particle analyzer (1064, Cilas), according to the Folk and Ward (1957) classification.

Figure 4 – Map of location and distribution of sampling sites*



*Prepared by the author adapted from Google Earth

For the quantification of organic carbon and total nitrogen, the samples initially decarbonated and subjected to the elemental analyzer (CN628, LECO Corporation). The tests were carried out in triplicates and the blanks were checked to detect possible contaminants in addition reliability to the results obtained.

4.2.3 Metals quantification

The extraction of chemical elements was carried out using the partial digestion methodology applied in a microwave system MARS 6 (CEM, Matthews, NC, EUA). For this purpose, 1 g of lyophilized sediment (<2 mm) was transferred to tubes and added 5 mL of concentrated HNO_3, and 5 mL of ultrapure water was added. After 20 min the tubes were closed and transferred to the microwaves to digestion at 160º C with 20 min of time hold. The samples were filtered in membrane 0.45 µm (Merck Millipore, Massachusetts, EUA) and volumed with pure water in the 50 mL volumetric flasks. Analytical blanks were performed to verify possible contaminants during the sample preparation. Certified sediment samples (NIST 1646a – Estuarine Sediment) were subjected to the same extraction process to the reliability of the results. The extracts were analyzed by optical emission spectrophotometry with inductively coupled plasma – ICP OES (700 series model, Agilent Technologies) and the contents of Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti, V, and Zn were determined.

4.2.4 Sediment pollution assessment

The geoaccumulation index (Igeo) propused by Müller (1981) is a tool for evaluate the level of contaminantion in sediments calculated using the Eq. (1)

$$Igeo = log2\left(\frac{Cn}{1.5 \times Bn}\right) \tag{1}$$

where Cn is concentration of metal in sediment; 1.5 is factor used to correct possibles background variations caused by lithogenic effects (KASTRATOVIC et al., 2016); and Bn is the background value for the metal, being in this paper the values proposed in previous studies. Igeo makes it possible to define seven pollution categories (KIM et al., 2018), according to Table 1.

Table 1 – The geoaccumulation index (Igeo) scale

Igeo	**Class**	**Pollution intensity**
< 0	0	Unpolluted
0 – 1	1	Unpolluted to moderately polluted
1 – 2	2	Moderately polluted
2 – 3	3	Moderately to seriously polluted
3 – 4	4	Seriously polluted
4 – 5	5	Seriously to very seriously polluted
5 – 6	6	Very seriously polluted

The Enrichment factor (EF) was calculated because it is an estimate that makes it possible to differentiate metals of anthropic origin from those of natural processes, evaluating the anthropic contribution. For this, Fe or Al is used as a normalizing element (CHEN et al., 2007). In the present paper, Al was used because of its low natural mobility in the environment using the Eq. (2):

$$EF = \frac{\left(\frac{CM}{CAl}\right) sample}{\left(\frac{CM}{CAl}\right) \ background}$$

(2)

EF < 1 indicates no enrichment; EF < 3 is a minor enrichment; EF = 3 – 5 is a moderate enrichment; EF = 5 – 10 is a moderately severe enrichment; EF = 10 – 25 is a severe enrichment; EF = 25 – 50 is a very severe enrichment; and EF > 50 is a extreme severe enrichment.

The sediment quality guidelines (SQGs) are important tools for measuring contamination in marine and estuarine sediments (LONG et al., 1995). Two sets of SQGs developed for marine ecosystems were applied in this study to assess the ecotoxicological risks of metals in sediments: (a) the effect range low (ERL)/effect range median (ERM) and (b) the threshold effect level (TEL)/probable effect level (PEL) values. Low range values (i.e., ERLs or TELs) are concentrations below which adverse effects on the sediment fauna would be rare. On the other hand, ERMs and PELs represent chemical concentrations above which adverse effects are likely to occur (JAMSHIDI; BASTAMI, 2016; SANTOS et al., 2020).

Another parameter used to assess contamination is Nemerow Synthetic Pollution (P_N). Unlike Igeo and EF, the P_N assesses the contribution of each contaminant separately, being an integrated index that considers the degree of contribution of all contaminants in the same sample (CHEN et al. 2015; BRITO et al., 2020). The Nemerow synthetic pollution index (PN) was calculated using the Eq. (3):

$$P_N = \sqrt{\frac{P_{MAX}^2 + P_{AVE}^2}{2}}$$

(3)

which P_N is the Nemerow Synthetic Pollution index for all evaluated samples; P_{MAX} is the maximum single factor pollution index for all samples; and P_{AVE} represents the arithmetic mean of the single factor pollution indices for all samples. Heavy metal pollution was rated at five degrees based on the Nemerow index: $P_N < 0.7$, safe; $0.7 < P_N < 1.0$, necessary precaution; $1.0 < P_N < 2.0$, light pollution; $2.0 < P_N < 3.0$, moderate pollution; and $P_N < 3.0$, serious pollution (ZHAO; LI, 2013).

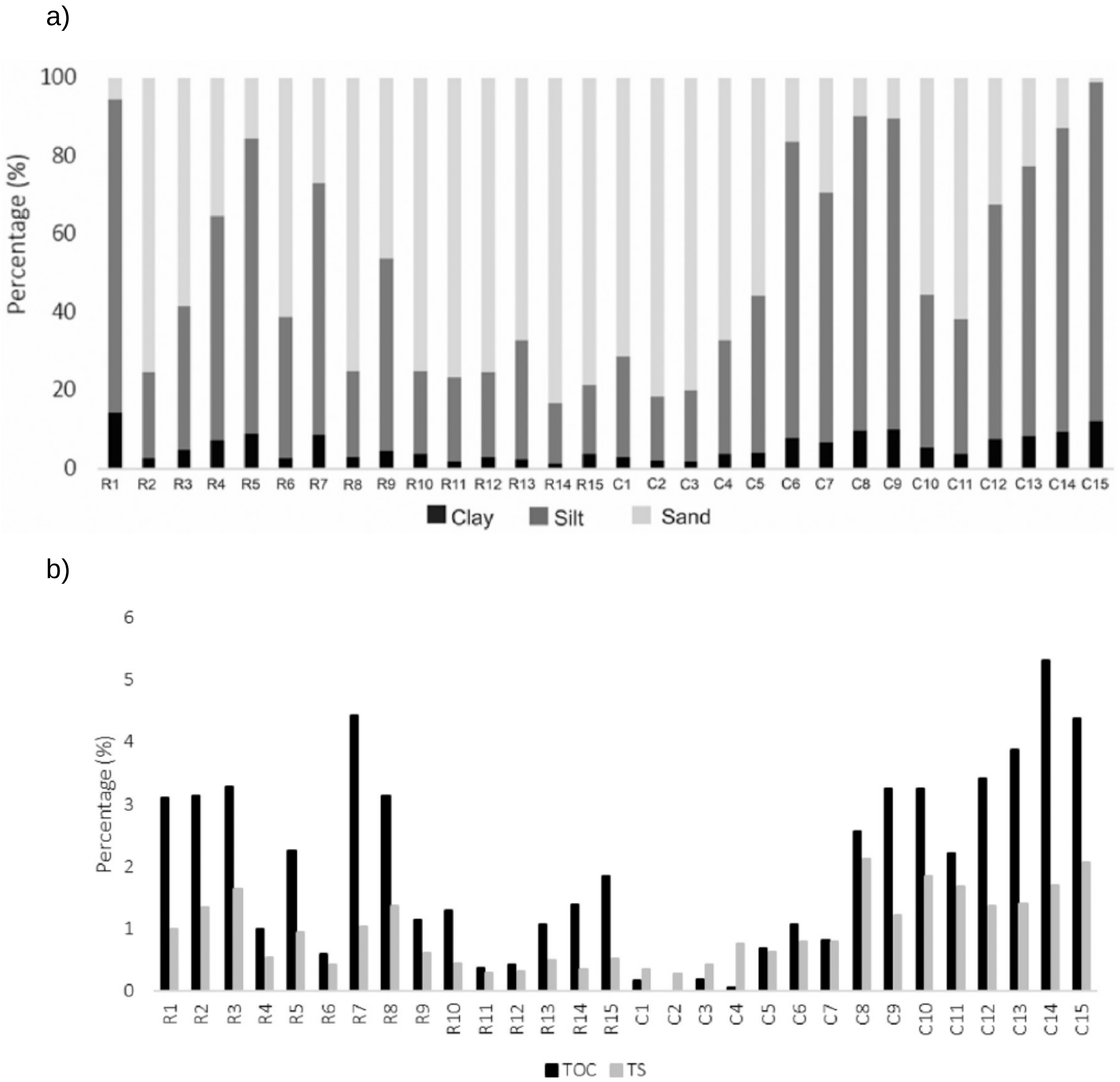
4.3 RESULTS AND DISCUSSION

The values of the particle size, and nutrient parameters (total organic carbon, total nitrogen and phosphorus available) obtained are shown in Figure 5.

In general, Caboto Beach had a greater sum of the silt-clay fraction (< 63 µm) when compared to the São Paulo river. In the first, the average of the silt-clay fraction was 59.53% (± 28.12%), varying between 18.39 and 98.94%. The highest proportions were found in C15, C8, C9, and C6 (98.94%, 90.31%, 89.60%, and 83.72%, respectively). In the São Paulo River, the average silt-clay fraction was 42.99% (± 25.11%), varying between 11.88% and 94.44%. The highest values were found in R1, R5, R7, R4 (94.44%, 84.57%, 72.98%, and 64.65%, respectively). The predominance of fine particles is an important factor in the environmental analysis due to the greater capacity for adsorption to metals and organic matter (SALOMONS; FÖRSTNER, 1984; BAYEN, 2012; ZHARA et al., 2014).

Caboto Beach had a higher average value of total organic carbon (TOC) than the São Paulo River, these being 2.08% (± 1.77%) and 1.89% (± 1.25%), respectively. TOC ranged between 0.42% and 4.43% in São Paulo River, and between the limit of quantification (0.04%) and 5.32% in Caboto Beach. Organic matter has the capacity to be easily incorporated into the silt-clay fraction, being carried by water bodies and deposited at the bottom of the river, estuary, or ocean (PEREIRA, 2001), as well as interacting with iron and manganese oxides, which together with acids humic and fulvic act as binders and geochemical support (BELO et al., 2010).

Figure 51 – Grain size fractions and nutrients values of mangrove sediments in São Paulo River and Caboto Beach. (a) grain size; (b) TOC and TS



Although mangroves are typically enriched with organic matter, the low values found in the present study are due to the high hydrodynamic potential of the region and are similar to the values found by Pereira et al. (2015) at the mouth of the Paraguaçu River, northwest portion of TSB, ranging between 0.38% and 3.00%. Total nitrogen (TN) was below the limit of quantification (0.1%) in 14 points in São Paulo River and in 12 points in Caboto Beach, being detected only in R7 (TN 0.15%) and in C 13 (TN 0.1%), C 14 (TN 0.21%) and C 15 (TN 0.33%). At these points, there is a greater decomposition of organic matter, since this is the main source of nitrogen in the environment (SERNA et al., 2014). The TOC and TN values are in line with those found in previous studies carried out in the Subaé River and São Paulo River estuaries, which showed values between 0.10 to 0.33% (COSTA et al., 2011). The average levels of P ava were higher in Caboto Beach, (257.37 ± 116.35 mg kg^{-1}), ranging between 14.6 to 385.86 mg kg^{-1}, than in São Paulo River (171.97 ± 122.89 $mg.kg^{-1}$), which varied between 1.07 and 441.61 mg kg^{-1}, respectively. In a previous study in Aratu Bay, 9 km from Caboto Beach, concentrations between 0.01 and 0.07% of total phosphorus were found (LEÃO et al., 2018), which is consistent with the oligotrophic environment as reported in a study in the estuary of the Paraguaçu River (PEREIRA et al., 2015). The average values of total sulfur (TS) were higher in Caboto Beach (0.75% ± 0.43%), ranging between 0.3 and 1.65% than in São Paulo River (1.16% ± 0.63%). TSB mangroves are abundant in sulfides, an important parameter for assessing the availability of metals for plants and the water column (LEÃO et al., 2018; SANTOS et al., 2020).

Among the elements analyzed, As and Cd were below the LOQ (limit of quantification) in all samples. In a previous study developed by Santos et al. (2019), As was detected in Caboto Beach in concentrations ranging from 8.59 to 10.01 $mg.kg^{-1}$, while Cd was below LOQ (0.25 mg kg^{-1}), confirming that this area is not polluted by this metal. The elements Al, Cr, Cu, Fe, Mn, Ni, Pb. Ti. V and Zn had the highest concentrations in Caboto Beach, while only Ba had the highest concentration in the São Paulo River (Table 2).

The Ba concentration ranged in São Paulo River and Caboto Beach respectively 17.53 - 210 and 53.08 - 98.19 $mg.kg^{-1}$, being found above TEL in R1, R2, R3, R4, R5, and R7, near the Landulpho Alves Mataripe Refinery (RLAM). Other authors found 68.76 $mg.kg^{-1}$ of Ba in the western portion of TSB (Pereira et al., 2015), and 102 and 73 $mg.kg^{-1}$ in the central and eastern portion of the bay, respectively (SANTOS, 2016).

Table 2 – Metal and As concentration in mg.kg^{-1} in sediments of the all sites. Values over SQGs are marked in hold

Site	Al	Ba	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Ti	V	Zn	As
R1	55595	**210**	< QL	**66.01**	**49.04**	22869	162.8	7.47	23.19	287.06	75.83	76.16	< QL
R2	44598	**177.8**	< QL	**56.15**	**51.47**	22888	176.5	5.94	19.42	189.9	63.99	73.85	< QL
R3	48550	**175.6**	< QL	**57.32**	**49.88**	22451	173.9	4.43	13.82	254	65.7	67.19	< QL
R4	32271	**165.1**	< QL	38.58	**28.81**	17547	141.8	< QL	6.73	218.4	43.96	42.7	< QL
R5	37973	**135.9**	< QL	46.99	**56.02**	20378	171.6	2.82	10.28	206.6	51.54	66.62	< QL
R6	18752	56.27	< QL	23.91	**23.38**	12627	115.46	< QL	< 2	134.83	25.23	31.74	< QL
R7	52727	**161.9**	< QL	**65.52**	**63.13**	23545	292.9	9.98	27.15	284.5	71.45	77.12	< QL
R8	29138	85.73	< QL	47.58	**52.58**	27128	167.1	3.46	10.61	102.1	50.1	74.39	< QL
R9	19355	56.71	< QL	29.18	**24.5**	16890	124.7	< QL	< QL	151	31.91	40.24	< QL
R10	14530	48.83	< QL	22.12	**18.98**	11889	73.58	< QL	< QL	149.8	22.92	24.28	< QL
R11	10865	38.33	< QL	19.22	7.58	11399	93.1	< QL	< QL	103.03	20.14	23.94	< QL
R12	10085	42.86	< QL	17.08	13.2	8247	56.09	< QL	< QL	92.6	16.41	20.67	< QL
R13	19710	66.91	< QL	27.84	**26.87**	15308	128.2	< QL	< QL	165.8	31.5	33.43	< QL
R14	9408	17.53	< QL	15.14	7.81	7595	48.38	< QL	< QL	114.1	16.3	20.33	< QL
R15	9962	25.4	< QL	17.14	13.96	8446	65.8	< QL	< QL	100.1	16.7	18.07	< QL
C1	17101	98.19	< QL	24.95	**19.22**	14485	97.16	< QL	< QL	165.4	28.43	30.44	< QL
C2	9938	86.99	< QL	16.62	12.58	9851	70.03	< QL	< QL	92.06	17.29	19.88	< QL
C3	9352	53.08	< QL	17.96	13.23	11813	85.09	< QL	< QL	71.1	19.38	21.18	< QL
C4	44366	83.3	< QL	**64.54**	**97.54**	28209	245.7	12.07	24.09	291.1	73.2	78.9	< QL
C5	26367	61.65	< QL	37.47	**48.48**	20870	259.4	< QL	7.44	215.3	41.55	47.65	< QL
C6	31399	66.38	< QL	45.86	**71.33**	25325	269.1	3.48	18.02	222.6	51.61	59.47	< QL
C7	32602	68.75	< QL	46.72	**70.67**	25102	291.1	4.36	11.69	226.5	52.53	60.03	< QL
C8	38766	71.4	< QL	**57.46**	**50.25**	29467	357.93	8.73	13.74	256.73	68.01	67.76	< QL
C9	40972	76.76	< QL	**60.87**	**92.71**	29189	323.3	10.16	22.18	262.3	69.11	74.59	< QL
C10	37298	68.66	< QL	**56.16**	**95.49**	28552	322.5	8.00	17.66	230.9	65.39	70.54	< QL
C11	40693	79.39	< QL	**58.15**	**83.71**	28626	293.7	7.94	22.04	285.1	67.93	70.26	< QL
C12	43374	84.59	< QL	**61.62**	**93.57**	29021	309.9	9.51	22.15	294.2	70.15	70.12	< QL
C13	40591	75.29	< QL	**59.19**	**92.93**	28191	335	7.39	21.76	283.1	67.34	69.95	< QL
C14	35309	58.92	< QL	**56.49**	**93.15**	28495	297.1	8.99	19.54	160.4	63.71	76.61	< QL
C15	39298	70.92	< QL	**59.29**	**95.12**	29910	319.1	9.09	21.22	228.46	66.71	76.79	< QL
TEL[a]	-	130.10	0.68	52.3	18.7	-	-	15.9	30.24	-	-	124	7.24
ERL[a]	-	-	1.20	81	34	-	-	20.9	46.7	-	-	150	8.2
PEL[a]	-	-	4.21	160	108	-	-	42.8	112	-	-	271	41.6
ERM[a]	-	-	9.60	370	270	-	-	51.6	218	-	-	410	70

The highest concentrations of Cu, Fe, Mn, Ni, and Pb (63.13; 23545; 292.9; 9.98; 27.15 mg.kg^{-1}, respectively) in the São Paulo River were found in R7, close to the RLAM oil extraction and refining plant, while the lowest concentrations were found in the estuary at R14 in the following concentrations: 7.81; 7595; 48.38; <LOQ; < LOQ mg.kg^{-1}, respectively. In a previous study, Santos et al. (2019) described for these same metals in samples collected near RLAM in the São Paulo River the concentrations: 12.2; 8400; 48; 5.77 and 5.59 mg.kg^{-1}, respectively. None of the analyzed metals exceeded the PEL limit, however, PEL and ERL were extrapolated at some points. In addition to Ba, Cr extrapolated TEL at 4 points in the São Paulo river and 9 points in the Caboto beach, while Cu extrapolated TEL at 11 and 13 points in São Paulo river and Caboto Beach, respectively. In São Paulo River, the points whose metals have exceeded TEL are located in the first half of the river course. Regarding the ERL guide value, only Cu exceeded São Paulo River at 6 points and Caboto Beach at 12 points. This metal, although essential to metabolism when present in the human body in high concentrations can irritate mucous membranes (MALAVOLTA, 1994). Although most of the elements analyzed did not exceed the reference values for sediment quality as described, it is noteworthy that the V was in relatively high concentrations concerning the background of the area, especially in Caboto Beach and in the initial half in São Paulo River. Points R14 and C2 showed the lowest concentrations of metals, as well as the lowest percentages of the silt-clay granulometric fraction, preferred for the adsorption of metals.

The calculated Enrichment Factor showed that Pb was the only metal that had no anthropogenic enrichment (EF <1) at any point and that V had a minor enrichment in C3 (EF 1.04). Ba had minor enrichment at all points on the São Paulo River and 7 points in Caboto Beach. In the latter, moderate enrichment for Ba was found in C1 (EF 3.08), C2 (EF 4.70), and C3 (EF 3.05). Cr had minor enrichment at all points and Cu had moderate enrichment at R8 (EF 3.34), C9 (EF 4.19), C10 (4.75), C11 (EF 3.81), C12 (EF 4.00), C13 (EF 4.24), C 14 (EF 4.89), C15 (4.49), and minor enrichment in the other samples. Fe had minor enrichment only in R11 (EF 1.10), C2 (EF 1.04), and C3 (EF 1.32). Mn had no enrichment in R1, R2, and R3 and minor enrichment in the others samples, while Ni showed minor enrichment in C4 (EF 1.30), C8 (EF 1.08), C9 (EF 1.18), C10 (EF 1.02), C12 (EF 1.05), C14 (EF 1.22) and C15 (EF 1.10) and no the enrichment in the other points. Ti had minor enrichment in R9 (EF 1.02), R10 (EF 1.35), R11 (EF 1.24), R12 (EF 1.20), R13 (EF 1.10), R14 (EF 1, 59) and R15 (EF 1.59) in

São Paulo River and in C1 (EF 1.27), C2 (EF 1.21), C3 (EF 1.10) and C5 (EF 1.07) in Caboto Beach. Zn had moderately severe enrichment in all samples from Praia de Caboto, in 10 samples from the São Paulo River, and moderate enrichment in the other samples. In previous studies, Cu enrichment was reported in the region of Porto de Aratu, near Caboto Beach related to port activities (Da ROCHA et al., 2012) and the burning of diesel oil by ships (PEREIRA et al., 2007). In the eastern and central portion of TSB, Cu had no enrichment and Zn had moderately enrichment at the mouth of the Jaguaripe River (PEREIRA et al., 2015). There are no previous studies of EF for Ti in TSB in the literature for comparison with this paper. The EF calculation was not performed for Cd and As because their concentrations were below LOQ in all samples (Table 3).

Although Igeo was developed to assess river sediments (ZHAO; Li, 2013), it has been widely used for other environments, including mangrove ecosystems (VEERASINGAM et al., 2015). This index differs from the enrichment factor by using the logarithmic function and by multiplying the area's background by 1.5 (ABRAHIM; PARKER, 2008). The Igeo calculated in this study suggested greater enrichment of metals than that obtained by EF. Except for Cd and As, all other metals had some pollution index, as shown in Table 4.

Table 3 – Enrichment factors and Nemerow synthetic pollution index for selected elements (by site) in surface sediments of São Paulo River and Caboto Beach.

Sites	**Enrichment factors**										**PIN**
	Ba	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Ti**	**V**	**Zn**	
R1	2.03	1.83	1.64	0.43	0.67	0.64	0.21	0.68	0.69	4.64	20.60
R2	2.14	1.94	2.14	0.54	0.90	0.64	0.22	0.56	0.72	5.61	19.88
R3	1.94	1.82	1.91	0.49	0.82	0.44	0.14	0.69	0.68	4.69	18.22
R4	2.75	1.84	1.66	0.57	1.00	0.00	0.11	0.89	0.68	4.48	11.72
R5	1.92	1.91	2.74	0.56	1.03	0.36	0.14	0.72	0.68	5.94	17.95
R6	1.61	1.96	2.31	0.71	1.40	< QL	< QL	0.95	0.68	5.74	8.59
R7	1.65	1.91	2.22	0.47	1.27	0.91	0.26	0.71	0.68	4.96	20.94
R8	1.58	2.52	3.35	0.98	1.31	0.57	0.19	0.46	0.86	8.65	19.77
R9	1.57	2.32	2.35	0.92	1.47	< QL	< QL	1.03	0.83	7.04	10.78
R10	1.81	2.35	2.42	0.86	1.16	< QL	< QL	1.36	0.79	5.66	6.63
R11	1.90	2.73	1.29	1.10	1.95	< QL	< QL	1.25	0.93	7.47	6.40
R12	2.28	2.61	2.43	0.86	1.27	< QL	< QL	1.21	0.82	6.94	5.60
R13	1.82	2.18	2.53	0.82	1.48	< QL	< QL	1.11	0.80	5.75	9.10
R14	1.00	2.48	1.54	0.85	1.17	< QL	< QL	1.60	0.87	7.32	5.43
R15	1.37	2.65	2.60	0.89	1.51	< QL	< QL	1.32	0.84	6.15	4.94
C1	3.09	2.25	2.09	0.89	1.30	< QL	< QL	1.27	0.84	6.03	8.29
C2	4.70	2.58	2.35	1.04	1.61	< QL	< QL	1.22	0.87	6.78	5.47
C3	3.05	2.96	2.63	1.33	2.08	< QL	< QL	1.00	1.04	7.67	5.75
C4	1.01	2.24	4.08	0.67	1.26	1.30	0.28	0.86	0.83	6.03	21.44
C5	1.26	2.19	3.41	0.83	2.24	< QL	0.14	1.07	0.79	6.12	12.99
C6	1.14	2.25	4.22	0.85	1.95	0.53	0.29	0.93	0.83	6.42	16.20
C7	1.13	2.21	4.02	0.81	2.04	0.64	0.18	0.91	0.81	6.24	16.36
C8	0.99	2.28	2.41	0.80	2.11	1.08	0.18	0.87	0.88	5.92	18.30
C9	1.01	2.29	4.20	0.75	1.80	1.19	0.28	0.84	0.85	6.17	20.32
C10	0.99	2.32	4.75	0.81	1.97	1.03	0.24	0.81	0.88	6.41	19.26
C11	1.05	2.20	3.82	0.74	1.65	0.94	0.28	0.92	0.84	5.85	19.15
C12	1.05	2.19	4.00	0.70	1.63	1.05	0.26	0.89	0.81	5.48	19.23
C13	1.00	2.25	4.25	0.73	1.88	0.87	0.27	0.92	0.83	5.84	19.16
C14	0.90	2.46	4.90	0.85	1.92	1.22	0.28	0.60	0.91	7.35	20.70
C15	0.97	2.32	4.49	0.80	1.85	1.11	0.27	0.76	0.85	6.62	20.84

Table 4 – Geoaccumulation indices for selected elements (by site) in surface sediments of São Paulo River and Caboto Beach.

Sites	Geoaccumulation index									
	Al	**Ba**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Ti**	**V**	**Zn**
R1	1.99	3.01	2.86	2.70	0.78	1.40	1.35	0.60	1.43	1.44	4.20
R2	1.67	2.77	2.62	2.77	0.78	1.52	1.02	0.34	0.83	1.20	4.16
R3	1.79	2.75	2.65	2.72	0.75	1.50	0.60	-0.15	1.25	1.24	4.02
R4	1.20	2.66	2.08	1.93	0.40	1.21	<QL	-1.19	1.03	0.66	3.37
R5	1.44	2.38	2.37	2.89	0.61	1.48	-0.05	-0.58	0.95	0.89	4.01
R6	0.42	1.11	1.39	1.63	-0.08	0.91	<QL	<QL	0.34	-0.15	2.94
R7	1.91	2.63	2.85	3.06	0.82	2.25	1.77	0.83	1.42	1.36	4.22
R8	1.05	1.72	2.39	2.80	1.03	1.44	0.24	-0.53	-0.06	0.84	4.17
R9	0.46	1.12	1.68	1.70	0.34	1.02	<QL	<QL	0.50	0.19	3.28
R10	0.05	0.90	1.28	1.33	-0.16	0.26	<QL	<QL	0.49	-0.28	2.55
R11	-0.37	0.55	1.08	0.00	-0.23	0.60	<QL	<QL	-0.05	-0.47	2.53
R12	-0.48	0.72	0.91	0.80	-0.69	-0.13	<QL	<QL	-0.20	-0.77	2.32
R13	0.49	1.36	1.61	1.83	0.20	1.06	<QL	<QL	0.64	0.18	3.01
R14	-0.58	-0.57	0.73	0.05	-0.81	-0.35	<QL	<QL	0.10	-0.78	2.30
R15	-0.49	-0.04	0.91	0.88	-0.66	0.10	<QL	<QL	-0.09	-0.74	2.13
C1	0.29	1.91	1.45	1.35	0.12	0.66	<QL	<QL	0.63	0.03	2.88
C2	-0.50	1.74	0.87	0.73	-0.44	0.19	<QL	<QL	-0.21	-0.69	2.26
C3	-0.58	1.02	0.98	0.81	-0.17	0.47	<QL	<QL	-0.58	-0.53	2.35
C4	1.66	1.67	2.83	3.69	1.08	2.00	2.04	0.65	1.45	1.39	4.25
C5	0.91	1.24	2.04	2.68	0.65	2.08	<QL	-1.04	1.01	0.57	3.52
C6	1.16	1.35	2.33	3.24	0.93	2.13	0.25	0.23	1.06	0.89	3.84
C7	1.22	1.40	2.36	3.22	0.91	2.24	0.58	-0.39	1.09	0.91	3.86
C8	1.47	1.45	2.66	2.73	1.14	2.54	1.58	-0.16	1.27	1.29	4.03
C9	1.55	1.56	2.74	3.62	1.13	2.39	1.80	0.53	1.30	1.31	4.17
C10	1.41	1.40	2.62	3.66	1.10	2.39	1.45	0.21	1.11	1.23	4.09
C11	1.54	1.60	2.68	3.47	1.10	2.26	1.44	0.53	1.42	1.28	4.09
C12	1.63	1.70	2.76	3.63	1.12	2.33	1.70	0.53	1.46	1.33	4.08
C13	1.53	1.53	2.70	3.62	1.08	2.45	1.34	0.51	1.41	1.27	4.08
C14	1.33	1.17	2.63	3.62	1.10	2.27	1.62	0.35	0.59	1.19	4.21
C15	1.49	1.44	2.70	3.65	1.17	2.38	1.64	0.47	1.10	1.26	4.21

All samples had some pollution by Cr, Cu, and Zn, especially the samples from R1, R2, R3, R5, R7, R8 and C9, C10, C11, C12, C13, C14, and C15. Moderate pollution for Al was found in 7 samples from São Paulo River and 11 samples from Caboto Beach, which only R11, R12, R14, R15, C2, and C3 were not polluted by this metal. Ba had moderately highly polluted in 7 samples from São Paulo River and 7 samples from Caboto Beach. Fe had moderately polluted only at R8 (I_{geo} 1.03) in the São Paulo River and at 9 samples from Caboto Beach and unpolluted to moderately polluted in 8 and 4 samples from these locations, respectively. Mn had moderately to highly polluted only R7 (I_{geo} 2.25) and in the last 10 samples from Caboto Beach and moderately polluted in 8 and 1 samples from São Paulo River and Caboto Beach, respectively. For Ni, only C4 (I_{geo} 2.04) had moderately to highly polluted and R1 (I_{geo} 1.35), R2 (I_{geo} 1.02), R7 (I_{geo} 1.77), C8 (I_{geo} 1.58), C9 (I_{geo} 1.80), C10 (I_{geo} 1.45), C11 (I_{geo} 1.44), C12 (I_{geo} 1.70), C13 (I_{geo} 1.43), C14 (I_{geo} 1.62) and C15 (I_{geo} 1.64) had moderately polluted, while Pb had unpolluted to moderately polluted in 3 and 9 samples from São Paulo River and Caboto Beach, respectively. Ti had moderately polluted at R1 (I_{geo} 1.43), R3 (I_{geo} 1.25), R4 (I_{geo} 1.03), R7 (I_{geo} 1.42), and in the last 11 samples from Caboto Beach. The V had in São Paulo River 4 samples moderately polluted and 5 samples unpolluted to moderately polluted, while in Caboto Beach V had 9 and 3 samples moderately polluted and unpolluted to moderately polluted, respectively. The highest pollution index was for Zn, which had highly to very polluted in R1 (I_{geo} 4.20), R2 (I_{geo} 4.15), R3 (I_{geo} 4.02), R5 (I_{geo} 4.00), R7 (I_{geo} 4.21), R8 (I_{geo} 4.16); highly polluted in R4 (I_{geo} 3.36), R9 (I_{geo} 3.28), R13 (I_{geo} 3.01), C5 (I_{geo} 3.52) C6 (I_{geo} 3.84) and C7 (I_{geo} 3.85). In R6 (I_{geo} 2.93), R10 (I_{geo} 2.55), R11 (I_{geo} 2.53), R12 (I_{geo} 2.31), R14 (I_{geo} 2.29), R15 (I_{geo} 2.12), C1 (I_{geo} 2.87), C2 (I_{geo} 2.26), and C3 (I_{geo} 2.35) Zn had moderately to highly polluted. It is noted that the pollution in the Todos os Santos Bay is not homogeneous, since in the western portion of the bay Pereira et al. (2015) found higher geoaccumulation rates for Al (I_{geo} 3.41), Mn (I_{geo} 4.28), and lower for Ba (I_{geo} 1.84), Cr (I_{geo} 1.48), Cu (I_{geo} 0.07), Fe (I_{geo} -0.82), Ni (I_{geo} 1.45), Pb (I_{geo} -1.75), V (I_{geo} -0.86) and Zn (I_{geo} 3.28), while Boaventura et al. (2011) studying mangrove sediments in Madre de Deus Beach found lower rates for Cr (I_{geo} 0.69), Cu (I_{geo} 2.35), Fe (I_{geo} 0.7), Mn (I_{geo} 1.18) and Zn (I_{geo} 1.44). In other locations outside of TSB, Masutti and Panitz (1999) found geoaccumulation rates lower than the present study for Al (I_{geo} -1.76), Cu (Igeo 0.34), Fe (I_{geo} -0.84), Mn (I_{geo} -1.50), Ni (I_{geo} -1.55) and Pb (I_{geo} 0.58), and higher for Cd in the Itacorubi mangrove, Santa Catarina state. Rios (2018) studying the Jaguaribe

River in the state of Ceará, found higher Igeo for Al (I_{geo} 4.8), Cu (I_{geo} 4.1), and Zn (I_{geo} 4.6) and lower for Ba (I_{geo} 0.4). Comparing to international studies, Alharbi et al. (2019) found higher rates for Cr (I_{geo} 3.21), Cu (I_{geo} 4.83), Ni (I_{geo} 3.56), Pb (I_{geo} 5.57), and Zn (Igeo 5.38) in Yanbu Coast, Red Sea, Saudi Arabia, and Shi et al. (2019) found lower rates for Cr (I_{geo} 0.04), Cu (I_{geo} 0.09), Ni (I_{geo} 0.05) and Pb (I_{geo} 0.19) in mangroves on the Southeast coast of China. The pollution by metals in the present study indicated by the Igeo and the EF is justified by the existence of potential polluting sources in the region, as described in previous works (ORGE et al., 2000; QUEIROZ; CELINO, 2008; BRITO et al., 2020).

Similar to I_{geo}, this index was more sensitive than EF, indicating that the sediments of the São Paulo River are heavily and moderately polluted at 6 and 9 points, respectively, while the Caboto Beach sediments are heavily and moderately polluted at 11 and 4 points, respectively. These data confirm the pollution by various elements in all samples analyzed in São Paulo River and Caboto Beach. Thus, the results found here to corroborate that the mangrove sediments of Caboto Beach can no longer serve as a control for pollution by metals, as occurred in previous studies conducted by Lopes et al. (2009).

The Spearman's rank was performed between metals (Al, Ba, Cr, Cu, Fe, Mn, Ni, Pb, Ti, V and Zn), nutrients (TOC, P_{ava}, TS) and grain size fractions (CS, MS, FS, VFS, ST and CL) to check the interaction between these parameters (Table 5). This nonparametric test was carried out after the results of non-normally distributed data given by Shapiro-Wilk test. As and Cd were not included as variables on the statistical analysis because they have < LOQ in all samples. For the “r” value, the Santos (2007) classificartion was used. A significant and positive relationship (> 0.8) suggests a similar origin and/or geochemical behavior of the elements.

Table 5 – Spearman's correlations coefficients (r) among nutrients, grain size and metals variables

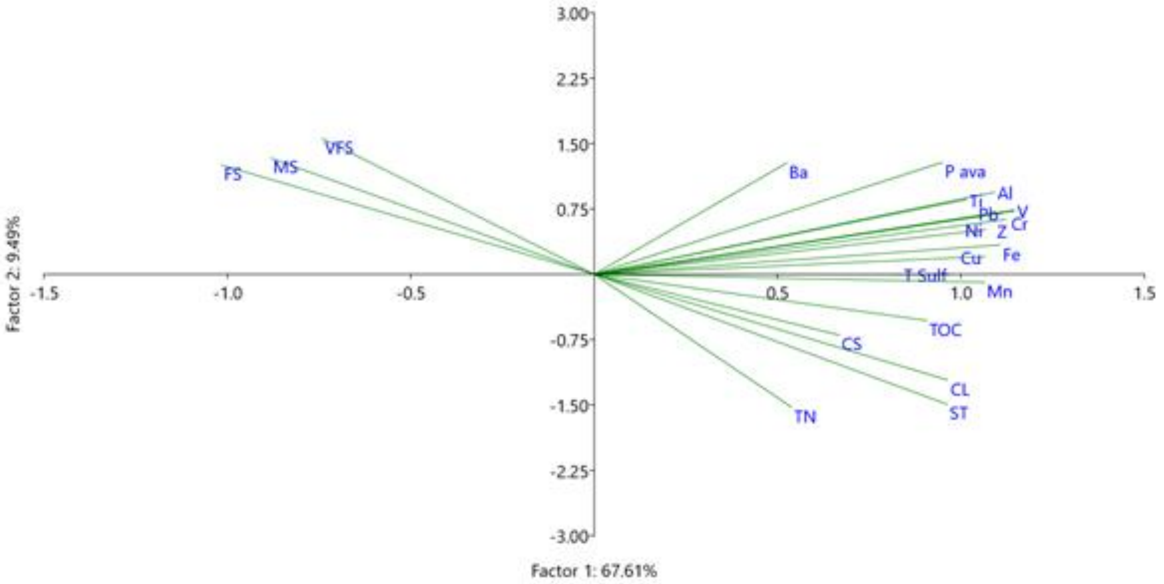
	TOC	TN	P ava	Sulf T	CS	MS	FS	VFS	ST	CL	Al	Ba	Cr	Cu	Fe	Mn	Ni	Pb	Ti	V	Z
TOC																					
TN	0.59																				
P ava	0.43	0.19																			
Sulf T	**0.82**	0.43	0.62																		
CS	0.46	0.05	0.42	0.64																	
MS	-0.54	-0.44	-0.45	-0.56	-0.49																
FS	-0.61	-0.46	-0.55	-0.71	-0.64	**0.94**															
VFS	-0.35	-0.29	-0.33	-0.49	-0.59	0.40	0.61														
ST	0.55	0.45	0.51	0.67	0.55	**-0.94**	**-0.98**	-0.58													
CL	0.63	0.46	0.56	0.69	0.52	**-0.89**	**-0.92**	-0.60	**0.92**												
Al	0.64	0.30	**0.82**	0.76	0.46	-0.59	-0.69	-0.31	0.65	0.68											
Ba	0.29	0.05	0.59	0.40	0.32	-0.21	-0.38	-0.31	0.33	0.37	0.72										
Cr	0.66	0.40	**0.83**	**0.80**	0.52	-0.64	-0.76	-0.45	0.72	0.75	**0.95**	0.62									
Cu	0.60	0.42	0.73	**0.82**	0.63	-0.61	-0.71	-0.48	0.66	0.66	0.76	0.40	**0.84**								
Fe	0.62	0.37	0.74	**0.89**	0.64	-0.61	-0.76	-0.58	0.73	0.69	0.76	0.41	**0.86**	**0.92**							
Mn	0.64	0.44	0.75	**0.87**	0.67	-0.69	-0.81	-0.54	0.78	0.72	0.75	0.36	**0.82**	**0.88**	**0.95**						
Ni	0.66	0.44	0.78	0.80	0.50	-0.56	-0.66	-0.48	0.63	0.68	**0.85**	0.45	**0.92**	**0.87**	**0.88**	**0.83**					
Pb	0.63	0.42	0.85	0.75	0.47	-0.59	-0.68	-0.45	0.64	0.69	**0.91**	0.56	**0.95**	**0.85**	**0.83**	**0.80**	**0.94**				
Ti	0.48	0.21	**0.85**	0.67	0.45	-0.66	-0.70	-0.29	0.67	0.68	**0.89**	0.55	**0.90**	0.77	0.77	0.78	**0.80**	**0.86**			
V	0.60	0.32	**0.86**	0.79	0.53	-0.64	-0.75	-0.44	0.71	0.73	**0.95**	0.63	**0.99**	**0.83**	**0.87**	**0.84**	**0.91**	**0.95**	**0.93**		
Z	0.65	0.47	0.74	**0.81**	0.47	-0.53	-0.69	-0.43	0.65	0.66	**0.88**	0.56	**0.94**	**0.86**	**0.86**	0.79	**0.90**	**0.91**	0.75	**0.91**	

Significant correlations (positive or negative) are marked in hold ($p < 0,05$).

There was a significant moderate correlation between the finer granulometric fractions (silt-clay) with all most metals (Al, Cr, Cu, Fe, Mn, Ni, Ti, V, and Zn) and with nutrients (TOC, P_{ava}, TS) since the large surface area favors the process of adsorption and cationic exchanges for metallic pollutants (SONG et al., 2014). Al had a strong positive correlation with Cr, Ni, Pb, Ti, V, and Zn and moderate with Ba, Fe Cu, and Mn, while Fe had a strong positive correlation with Cr, Cu, Mn, Ni, Pb, V, and Zn, what makes these two metals normalizers for geochemical calculations (MATTHAI; BIRCH, 2001). Al, Fe, and Mn showed a strong positive correlation with Cr, Cu, Ni, Pb, and V, being the main geochemical carriers of these elements, especially in the form of Fe and Mn oxyhydroxides (PATCHINEELAM, 1999). The abundance of kaolinite ($(Al_2Si_2O_5(OH)_4$) and goethite ($FeO(OH)$), in the study area, makes this environment vulnerable to enrichment by metallic elements. The significant strong correlation between Cr, Cu, Ni, Pb, V, and Zn suggests that they have similar sources and mechanisms of transport (MWANAMOKI et al., 2014). The strong correlation between Pb and Zn is in accordance with that described by Pereira et al. (2015), in which the sources of these elements are within TSB being transported by seawater. Ba despite having significant correlations with other metals, this was only moderate. Comparing São Paulo River with Caboto Beach, there was a significant difference between the areas for the concentrations of metals Cu, Fe, Mn, Ni, and Pb, which were higher in Caboto Beach, a place with a higher percentage of fine grains (silt-clay).

Principal component analysis (PCA) was performed because it is a mathematical formula that makes it possible to reduce the size of the data (MEYERS et al, 2006; Da SILVA JÚNIOR et al., 2020), identifying patterns that highlight their similarities and differences (ESPÍRITO SANTO, 2012), having been widely used in the identification of patterns of environmental contamination (YANG et al., 2015). The PCA results for nutrients, granulometry, and metals are shown in Figure 6.

Figure 6 – Graph of the PCA and their respective correlations with the variables studied on superficial mangrove sediments in São Paulo River and Caboto Beach



Legend: FS – fine sand; MS – medium sand; VFS – very fine sand; TN – total nitrogen; ST – silt; CL – clay

The first two components explained the accumulated variance data of 77.10%: (Factor 1: 67.61%; Factor 2: 9.49%). The PCA confirms the tendency of the silt-clay fraction and organic matter to act in the control and distribution of metals (BAYEN, 2012) and the divergence as to the origin and behavior of Ba since the load factor for this metal was 0.444 in PC1 and 0.405 in PC2. The higher concentration of Ba in the São Paulo River may be related to the proximity to the Landulpho Alves refinery, since barite is used in the drilling of oil wells (LUZ; BALTAR, 2008). During exploration, seawater, rich in carbonate and sulfate, is used to maintain pressure in the reservoir (BECKER, 1998; RIBEIRO et al., 2013). This water when associated with geology rich in Ba, Ca and Sr form precipitates that are embedded in the pipes (AK et al., 2012), being subsequently removed by mechanical or chemical means (NARS-EL-DIN et al., 2004) and discharged as effluents.

4.4 CONCLUSIONS

This study showed that both areas are polluted by metals due to oil and port activities. The São Paulo River was more polluted in the most distant part of the estuary while Caboto beach had more homogeneous pollution. The parameters used showed strong pollution by Cu and Zn in both areas, however, although Zn has greater anthropic enrichment, the concentrations of Cu offer more risks to the biota. It was not possible to affirm that Ba does not offer risks to the biota, since the concentrations found were above the TEL threshold in the São Paulo River, but it seems to have a geochemical and transport behavior different from the classic precipitation for clay minerals and Fe and Mn oxides. In effluents from the oil industry, the release of Fe and Pb is common (PINHEIRO et al., 2020), however, our samples are weakly enriched by Fe and no evidence of pollution for Pb was found. For future studies, we suggest a further investigation of the mechanisms of Ba transport through isotopic analysis; monitor the mangrove on a seasonal basis, checking the variability of metal concentration throughout the year; proceed with the collection of depositions to determine the chronology of the pollution of the northeast portion of Todos os Santos Bay, as well as to investigate the bioaccumulation and the damages caused directly in living organisms collected in the vicinity of the oil complex.

5 INFLUENCE OF OIL REFINING-DERIVED-METALS ON THE MICROBIOME TAXONOMIC AND FUNCTIONAL COMPOSITION OF POLLUTED MANGROVE SEDIMENTS

ABSTRACT

Mangroves are complex ecosystems with high productivity, specially for microbial diversity responsible for the cycling of nutrients and interactions that enable the installation of more complex organisms. Due to the presence of organic matter and predominantly fine granulometry, it is sensitive to the adsorption of metals capable of causing changes in the composition of communities and adverse effects to biota and humans. In the northeastern area of the Todos os Santos Bay, Bahia, Brazil, oil spills are recurrent related to the presence of the Landulpho Alves Mataripe - RLAM refinery, the first to start operating in Brazil in the 1950s. Thus, the main goal of this study was to evaluate the impact of metals Al, Ba, Cr, Cd, Cu, Fe, Mn, Ni, Ob, Ti, V, Zn and As elements on the composition and functioning of the Mangrove microbiome in two areas near of the refinery. For this purpose, surface sediments were collected in an area close to the refinery (São Paulo River) and antother more distant (Caboto Beach) for the metal analysis and sequencing of the 16S rRNA gene through the Illumina MiSeq platform. Both areas are impacted by metals of anthropogenic origin, with the highest concentrations in sediments from Caboto Beach. Proteobacteria, Firmicutes and Bactedoidetes were the dominant phyla in both areas, however, Caboto Beach had greater richness and diversity of DNA sequences. Beetwen the metals only Cu was positively correlated to the Caboto Beach samples. Evidence suggests that organic pollutants evaluated not have contributed to the differences in diversity indices between the two areas. Regarding the functional aspects, the areas differ little, however, the metabolic routes and resistance to metals were related to different organisms, highlighting *Mycobacterium* in the São Paulo River and *Desulfosarcina* in Caboto Beach, confirming the functional redundancy typically associated with microorganisms.

Keywords: Sequencing 16S, mangrove, Todos os Santos Bay, petroleum.

5.1 INTRODUCTION

Mangroves are ecosystems distributed in tropical and subtropical regions, occupying between 60 and 70% of the world’s coastline, which are essential for protecting and maintaining sea levels (DUKE et al., 2006). Approximately 4.1 million hectares in the Americas are covered by mangroves at the interface between ocean and continental waters (GHOSH et al., 2010). Such areas present particular conditions for the biota due to the predominant anoxia in the sediments, with wide variations in salinity and temperature rates throughout the day as a result of the tidal regime

(TAKETANI et al., 2010). Brazilian coast presents one of the largest mangrove areas on the planet (ASSUNÇÃO et al., 2017), and it is distributed from North to South of the country (CURY et al., 2002).

As one of the most productive ecosystems in the world (SHEAVES, 2005; SARAVANAKUMAR et al., 2016), mangroves are important hotspots due to the rich diversity of the microbiological community (ANDREOTE et al., 2012), which is mainly composed of bacteria and archaea, responsible for biogeochemical cycles, energy production and biofilms that provide protection for larger organisms (ZHANG et al., 2018). Thus, microorganisms play a fundamental role in the maintenance and functioning of mangroves, contributing to environmental conservation and recovery (ALONGI, 2008), providing nutrients from the decomposition of organic matter, mainly nitrogen and phosphorus, essential for the development of mangrove seedlings (HOLGUIN et al., 2001). Despite their great importance, about 35% of mangroves have been destroyed in the last two decades (VALIELA et al., 2001; FELLER et al., 2010) as a result of anthropogenic activities related to aquaculture (ILMAN et al., 2016), urbanization (TUHOLSKE et al., 2017), deforestation (RICHARDS; FRIESS, 2015), agriculture, effluent disposal, mining, waste disposal and oil exploration (PEIXOTO et al., 2011; VAN LAVIEREN et al., 2012). Exposure to pollutants impacts the bacterial diversity that constitutes the main pathway for nutrient cycling through the decomposition of lignocellulosic materials, and the production of different enzymes with proteolytic, amylolytic and cellulolytic activities (THATOI et al., 2013).

Regarding oil exploration, Todos os Santos Bay – BTS, is know worldwide for the presence of the oldest refinery in Brazil, intalled since the 1950s (NASCIMENTO et al., 2017). Surrounded by the third largest metropolitan area in Brazil with approximately 3.9 million inhabitants in 15 cities (IBGE, 2017), BTS has experienced impacts by anthropogenic action from urbanization, port and industrial activities and especially oil exploration (VENTURINI et al., 2008; WAGENER et al., 2010; NASCIMENTO et al., 2017), which has fostered impact studies on different environmental matrices. Among these studies, there are those by Brito et al. (2016) and Pereira et al., (2015), which described trace metals in seaweed and surface sediments, respectively; from Andrade et al. (2017) which described the chronology of contamination by trace elements; from Martins et al. (2020) which found high concentrations of polycyclic aromatic hydrocarbons in oysters collected in region of Madre de Deus, close to RLAM and the estuary of the Paraguasçu River; from Almedia et al. (2018) which described the sources, hydrodynamics and geochemical parameters that influence the distribution of polycyclic aromatic hydrocarbons in BTS; those by Costa (2006), Silva (2006), Silva (2007), Felizzola (2007), Barros et al. (2008), Costa (2008), Queiroz and Celino (2008) and Hatje et al. (2009) which described contamination by metals in sediments. These studies point to chronic contamination in BTS, especially in the north and northeast, where the Subaé and Paraguaçu rivers drain. The Subaé River, which drains 655 km^2 of area over 55 km in length (INEMA, 2015), has already been identified as the main source of trace metals for BTS, especially Cd, Pb and Zn from Plumbum Mineração e Metalúrgica S.A., a metallurgical company that operated between 1960 and 1993 introducting large quantities of these elements into the river and into the atmosphere, imposing persistent toxicological impacts on the biota and on human health (HATJE et al., 2009). The Paraguaçu River is the main tributary of the BTS and the most important water system of the Bahia state (REIS-FILHO et al., 2010), whose estuary is influenced by tidal currents and the control of the water flow of the Pedra do Cavalo dam, 40 km upstream from the mouth (MESTRINHO, 1998). The presence of a shipyard increases naval traffic and the possibility of contamination by oil and metals in the Paraguaçu River estuary (MOREIRA et al., 2013), whose area presents strong pollution for Mn and moderate for Zn and Hg (PEREIRA et al., 2015).

Although microorganisms may be able to mitigate the effects caused by pollutants (DIAS, 2010), many are sensitive to environmental variations, serving as bioindicators of soil and sediment quality (SCHLOTER et al., 2003). For example, Ag, Cd, Cu, Pb and Zn affect the antioxidant system causing oxidative stress (WU et al., 2016; GILLAN et al., 2005); Cr and Se inhibit growth rates (CERVANTES et al., 2001; DIXIT et al., 2015); Hg denatures proteins and causes damage to the cell membrane (FASHOLA et al., 2016); and the As deactivates important enzymes (SANKARAMMAL et al., 2014). Thus the introduction of such pollutants can cause alterations in microbial abundance and diversity. On the other hand, the specific functions of different microorganisms under different conditions are not yet fully understood (KESHRI et al., 2015), which has stimulated the development of new studies of microbial communities through culture independent methodology, such as High-Throughput Sequencing from environmental samples. In this regard, we highlight the work of Araújo et al. (2017) on the microbiome diversity along the Cerrado gradient; those of mangrove microbiome under anthropic influences (CABRAL et al., 2016; COTTA et al., 2020; SOUZA et al., 2020; COLARES; MELO, 2013; RIGONATO et al., 2018; CABRAL et al., 2019); those from Tavares et al. (2016) about microbiome in port area; from Wilkins et al. (2018) about microbiome in hot springs; and from Igiri et al. (2018) about metal effects on the microbiome. A study by Paes (2008) using T-RFLP and DNA microarrays observed differences in structure and composition microbiome at differents sites within the BTS. Thus, the main goal of this study was to evaluate the impact of metals and arsenic on the taxonomic and funcional structure on the microbiome of two mangroves close to an oil refinery in the Todos os Santos Bay through High-Throughput Sequencing.

5.2 MATERIALS AND METHODS

5.2.1 Study area

Todos os Santos Bay is located on the edges of Salvador city, the third largest in Brazil. It has a total area of approximately 1122 km^2, the second largest bay in the Brazil (HATJE; ANDRADE, 2009), of which 152 Km^2 is covered by mangrove forest (MANSO et al., 2008). A large sea port is located at BTS being responsible for the flow of products from the petrochemical industry through the Salvador channel and the Itaparica channel (MARTA-ALMEIDA et al., 2017). Tides are semidiurnal and essentially oceanic, and reflect the discharge of the three main tributaries, the Jaguaripe, Subaé and Paraguaçu rivers (COSTA et al., 2015). The climate is tropical humid with an average temperature of 28.3 °C, determining a constantly green vegetation and medium-sized trees (BAHIA, 1994). Summer is dry with higher temperatures while winter is rainy (CIRANO; LESSA, 2007), especially between April and June (CARVALHO, 2007), where the average annual rainfall is close to 2100 mm (COSTA et al., 2015).

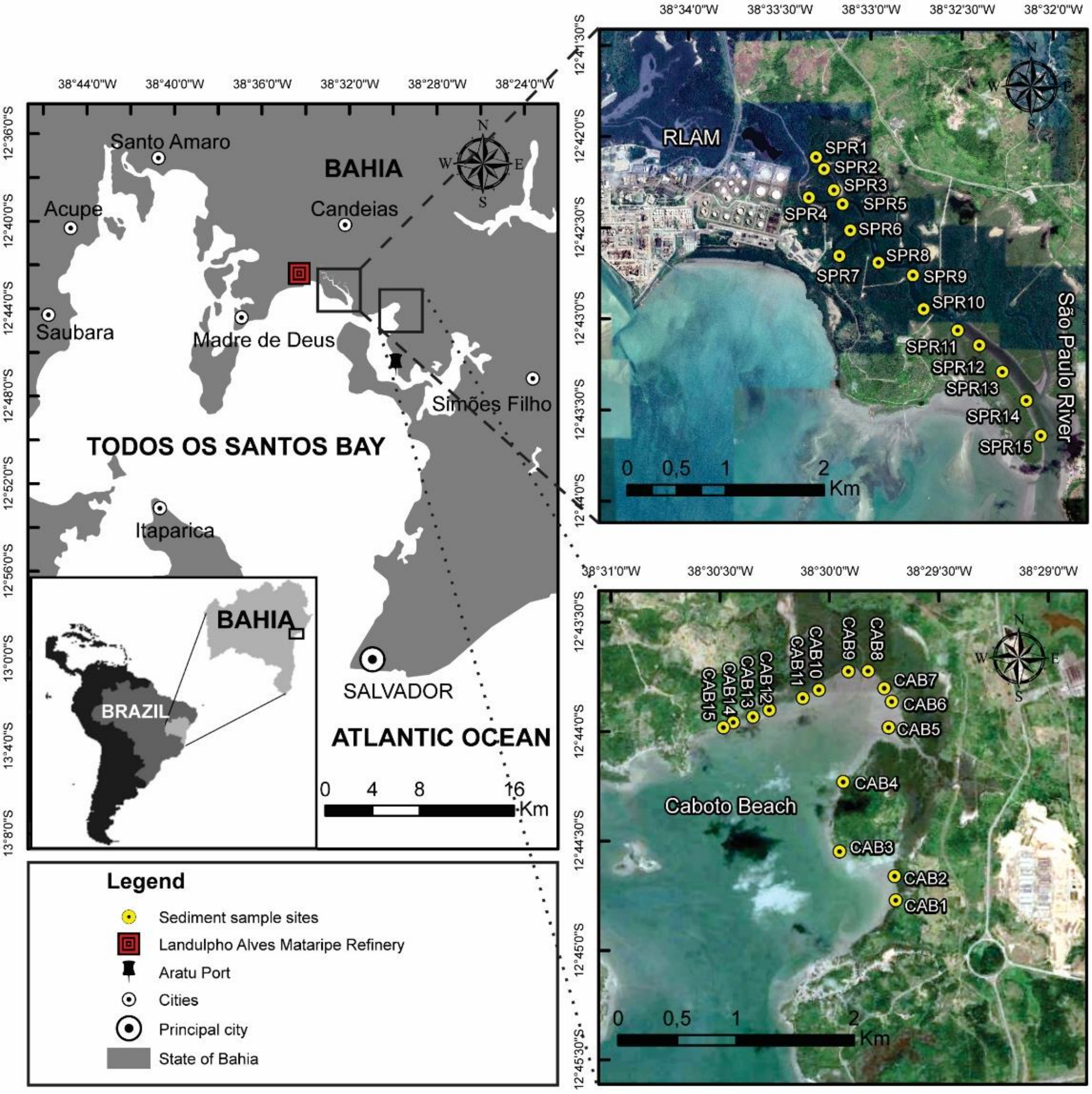
5.2.2 Sampling

Thirty collection points were distributed in two areas in the northeast portion of BTS (Figure 7) and georeferenced through Global Position System with Garmin's GPSMAP 64 device. 15 samples were collected along the course of the São Paulo River (SPR1 to SPR15) near the Petrochemical Complex, an area considered contaminated by oil activity (HATJE et al., 2009) and 15 points were distributed around

Caboto Beach (CAB1 to CAB15), in Candeias-BA, an area considered control point in previous studies for metal contamination (LOPES et al., 2009; PAES, 2008). The area of the São Paulo River basin is protect from wave action, with a predominance of clay sediments and constantly affected by chemical pollutants (QUEIROZ; CELINO, 2008), among which metals and hydrocarbons from oil stand out (MOREIRA, 2011; SILVA, 2011; NASCIMENTO, 2017). It has a drainage area of 37 km^2 in 17 km in length and flow of $0.3m^3.s^{-1}$ (BAHIA, 2004) surrounded by mangroves, having its source in the municipality of Candeias, flowing into the left bank of the district of Passé. Several oil wells are distributed throughout the São Paulo River basin, and several blow-out accidents that resulted in contamination by crude oil have been recorded (BAHIA, 2002; JESUS, 2011). The Caboto region comprises a district belonging to Candeias, with a population close to 2000 people, with just over 800 considered as urban population (BENEVIDES et al., 2015). In the infralittoral portion, Caboto Beach presents sandy-clay substrate with small rocky conglomerates, while in the supralittoral portion the substrate presents more sandy granulometry with material of biological origin, such as coral skeletons and shells, in addition to the water course that creates a small estuary (DUARTE, 2013).

The samples were collected in the dry period in November 2018 from the surface layer of the sediments (0 – 15 cm) and immediately transferred to glass vials packed in an ice box for transportation to the Petroleum Studies Laboratory (LEPETRO) of the Geosciences Institute at the Federal University of Bahia (UFBA). A portion of the samples was kept at a temperature of -20 ºC to be sent to the Microbial Ecology Laboratory (LEMBIOTECH) at the Federal University of Ceará for DNA analysis, and another portion was dried through the freeze dryer (Liotop L101), sieved at 2 mm and homogenized for chemical analysis.

Figure 7 – Distribuition of sediments sampling sites in São Paulo River (SPR1 – SPR15) and Caboto Beach (CAB1 – CAB15) in Todos os Santos Bay, Bahia, Brazil**



**Prepared by the author adapted from Google Earth

5.2.3 Geochemical analysis

Physicochemical parameters such as hydrogen potential (pH), redox potential (Eh), temperature, dissolved oxygen, conductivity, total dissolved solids, salinity and turbidity were measured in situ in both the marine and estuarine water around the collection sites through multiparameter probe Horiba D-54, according to Garcia et al. (2014).

We analyzed the following nutrients: available phosphorus, total sulfur, total organic carbon and total nitrogen. For the determination of inorganic phosphorus, 0.4 g of the sample was transferred to a 50 mL graduated test tube, added with 10 mL of 1.mol.L^{-1} HCl and taken to the shaking table for 16 hours. The products were transferred to test tubes and centrifuged for 15 minutes at 3000 RPM. 1 ml aliquots were removed from each sample, with 10 ml of deionized water, 0.8 ml of molybdate acid solution + tartrate and 0.2 ml of 0.1 g.mL^{-1} ascorbic acid added. After mixed and left to stand for 10 min, the samples were analyzed on a spectrophotometer (Cary 60) at 880 nm. Total nitrogen, total sulfur and total organic carbon were determined from 1 g of samples previously decarbonated by combustion (950 ºC) by the LECO 628 S and CN 680 Elemental Analyzer with precision <1 ppm and 0.10% LDM.

5.2.4 Particle grain size

The analysis of particle size followed the methodology by Garcia et al. (2014). After disintegrating the clods and sieving in 10 mesh (2 mm), 3 g aliquots of sediment were placed in a porcelain crucible and subjected to a temperature of 450 ºC for 3 h in the muffle, in order to calcinate the organic matter. After cooling, 1.5 g of each sample was sieved at 35 mesh (500 µm) and transferred to 50 mL Falcon tubes where 10 mL of 0.1 mol.L^{-1} sodium hexametaphosphate dispersant was added and poured into the transfer for 4 h, in order to avoid flocculation. The determination was made through the particle analyzer with laser diffraction model Cilas 1064, according to the classification provided by Folk and Ward (1957).

5.2.5 Determination of metals

The metals were quantified after the partial acid digestion of each sample according to the USEPA method 3051A. For this purpose, 1 g aliquots of sediment were transferred to teflon tubes and 10 mL of ultrapure HNO_3 (Merck-Darmstadt, Germany) and 10 mL of ultrapure water obtained through the Milli-Q system, for digestion in the Provecto DGT 100 microwave oven, were added for digestion at 160º and hold time of 20 minutes. The samples were then transferred to 50 mL flasks, filtered on Whattman paper, and later washed with ultra pure water. The quantification of the elements As, Ba, Cd, Cr, Cu, Fe, Mn, Ni, Ti, Pb, V and Zn was performed using

Inductive Coupled Plasma Optical Emission Spectrophotometry (ICP-OES – Agilent Technologies 700 series model), expressed in mg.Kg^{-1} of dry sediment. Through the same procedure, analyses of analytical blanks were performed in order to correct for possible interferences. All materials used were previously decontaminated by immersion in HNO_3 solution for 24 hours and rinsed several times with ultra pure water. Triplicates were performed in 20% of the samples. To ensure quality control, STSD-4 Certified Reference Material method (estuarine sediment) was used.

5.2.6 DNA extraction and preparation of the metagenomic library

For DNA analysis, 0.5 g of each sediment sample (wet weight) was used for DNA extraction through the PowerLyzer PowerSoil DNA Isolation Kit (MoBIO laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The extractions were performed in triplicates and the quantity and concentration of the extracted DNA was estimated using Nanodrop 1000 (Thermo Scientific, Waltham, MA, USA). A library of amplicons of the V4 region of the 16S rRNA gene was prepared as described by the manufacturer (ILLUMINA, 2013) using specific primers for that region (515F / 806R) (CAPORASO et al., 2011). For the first stage of amplification, a reaction was carried out with 25 µL of six different compounds: 14,8 µL of nuclease-free water (Certified Nuclease-free, Promega, Madison, WI, USA), 2.5 µL of 10x High Fidelity PCR Buffer (Invitrogen, Carlsbad, CA, USA), 1,0 µL of 50 mM MgSO4, 0.5 µL of each primer (10 µM concentration, 200 µM final concentration), 1,0 unit of Platinum Taq polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), and 4,0 µL of DNA template (10 ng). For PCR, the DNA was denatured at 94 ºC for 4 minutes with 25 cycles at 94 ºC for 45 s, then 60 ºC for 60 s and 72 ºC for 2 minutes, with a final extension of 10 minutes at 72 ºC. In the following stage, the unique pair of Illumina Nextera XT indexes (Illumina, San Diego, CA) was added at both ends of the amplified product. Each 50 µl of the reaction content consisted of 23.5 µl of nuclease-free water (Certified Nuclease-free, Promega, Madison, WI, USA), 5.0 µL of 10x High Fidelity (Invitrogen, Carlsbad, CA, USA), 1.0 unit of Platinum Taq polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), and 5.0 µL of the products obtained from the previous PCR. For this second PCR, the initial DNA denaturation occurred at 95 ºC for 3 minutes, then 8 cycles at 95 ºC for 30 s, 55 ºC for 30 s, and 72 ºC for 30 minutes, with final extension at 72 ºC for 5 minutes.

PCR products were purified with Agencourt AMPure XP-PCR purification beads (Beckman Coulter, Brea, CA, USA) following the manufacturer's protocol and quantified with dsDNA BR assay Kit (Invitrogen, Carlsbad, CA, USA) on the Qubit fluorometer 2.0. After quantification, a pool was performed with different volumes from each library in a tube in which each sample was represented equally. After quantification, the pool molarity was determined and diluted to 2 nM, denatured and diluted to a final concentration of 8.0 pM with 20% PhiX (Illumina, San Diego, CA, USA) spike for sequencing on Illumina MiSeq (Illumina, San Diego, CA, USA).

5.2.7 Sequence data processing and functional analysis

The data resulting from the sequencing was analyzed using bioinformatics tools, as follows. Illumina adapter sequences were trimmed from the already demultiplexed raw fastq files using Cutadapt v1.8 (Martin, 2011) in paired-end mode, and the quality of the reads was assessed using FastQC v.0.11.8 (Andrews, 2012) and vsearch v2.10.4 (Rognes, 2016). Subsequent analyses were performed in the R v3.5.3 environment (R Development Core Team, 2016), following we used the DADA2 v1.11.1 package (CALLAHAN, 2016) pipeline as suggested by the authors, adjusting the parameters to our data. The product was a table of non-chimeric amplicon sequence variants (ASVs) (CALLAHAN, 2017), which records the number of times each ASV (sequences differing by as little as one nucleotide) was observed in each sample. DADA2 identifies more real variants and outputs fewer spurious sequences than traditional clustering methods using operational taxonomic units (OTUs) (CALLAHAN, 2016). Taxonomy assignment and removal of non-bacterial sequences was performed against the SILVA nr v.132 reference database (YILMAZ, 2014).

Functional analysis was performed using PICRUSt2 (version 2.3.0) with defalt settings. The predicted genes were classified by alinging to the KO (Genomes Orthology) databases. This technique has an accuracy above 85% and is a much cheaper method than a complete metagenomic sequencing, being used in several studies of microbial ecology (DOUGLAS et al., 2018; LOUCA et al., 2018).

5.2.8 Statistical analysis

Alpha diversity estimators were calculated and tested for normality by Shapiro-Wilk test. As Shannon, Observed ASV and Chao1 were shown to be parametric ($p > 0.05$), one-way analysis of variance and Tukey's honest significance difference (HSD) *post-hoc* test was used for multiple comparisons of means at a 95% confidence interval. For Simpson index, we used Kruskal-Wallis non-parametric test. To estimate how representative our samples were of the bacterial community, Good's coverage estimator was calculated for all samples as well as rarefaction curves. Samples were clustered by unweighted pair group method with arithmetic mean (UPGMA), a hierarchical clustering method based on the arithmetic mean, to determine clustering patterns across host species. UPGMA was used on Bray Curtis distances of mean Hellinger transformed ASV relative abundances at the genus level. UPGMA, Bray Curtis calculations and the resulting heatmap were completed using vegan package (Oksanen et al., 2015) in R environment (R Core Team, 2014). All plots were generated using R v3.5.3 environment (R Development Core Team, 2016). Statistical analysis of geochemical variables were performed in the software PAST (v. 2.17) in which the differences between sites were tested through Mann-Whitney test ($p < 0.05$).

5.3 RESULTS

In the field, 30 sediment samples were initially collected from the São Paulo River and Caboto Beach, which 15 from each area for geochemical analysis and DNA extraction and sequencing. However, the CAB 9 sample was discarded because it did not have quality that would allow a good molecular analysis. Thus, this work presents the results obtained from 29 samples, 15 from São Paulo River and 14 from Caboto Beach.

5.3.1 Environmental characteristics of SRP and CAB

The most of the environmental variables measured did not present normal distribution according to the Shapiro-Wilk test. Thus, Table 6 shows the minimum, maximum and median values obtained in each area. Samples with statistically significant differences are indicated in the table.

Table 6 – Physical-chemistry parameters and grain size sediments for São Paulo River (SPR) and Caboto Beach (CAB).

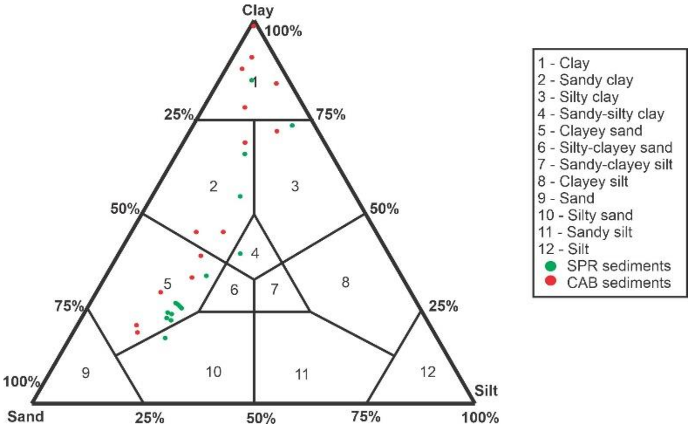
	SPR			CAB		
	Min.	Med.	Max.	Min.	Med.	Max.
Temperature (ºC) [a]	27.26	28.14	28.57	27.72	29.46	30.48
Ph	7.22	7.78	8.79	7.78	7.96	8.2
Eh (mV) [a]	-50	55	108	86	95	99
Condutivity (µS/cm) [a]	26	55.4	61.6	62	65	68
Turbidy (nTu) [a]	31.6	80.2	197	42.6	54	74
Dissolved oxygen (mg.L^{-1}) [a]	4.09	4.82	6.34	5.7	5.83	6.17
Total dissolved solid (mg. Kg^{-1}) [a]	16.1	33.3	37	37	43.75	56
Salinity (%)[a]	1.59	3.68	4.15	3.27	4.4	4.7
Total organic carbono (%)	0.37	1.38	4.43	0.28	1.08	2.13
Total nitrogen (%)	< LQ	< LQ	0.15	< LQ	< LQ	0.33
Total sulfur (%)	0.3	0.54	1.65	0.28	1.08	2.13
Phosphorus avalaible (mg.Kg^{-1})	1.07	134.13	441.61	14.6	292.79	362.66
Clay (%)	1.3	3.69	14.42	1.82	6.11	12.12
Silt (%)	15.58	30.64	80.02	16.15	50.26	86.82
Very fine sand (%) [a]	4.5	20.92	27.68	1.06	14.34	21.11
Fine sand (%)	0	29.08	38.54	0	13.02	38.62
Medium sand (%)	0	15	25.79	0	5.41	30.38
Coarse sand (%)[a]	0	0	7.91	0	1.74	16.81
Al (g.Kg^{-1})	9.4	19.7	55.5	9.3	36.3	44.3
Ba (mg.Kg^{-1})	17.53	66.91	210	53.08	71.16	98.19
Cr (mg.Kg^{-1})	15.14	29.18	66.01	16.61	56.33	64.54
Cu (mg.Kg^{-1}) [a]	7.58	26.87	63.13	12.98	77.52	97.94
Fe (g.Kg^{-1}) [a]	7.6	16.9	27.1	9.8	28.2	29.9
Mn (mg.Kg^{-1}) [a]	48.48	128.2	292.9	70.03	292.4	357.93
Ni (mg.Kg^{-1}) [a]	< LQ	< LQ	9.98	< LQ	7.67	12.07
Pb (mg.Kg^{-1})	< LQ	< LQ	27.15	< LQ	17.84	24.09
Ti (mg.Kg^{-1})	92.6	151	287.06	71.71	227.48	294.2
V (mg.Kg^{-1})	16.3	31.91	75.83	17.29	64.55	73.2
Zn (mg.Kg^{-1})	20.33	40.24	77.12	19.88	68.86	78.9

[a] Significantly diference between the two areas

As shown in Table 6, the concentration of Cu, Fe and Mn were higher in CAB than SPR sediments whereas Ni was higher in SPR sediments. Although the concentrations of Al, Ba, Cr, Ti, V and Zn were higher in CAB, they were not statistically significant ($p < 0.05$). The phisico-chemical parameters salinity, total dissolved solid and dissolved oxigen of water were higher in CAB. Both areas were not different for total nitrogen, phosphorus avalaible and total organic carbon of sediments.

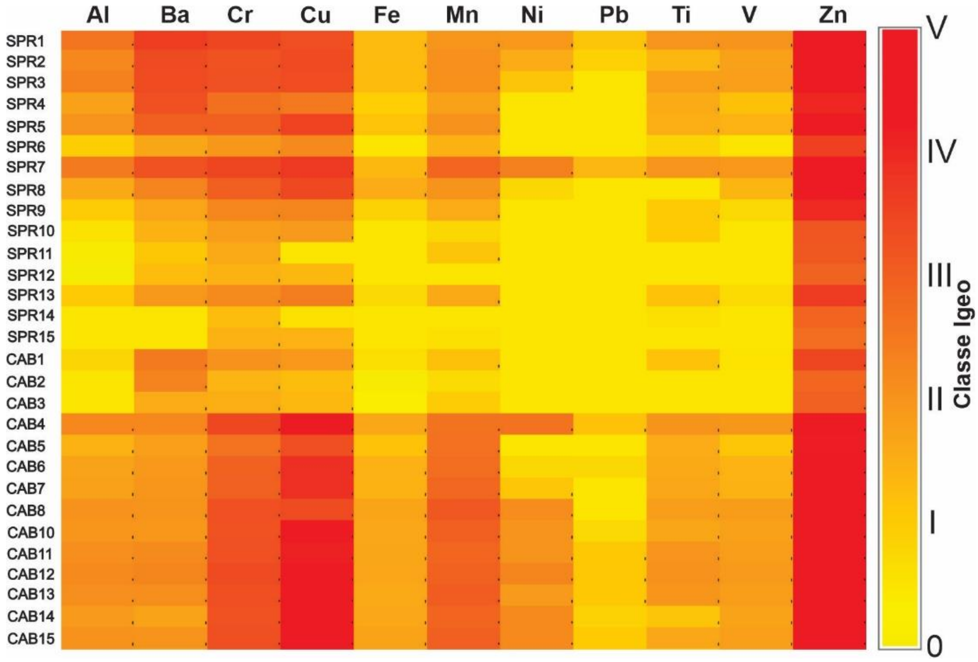
The particle grain size analysis showed a predominance of fine grains in CAB (<63 µm). In this area, ~36% of the sediments were classified as clayey, ~21% as sandy clay, ~7% as silty sandy clay and ~36% as clayey sand. In SPR, clayey sediments corresponded to only ~7% of the samples, while ~13% corresponded to sandy clay, ~7% to silty clay, ~7% to sandy silty clay, ~60% to clayey sand and ~7% to sand silica (Figure 8).

Figure 8 – Shepard diagram from sediments sites samples



To assess the enrichment of metals in sediment samples, the Geoaccumulation Index described by Müller (1981) was calculated. It comprises 6 pollution classes (0, I, II, III, IV and V) classified as unpolluted, unpolluted to moderately polluted, moderately polluted, moderately to highly polluted, highly polluted, highly to very highly polluted and very highly polluted, respectively. The Igeo classes in both areas for Al and Ba were I and II, respectively. For the other metals, CAB presented greater pollution, which class I was found for Pb; class II for Fe, Ni, Ti, and V; class III for Cr and Mn; class IV for Cu and class V for Zn, while SPR had class 0 for Ni and Pb; class I for Fe, Ti and V; class II for Cr and Cu; and class IV for Zn (Figure 9).

Figure 9 – Heatmap shows the classes of metal pollution in the samples according to Igeo proposed by Müller (1981)



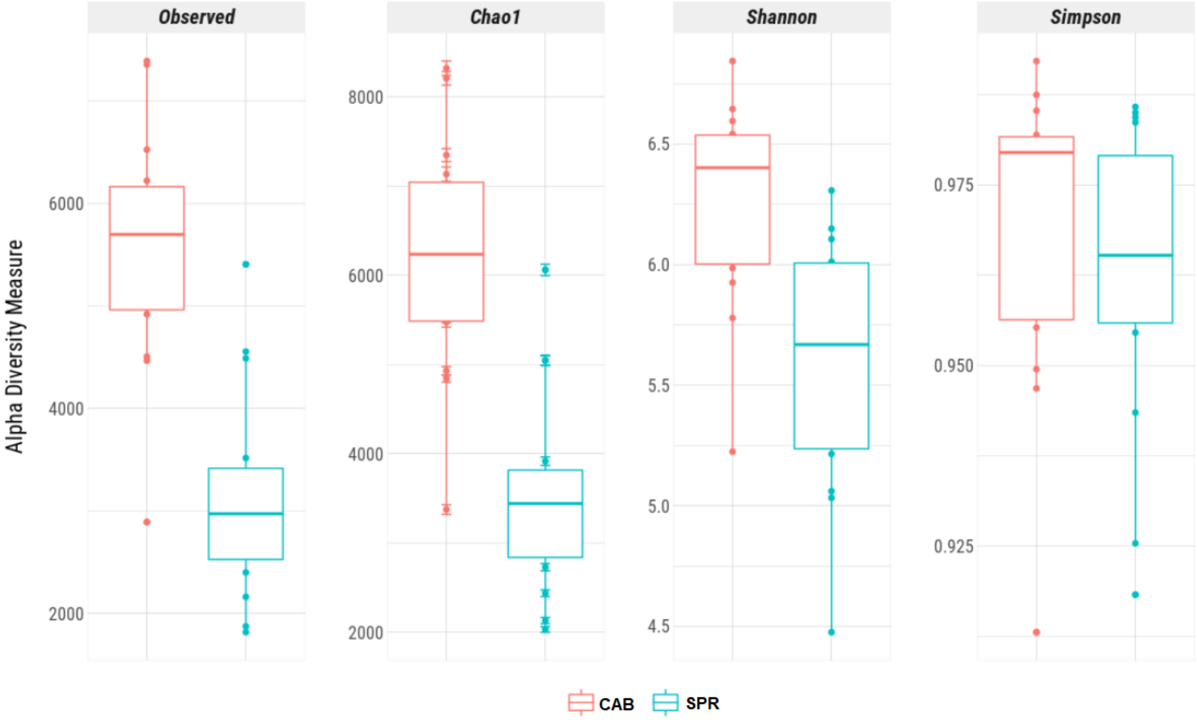
5.3.2 Overview of 16S rRNA sequencing

After filtering the low-quality reads and trimming the adapters and barcodes, 5.363.092 sequences were obtained from the 29 sediment samples. From them, we removed 80 Eukarya sequences and 10.525 Chloroplast sequences. No sequence was classified as Mitochondria. Samples were subsequently rarefied at 35.829 reads per sample to normalize read counts across the 29 samples. Average coverages of 0.9949 and 0.9962 were achieved in SPR and CAB, respectively, demonstrate a good coverage of diversity, with a total of 47111 ASVs from São Paulo River and 77999 ASVs from Caboto Beach.

5.3.3 Richness and diversity of the microbiome community

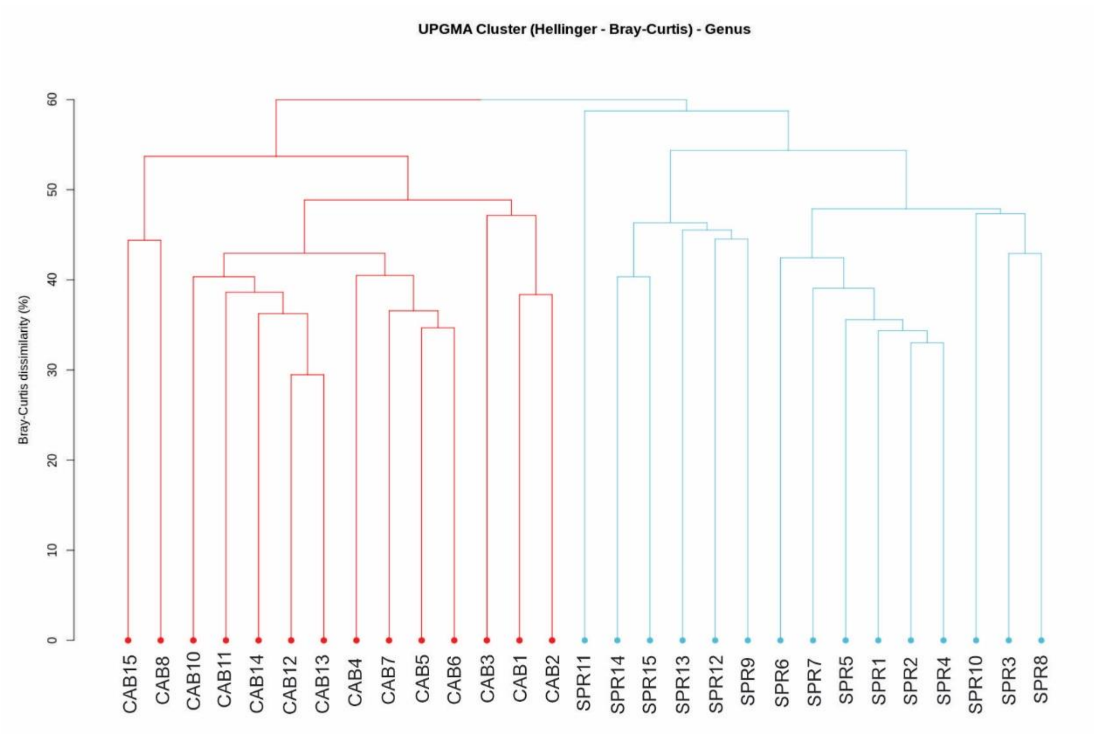
The sequencing of the 16S rRNA revealed that CAB had greater diversity and richness than SPR, which the amount of ASVs observed in the samples varied between 1816 and 5406 in SPR and between 2891 and 7390 in CAB, differing statistically according to the Tukey test ($p < 0.05$). The Ace and Chao1 indices were similar to the number of ASVs observed in both areas, indicating that the quantity of samples collected was satisfactory. In detail, Ace index and Chao1 in CAB varied between 3228.80 and 7981.88 and between 3373.34 and 8321.00, respectively, while in SPR the same indices varied between 2017.57 and 5875.19 and between 2028.75 and 6060.31, respectively. Similarly, Shannon index indicated greater diversity in CAB (Shannon index of 5.22 - 6.84) than in SPR (Shannon index of 4.47 - 6.30), differing statistically. Regarding the Simpson index, although this was higher in CAB (Simpson index of 0.913 - 0.992) than in SPR (Simpson index of 0.918 - 0.985), it did not differ statistically (Figure 10).

Figure 10 – Statistics diversity indexes from SPR and CAB samples



Although CAB has higher levels of alpha diversity, beta diversity was higher in SPR (βw = 2,941; βt = 6,870), showing greater heterogeneity between the collection points when compared to CAB (βw = 2,266; βt = 5,281). Tree Cluster based on dissimiliração Bray-Curtis sequencing of the 16S rRNA (Figure 11) shows greater genus dissimilarity in the SPR, probably associated with the geographical location of the point, given the existence of refinery and oil transportation pipelines, mainly in the middle reaches of the river.

Figure 11 – Cluster tree Bray-Curtis dissimilarity analisys from São Paulo River and Caboto Beach sediments at genus level



5.3.4 Taxonomic profile of SPR and CAB metagenomes

In both areas, the Bacteria domain was predominant, comprising ~ 94% of the sequences, while Archae comprised only ~ 1%. Sequence analyzes showed a total of 42 phyla (5 archae and 37 bacteria), 79 classes, 292 orders, 335 families and 548 genus.

Proteobacteria was the most abundant phylum in the Bacteria domain in SPR and CAB (51.45 and 60.16%, respectively), as also observed in other Brazilian mangroves (ANDREOTE et al., 2012; NOGUEIRA et al., 2015), followed by Firmicutes (21.83 and 9.13%, respectively), Bacteroidetes (7.07 and 7.04%, respectively) and Chloroflexi (6.33 and 6.59%, respectively). In SPR, the other phyla with an abundance above 1% were Actinobacteria (3.41%), Planctomycetes (3.08%), Acidobacteria (2.01%) and Epsilonbacteraeota (1.56%), while in CAB the other phyla with an abundance above 1% were Plancomycetes (3.85%), Epsilonbacteraeota (3.64%), Acidobacteria (2.22%), Actinobacteria (1.69%), Latescibacteria (1.07%) and Calditrichaeota (1.06%) (Figure 12A). These phyla, added to Fusobacteria and

Spirochaetes correspond to 89.79% of the total ASVs shared between the two areas. In the archae domain, the phyla Euryachaeota, Thaumarchaeota, Nanoarchaeota and Crenarchaeota were identified in both areas, of which Euryachaeota was the dominant phylum in SRP and CAB, corresponding to ~86% of ASVs. Among the exclusive phyla, Nitrospinae (Bacteria domain) was found only in SPR with 11 sequences of the genus *Nitrospina*, while Diapherotrites (Dominio Archae) was exclusive exclusively in CAB with 140 sequences.

At class level, Gammaproteobacteria was dominant in SRP and CAB (33.4 and 40.7%, respectively). In SRP the other classes with relative abundance above 1% were Clostridia (15.5%), Deltaproteobacteria (10.5%), Alphaproteobacteria (7.2%), Bacteroidia (6.5%), Bacilli (6%), Anaerolineae (5.4%), Planctomycetacia (2.6%), Acidimicrobial (2.4%), Campylobacteria (1.5%) and Thermoanaerobaculia (1.3%), while in CAB the other classes were Deltaproteobacteria (14.5%), Clostridia (8.4%), Bacteroidia (6.2%), Anaerolineae (5%), Alphaproteobacteria (4%), Campylobacteria (3.6%), Plancomycetacia (2.4%), Thermoanaerobaculia, Thermoleophilia and Dehalococcoidia (1.5%), Acidimicrobiia and Phycisphaerae (1.4% both), Thermplasmata (1.3%), and Latescibacteria and Calditrichia (1.0% both). The classes Iainarchaeia, Chlorobia, and Nitrospira were exclusive to CAB, while the classes Methanobacteria, Nitrospinia and Zetaproteobacteria were exclusive to SRP. Significantly enriched taxa (i.e., with a log10 odds ratio> 0.3 or <-0.3) were found in the samples. For example, Bacilli, Clostridia were highly enriched in the SPR samples; whereas Campylobacteria, Fusobacteriia, Phycispharaerae and Thermoplasmata were more abundant in the CAB samples (Figure 12B).

In SPR, 260 orders were detected, of which 14 were exclusive while in CAB 278 were detected, of which 32 were exclusive. The most abundant orders were Vibrionales (21.1% and 24.7%), Clostridiales (15.5% and 8.4%) and Desulfobacterales (6.0% and 6.9%) in SPR and CAB, respectively. The significantly enriched orders (i.e., with a log10 odds ratio > 0.3 or < -0.3) were Bacillales, Microtrichales, Rhizobiales and Sphingomonadales in SPR and Campylobacterales, Fusobacteriales, Oceanospirillales, Syntronphobacterales and Thiotrichales in CAB.

At the family level, the microbial taxa also showed distinct distribuition patterns in the diferent areas (i.e. São Paulo River vs Caboto Beach) (Figure 12C). For example, Arcobacteraceae, Fusobacteraceae, Nitrincolaceae were significantly enriched (i.e. with an odd ratio > 0.3) in the CAB mangrove sediment, whereas only

Planococcaceae was abundant (i.e. with na odd ratio < - 0.3) in the SPR sediments. In SPR and CAB, 295 and 305 families were found, respectively, of which 31 were exclusive to SPR and 38 exclusive to CAB.

At genus level, in SRP and CAB, respectively, 34.9% and 37.4% of sequenceas were not classifie. *Vibrio* was the most abundant genus in (12.0%), followed by *Clostridiisalibacter* (8.81%), *Catenococcus* (8.85%) and *Woesia* (2.02%) while in CAB the decrease abundance was *Catenococcus* (15.73%), *Vibrio* (6.79%), *Woesia* (3.52%) and *Clostridiisalibacter* 2.61%). 60 genus were exclusives to SPR and 96 exclusives to CAB. *Bacillus*, *Brassicibacter*, *Exiguobacterium*, *Romboutsia* e *Thioclava* were enriched (i.e. with na odd ratio < -0.8) in the SPR, while *Arcobacter*, *Marinomonas*, *Proprionigenium* were more enriched (with odds ratio of -1.52, -1.41, -1.25 and 2.92, respectively) in the CAB (Figure 21D).

5.3.5 Microbiome structure and function of SPR and CAB sediments

The results demonstrated that the number of total predicted functional genes was higher in CAB than SPR, and despiste this, both areas are very similar functionally. The predicted genes in SPR and CAB, respectively, were more closely associated with methane metabolism (31.57% and 33.20%), sulfur metabolism (16.39% and 16.87%), nitrogen metabolism (13.95% and 14.22%), photosynthesis (12.52% and 12.06%), heavy metal resistence or reduction (23.77% and 21.54) and PHA degradation (1.80% and 2.10%) (Figure 13A). Between heavy metals resistence or reduction genes, fives types of enzymes were abundat (> 0,05). They were ArsR family transcriptional regulator, P-type Cu^+ transporter, peptide/nickel transport system ATP-binding protein, peptide/nickel transport system permease protein and peptide/nickel transporte system substrate-binding protein (Figure 13B).

Figure 12 – (A) The composition of the microbial communities in the SRP and CAB sampling sites at the phylum level, (B) and a differencial analysis at classes level, (C) at family level, (D) and at genus level. For the differencial analysis, values are based-10 logarithm of the odds ratio. The positive and negative values indicate taxa that are more in CAB and SPR, respectively.

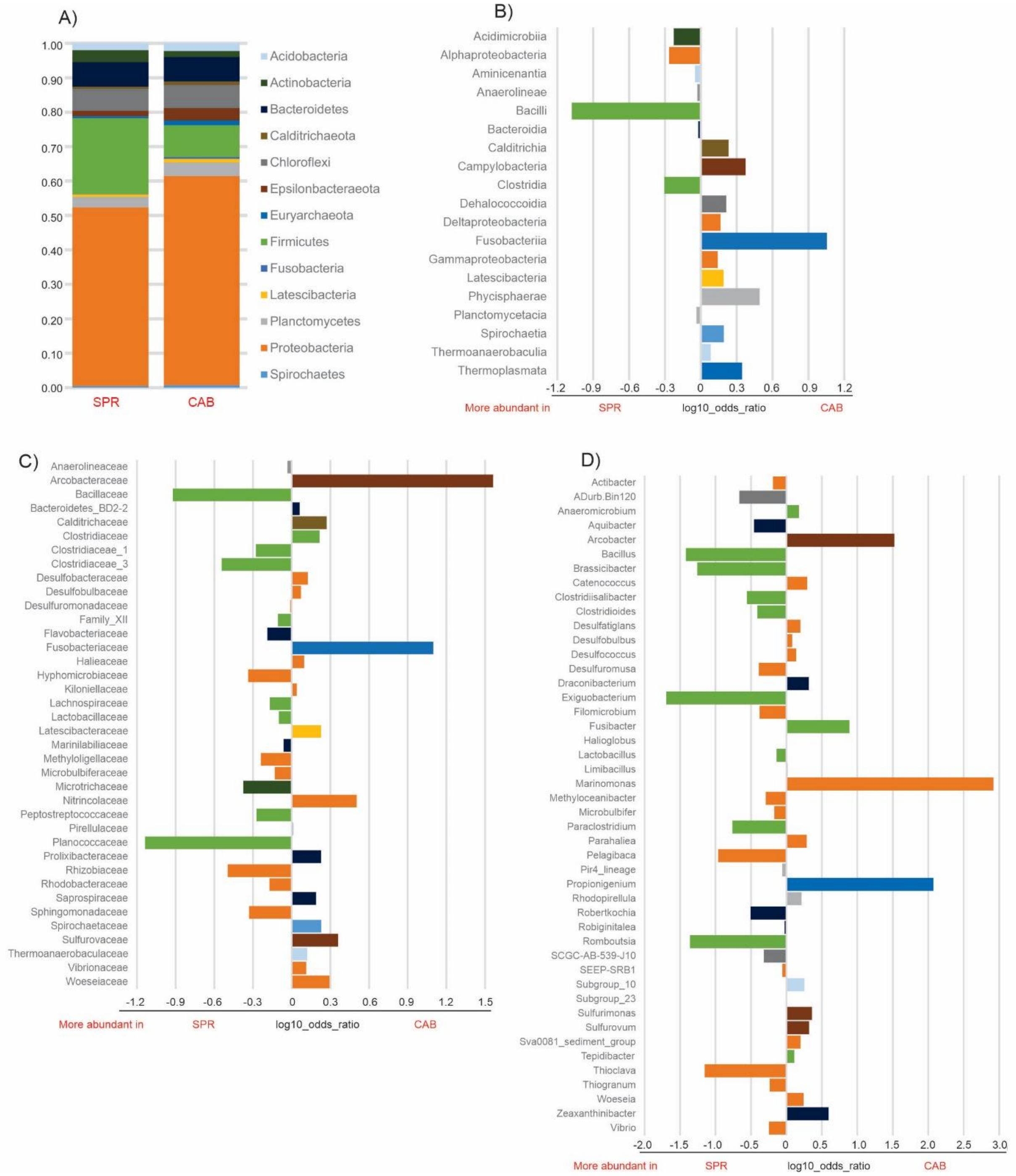
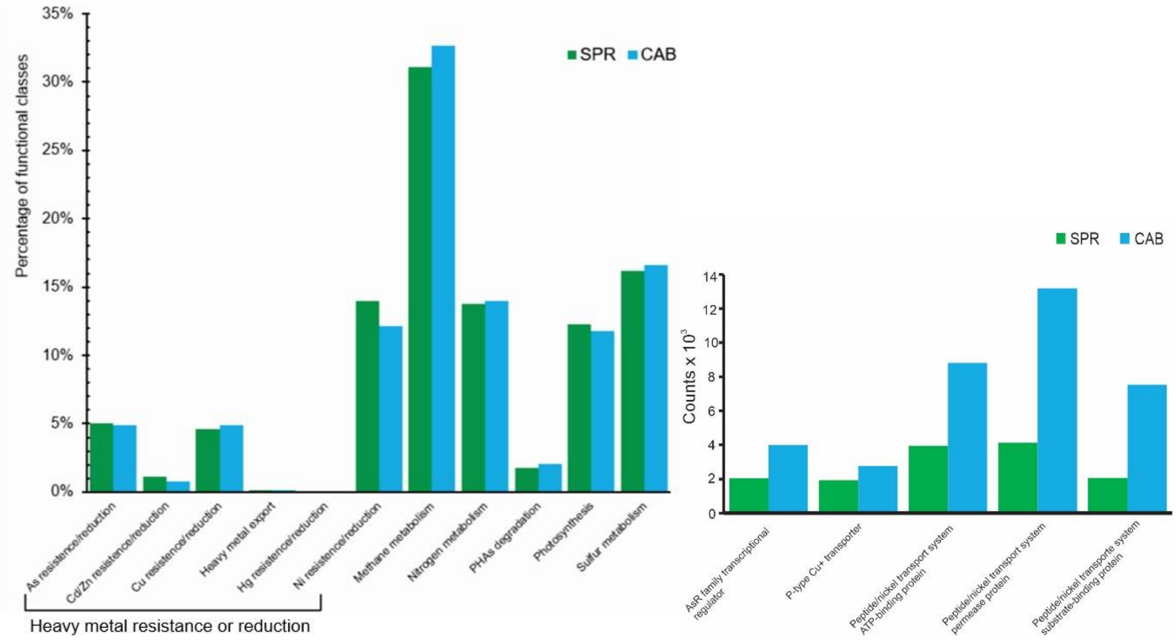


Figure 13 – (A) Clusteres of Orthologus Group annotatios from the São Paulo River and Caboto Beach areas. (B) Enzymes of heavy-metals resistance or reduction in the KEGG were predicted.



Twent phylum shared heavy-metal resistence and/or reduction genes in both areas, they were: Acidobacteria, Actinobacteria, Bacteroidetes, Calditrichaeota, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Epilonbactereota, Euryachaeota, Firmicutes, Fusobacteria, Halanaerobiaeota, Kiritimatiellaeota, Latescibacteria, Plactomycetes, Proteobacteria, Spirochaetes, Tenericutes, Thaumarchaeota e Verrucomicrobia. Beetwen these, Proteobacteria, Firmicutes and Bacteroidetes had the most varieties and counts of heavy metal resistance/reduction genes, including 282 genus, which 179 were Proteobacteria (68, 68 and 43 in Alpha, Gamma, and Deltabacteria, respectively), 69 genus were Firmicutes, and 34 genus were grouped in Bacteroidetes.

Genes to PHA degradation were found in both areas presente in 64 and 73 genus respectively. 12% of these were in *Mycobacterium* and 8% were in *Pseudoceanicola* in SPR while in CAB 15% were in *Marinobacterium*, 13 % were in *Desulfosarcina* and 5% were in *Desulfococcus* and *Desulfotitalea.*

5.3.6 Correlation between microbiome community and environmental parameters

In this paper, temperature, pH, Eh, salinity, total dissolved solids, total nitrogen, total sulfur, total organic carbono, avalaible phosporus and the elements Al, As, Ba, Cd, Cr, Cu, Fe, Mn, Pb, V, Ti and Zn were measured to assess the relative contribuition to microbiome composition. The redundancy analysis revealed a clear separation beetwen SPR and CAB, highlighting six parameters with major contributions to bacterial community (Figure 14.) Beetwen them, total nitrogen, total sulfur, dissolved oxygen and dissolved solids, in addition to the Cu metal were positively correlated with the CAB samples. Axes 1 and 2 of the RDA explain 28.95% and 20.36%, respectively, generating 49.31% of the accumulated proportion.

In addition, a Spearman's rank correlations analysis was carried out beetwen chemical variables and the abundance of the main phyla in the microbial community (Figure 15). Although no Strong correlation was found (< -0.8 or > 0.8) (SANTOS, 2007), Bacteroidetes exhibited a greater number of significant correlations ($p < 0.05$), these being in relation to Ba, Cu, Fe, Mn, Ni, Pb, Ti, Zn, V and avalaibe phosphorus, while Euryachaetoa had significant correlations only to Fe.

Figure 14 – Redundance analysis (RDA) of the microbial community, environmental parameters and samples

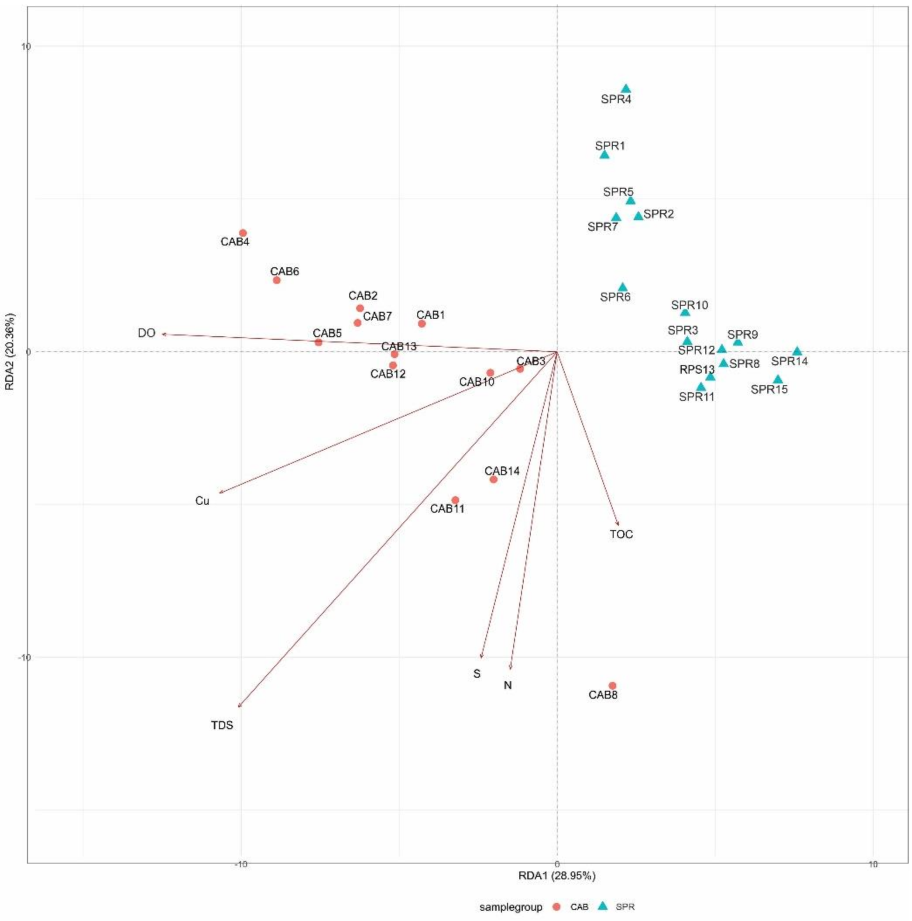
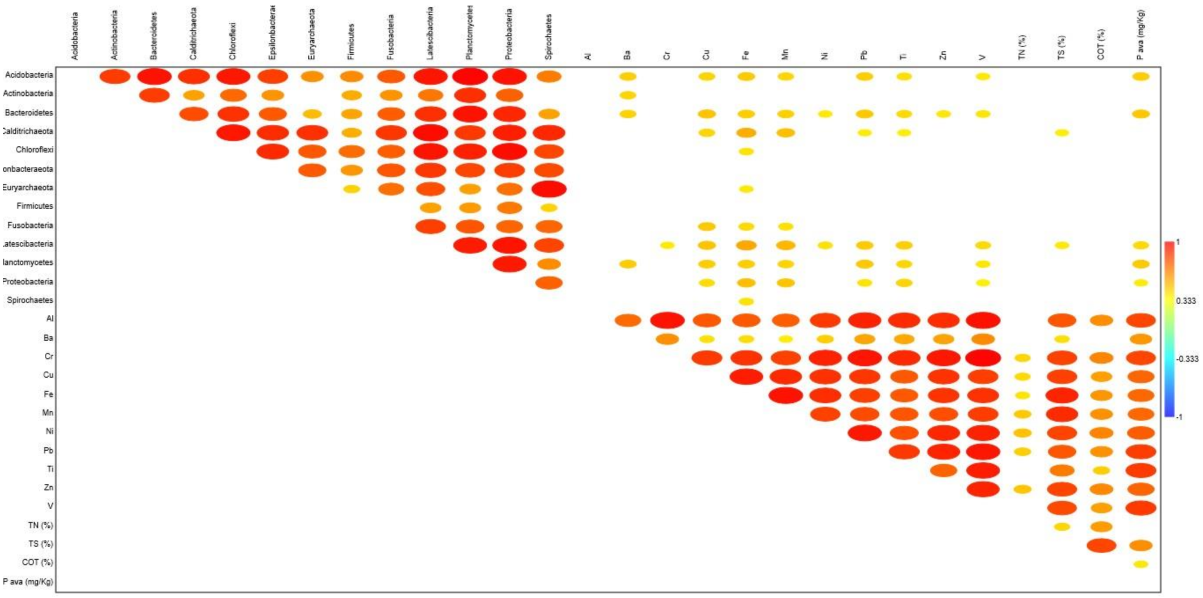


Figure 15 – Heatmap of the Spearman's rank correlation coefficients between microbes phyla and physical-chemical parameters. Only significant correlations (p < 0,05) are shown. The color grade and the proporcional circle size are proportional to the weight of correlation.



5.4 DISCUSSION

In the presente study, we analyzed the impacts of metal on the composition and structure of the microbiome of mangrove sediments in an area chronically impacted by the oil industry for over 60 years. Although it is known of the potential impact of these elements on microbial communities in rivers and estuaries over time, the full understanding of this phenomenon remains not fully understood (HEMME et al., 2010; AZARBAD et al., 2015; CHEN et al., 2018).

Most mangrove forests in northeastern Brazil are considered preserved because they are not inserted directly in urban areas as in other regions of the country (Nogueira et al., 2015), however, the study area of this article receives a constant supply of pollutants from the oil production, refining and transportation industry, in addition to the circulation of vessels and other potentially polluting enterprises.

The higher proportion of fine sediments in CAB (silt + clay) makes this area more sensitive to the adsorption of metals by the tendency of the silt-clay particle to retain pollutants as widely reported in the literature (SALOMONS; FÖSTER, 1984; KASTRATOVIC et al., 2016; ZHANG et al., 2016; MANJU et al., 2020; SANTOS et al., 2020). At the time of collection, CAB showed a higher concentration of total dissolved solids, particles that can act as colloidal transporters of metals, especially in the hydroxides and oxides forms (PINTILIE et al., 2007; SANTOS, 2015). Despite being greater in the SPR, no statistically significant difference was found between the total organic carbon of the areas, which plays an important role in controlling pollutants. (PEREIRA, 2010; WANG et al., 2006; OROS et al., 2007; CHEN; CHEN, 2011).

The analyzes performed showed metal pollution in both areas, but CAB samples were more polluted for the Cu, Fe, Mn and Ni. The metals Al, Ba, Cr, Pb, Ti, V and Zn did not show statistically significant differences between areas. Regarding biological aspects, CAB exhibited greater biomass and taxonomic richness when compared to SPR, whose Shannon-Weaver index showed a statistically significant difference, and the Simpson index, although higher in CAB, was not statistically significant, which is due the largest amount of rare species found in the mangrove sediments of Caboto Beach.

Several authors have demonstrated that the increase in the concentration of metals causes a reduction in biomass and microbial diversity (FLIESSBACH et al., 1994; CHANDER, et al., 1995; STEFANOWICZ et al., 2008; CHEN et al., 2018; LI et al., 2020), while others found no differences (TAVARES et al., 2016). In addition, Gillan et al. (2005) indicated that microbial communities in contaminated sediments can be as diverse as preserved sediments. In this study, Cu and the nutrients total sulfur and nitrogen seem to be strong contributors to the composition of the microbiome, according to RDA analysis. We hypothesized that possible organic pollutants - not measured in this study - also contributed to the difference in alphadiversity between the environments analyzed. Despiste the difference of TOC between areas was not statistically significant, although greater in SPR, this parameter indicates events of eutrophication and oil pollution (MEYER-REIL; KÖSTER, 2000; EVANS et al., 1990). Nascimento et al. (2017) found in this place concentrations of acenaphthene, antrhacene, benzo(a)anthracene and benzo(a)pyrene above TEL (threshold effects level) and dibenzo(ah)anthracene above PEL (probable effects level), compounds capable of causing reduction of biomass and microbial diversity associated with it (HENTATI et al., 2012; RAMADASS et al., 2015). In addition, this region has suffered frequent oil spills over the years (JESUS, 2011), and traces of oil in the mangroves surrounding refinery are common.

Regarding taxonomic data, the dominance of both areas by representatives of the phyla Proteobaceria, Firmicutes and Bacteroidetes is consistent with the dominance in other soils impacted by metals (CHEN et al., 2018; JANSSEN, 2006; ELLIS et al., 2003). In addition to these, an increase in the abundance of Chloroflexi in soils polluted by metals has been reported (AZARBAD et al., 2015; CHODAK et al., 2013). In the Proteobacteria phylum, the high abundance of Gammaproteobacteria in both areas is according to Dos Santos et al. (2010) who found dominance of this class both inpreserved mangroves and in experiments simulating oil spills, and diverging from Ellis et al. (2003) who found dominance of this class both in preserved mangroves and in experiments simulating oil spills, and diverging from Ellis et al. (2003). Deltaproteobacteria also showed great abundance, being a class of high prevalence and Brazilian and Indian mangroves (FERNANDES et al., 2014; Dos SANTOS et al., 2011). According to Liang et al. (2007), representatives of Deltaproteobacteria act in the anaerobic reduction of sulfur and sulfates. In fact, this group has an extensive metabolic spectrum, which includes a wide range of aerobicity, adaptation to temperature variation and the occurrence of photoautotrophism and chemoautotrophism (WILLIAMS et al., 2010).

In the Firmicutes phylum, both areas were dominated by Clostridia, however, the Bacilli class was more enriched in SPR (with an odd ratio of -1.07), especially for *Bacillu*s and *Exiguobacterium* phyla (with na odd ratio -1.41 and -1.69, respectively), petroleum indicator organisms as they act in the degradation of oil hydrocarbons (LUSTOSA et al., 2018; NWEKE; OKPOKWASILI, 2010). While *Exiguobacterium* use 4-chloro-2-nitrophenol (4C2NP) as the sole carbon source and energy (ARORA et al., 2012), *Bacilli,* especially *B. carboniphilus* e *B. oleivorans* are diesel oil degraders and tolerant to organic solvents (AZMATUNNISA et al., 2015).

In the Archae domain, the high proportion of organisms in the Euryarchaeota phylum diverged from studies in areas impacted by anthropic activities. For example, Lomakina et al. (2018) found similar abundances beetwen Euryachaeota and Thaumarchaeota in bottom sediments in an oil discharge area in lake in southern Siberia, while Dias (2012), Tavares (2016) and Chang et al. (2015) found predominance of Thaumarchaeota in mangroves from the São Paulo state, in bottom sediments from port area in northwastern Brazil, and sediments from hot springs in China, respectively. Although no significant difference was found for the total nitrogen concentrations between the areas, this parameter may have contributed to increase the abundance of Thaumarchaeota in Caboto Beach, since they are key organisms in the nitrogen cycle (PESTER et al., 2011; KOZLOWKI et al., 2016; KIMBLE et al., 2018) as the main responsible for ammonium oxidation (SCHELPER; NICOL, 2010) and release of N_2O (LÖSCHER et al., 2012).

Some rare phyla in our samples (< 1 %) were more abundant in other studies. For exemple, the Cyanobacteria phylum comprised only 0.1% of the assigned sequences in both areas, but it is abundant after frequent oil spills (IBAMA 2008; AL-THUKAIR et al., 2007; TAVARES, 2015), as well as members of the order Alteromonadales (Dos SANTOS et al., 2011), which assigned here only 0.2% and 0.5% of the strings in SPR and CAB, respectively. Cyanobacteria are more abundant in surface ocean waters, however, they tend to suffer population enrichment from environmental disturbances (ZWIRGLMAIER et al., 2008), and although they are not oil degraders, they act indirectly in this process, expanding the ecological niches favoring the growth of degrading organisms (SANCHÈZ et al., 2006).

As shown by the RDA analysis, the levels of total sulfur and total nitrogen were positively correlated with the CAB samples, suggesting that these parameters contributed to the shape of the microbiome. Sulfator-reducing bacteria of the

Desulfobacterales order were the main assigned to sulfur and nitrogen metabolism in CAB while in SPR they were Rhizobiales, but in different pathways. While Rhizobiales participates in the assimilation of sulfate to sulfite, covering nutritional needs, Desulfobacterales participates in the dissimilar reduction of sulfate, contributing about 50% of carbon remineralization in anoxic sediments (CASPI et al., 2010). In addition Desulfobacterales were the order with major abundance of genes for resistance to metals and PAHs.

In the present study, *Desulfosarcina*, *Crassaminicella* and *Silicimonas* in CAB, and *Cohaesibacter*, *Mycobacterium* and *Pelagibaca* in SPR were the genus that presented the greatest abundance of genes to metals resistance in CAB. In the literature, the genus commonly related to this process in environments with a history of contamination are *Rhodopirellula*, *Pseudomonas* e *Thiobacillus* (LAGE et al., 2012, HUANG et al., 2017; CHEN et al., 2018). In addition, *Desulfosarcina* e *Mycobacterium*, and *Marinobacterium*, *Desulfococcus* e *Desulfatitalea* had also the major abundance of genes to HPAs resiatance. *Desulfosarcina* e *Desulfococcus* are the main alkane degraders in marine infiltrations (KLEINDIENST et al., 2014), and *Mycobacterium* is a Strong PHAs degrader in contaminated mangroves (WILLUMSEN et al., 2001; HENNESSE et al., 2009; GUO et al., 2011), showing high potencial for use in the bioremediation of environments impacted by oil (BISHT et al., 2015; DUDHAGARA; DAVE, 2018). Dos Santos et al., 2011 found na increase in the *Marinobacterium* abundance, due to oil in controlled experiments (DOS SANTOS et al., 2011).

Among the genes for resistance to metals, the findings corroborate the adaptation of the microbiome to these pollutants, especially Ni, Cd/Zn, Cu e As. *Mycobacterium* in SPR (5%), *Caminicella* and *Crassaminicella* in CAB (3% both) were the main genus with Arsr Family transcriptional regulator genes (K03892), which work reducing As (V) to As (III) by the reductase arsenate enzyme and subsequent efflux of As (III) by membrane protein (WYSOCKI et al., 2003; JACOBSON et al., 2012). The main way of Cu resistance in the samples, exhibited mainly *Mycobacterium* in SPR and *Desulfosarcina* in CAB, occurs through active pumping mediated by ATPses, enzimes that require the prevoius conversion of Cu(II) para Cu(I) (SOLIOZ; STOYANOV, 2003) to guarantee the homeostasis of organisms when effluxing the metal and maintaining adequate concentrations on the cytoplasmic side (ARGÜELLO et al., 2012). In relation to Ni, this metal acts as a cofactor for several enzymes, but it is toxic in the form Ni^{+2} when there are flaws in the homeostatic mechanism of microorganisms (MACOMBER; HAUSINGER, 2011). The main genera here assigned to Ni resistance were Cohaesibacter in SPR and Desulfosarcina in CAB, while other studies of Ni resistance are assigned to *Cupriavidus*, *Klebsiella* (MACOMBER; HAUSINGER, 2016) and *Methylobacterium* (ALBOGHOBEISH et al., 2014).

In our study, the areas analyzed showed differences in richness and diversity, being significantly smaller around the oil refinery, however, the metabolic diversity and resistance to metals were very similar due to the functional redundancy that microbial communities normally have. Over time, these organisms adapt to prolonged stress due to contamination by metals (HEMME et al., 2010) through complexation, efflux and redox reactions, as observed in the counting of resistance genes.

The metagenomic comparison helps to elucidate issues related to Microbial Ecology (DELMONT et al., 2011), as well as promoting the implementation of more robust sequencing techniques in order to obtain a holistic view of the interaction between microbiome and environment, thus outlining new strategies for environmental monitoring and bioremediation of environments impacted by oil and industrial activities.

5.5 CONCLUSION

Our datasets indicate that both areas are polluted by metals, mainly CAB by Cu, Fe, Mn and Ni. Possible organic pollutants and the total sulfur and nitrogen nutrients probably shaped the microbial communities by the enrichment of some groups in different areas, favoring the occurrence of different ecological niches that increased the functional redundancy. Despiste the low funcional difference beetwen areas, the taxons responsible for the metabolic routes and resistance to metals and PAHs were diferentes. However, the microbiome of both areas are adapted and have mechanisms of resistance to metals. The mangrove nearest to oil refiney were pooer in diversity that the most distante, confirming the impact that this exploration has on the microbiome

For future studies, we suggest an approach based on metatranscriptomics and metabolomics, in addition to quantification of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons. In this way, knowledge about gene regulation at the transcriptional level of microorganisms in the face of the presence of oil will be expanded, contributing to elucidate the evolutionary mechanisms involved in the degreasing and resistance of oil components in order to encourage the implementation of new technologies for bioremediation of areas impacted by more efficient processes that have less impact on the environment.

6 CONLUSÃO FINAL

Ambas as áreas avaliadas nesse estudo se encontram com algum nível de poluição por metais de origem antropogênica especialmente em função das atividades petrolíferas, mas, diferente do esperado, os maiores índices de poluição foram registrados nas amostras de manguezal da Praia do Caboto. Por outro lado, deve-se considerar que os sedimentos da Praia do Caboto possuem maior proporção de grãos finos quando comparados aos sedimentos do Rio São Paulo, tornando-os mais propensos a adsorção de poluentes. O Rio São Paulo exibiu baixo nível de poluição para Al e Fe, moderada para Ba, Cr e Cu, alta para Zn e ausente para Ni e Pb, enquanto Praia do Caboto teve poluição baixa para Al, e Pb, moderada para Fe, Ni, Ti e V, moderada a alta para Cu e muita alta para Zn. Apesar do enriquecimento antropogênico, nenhum dos metais que possuem valores internacionais de referência extrapolou os limiares de riscos iminentes à biota (PEL), entretando, o Ba em amostras do Rio São Paulo e Cu e Zn em amostras de ambas as áreas estiveram acima do limiar TEL, referência abaixo da qual os efeitos adversos são raramentre encontrados. Em relação ao As e Cd, nenhuma das amostras está poluída por esses metais.

A análise taxonômica revelou diferenças de riqueza e diversidade entre as áreas, sendo maiores na Praia do Caboto, uma vez que apresentou maior quantidade de ASVs observadas, maiores índices de Shannon e Simpson e maior quantidade de táxons raros encontrados. As amostras colhidas no manguezal do Rio São Paulo apresentam distribuição mais heterogênea de metais, possivelmente relacionada à localização da refinaria, pois os pontos mais poluídos encontram-se mais próximos de tubulações da óelo. É muito provável que poluentes orgânicos estejam em maiores concentrações no manguezal do Rio São Paulo, também com distribuição heterogênea, justificando a maior betadiversidade nas amostras do Rio São Paulo. Ainda que os dados obtidos permitam afirmar que Praia do Caboto não possa ser considerado um manguezal pristine, a comparação com o também impactado Rio São Paulo possibilitou uma comparação interessante, pois embora ambos estejam poluídos pelos metais analisados, apresentam diferenças significativas quanto a presença de organismos raros.

Os microbiomas de ambas as áreas apresentam adaptações frente ao etstresse oxidativa provocado pelos elementos analisados, especialmente ao Ni, Cd/Az, Cu e As. Genes para resistência ao Ni corresponderam a aproximadamente

60% da abundância de genes para resistência a metais, o que sinaliza indiretamente poluição por petróleo uma vez que esse metal serve como indicar. Além do mais, mesmo que em concentrações abaixo de TEL, a maior porcentagem de genes para resistência a níquel no microbioma do Rio São Paulo indica que essa área experimenta mais impactos negativos pela presença do petróleo.

A grande quantidade de sequências não classificadas tanto em Caboto quanto no Rio São Paulo indica que ainda há muita biodiversidade bacteriana para ser descoberta, ou que o sequênciamento de nova geração, ainda que uma potente ferramenta para detectar organismos não cutiváveis por técnicas convencionais, não consegue ainda cobrir todo o espectro de vida microscópica. Devido a grande quantidade de amostras coletadas e sequências obtidas pelo sequênciamento do 16S rRNA durante o período de doutorado, muitas informações poderão ser obtidas a médio e longo prazo e disponibilizadas em futuras publicações, especialmente quanto a identificação de grupos raros ainda não descritos em manguezal sob influência do petróleo e associação com rotas metabólicas ou mecanimos de resistência a metais.

Assim, essa tese apresentou os resultados na forma de dois artigos submetidos a revistas científicas, contudo, o autor e colaboradores projetam pelo menos mais três publicações dentro dos dois próximos anos. Ademais, as bibliotecas metagenômicas estarão dispobilizadas em domínio público hospedadas no Nacional Center for Biotechnology Information – NCBI podendo servir para futuros estudos por outros grupos de pesquisadores. Además, para estudos futuros sugere-se a utilização da metatranscriptômica e metabolômica por permitir estudar em tempo real os mecanismos adaptativos da microbiota de manguezal frente às adversidades ambientais impostas pela contaminação por petróleo, o que fornecerá novas ferramentas tecnológicas e mesmo patentes aplicáveis à recuperação de ambientes impactados por atividades antrópicas.

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APÊNDICES

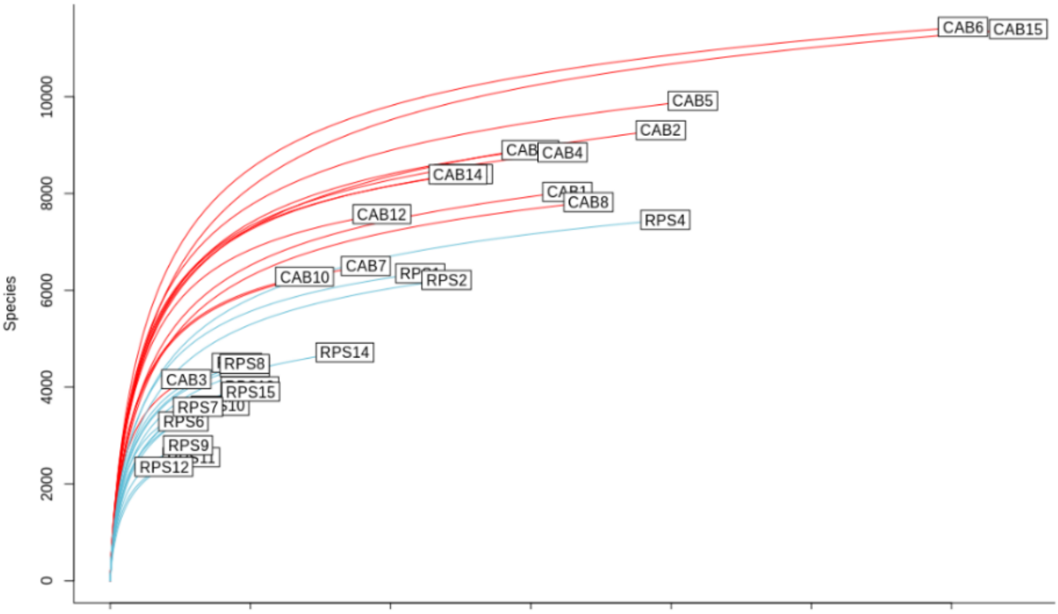
Tabela 7 – Coordenadas geográficas dos pontos de coleta de sedimentos superficiais

Rio São Paulo		Praia do Caboto	
SPR1	12º42’06”S 038º33’18”W	CAB1	12º44’46”S 038º29’41”W
SPR2	12º42’10”S 038º33’15”W	CAB2	12º44’39”S 038º29’42”W
SPR3	12º42’17”S 038º33’12”W	CAB3	12º44’32”S 038º29’57”W
SPR4	12º42’20”S 038º33’20”W	CAB4	12º44’13”S 038º29’56”W
SPR5	12º42’22”S 038º33’09”W	CAB5	12º43’58”S 038º29’43”W
SPR6	12º42’30”S 038º33’06”W	CAB6	12º43’51”S 038º29’42”W
SPR7	12º42’39”S 038º33’10”W	CAB7	12º43’48”S 038º29’44”W
SPR8	12º42’41”S 038º32’57”W	CAB8	12º43’43”S 038º29’44”W
SPR9	12º42’45”S 038º32’46”W	CAB9	12º43’43”S 030º29’54”W
SPR10	12º42’56”S 038º32’42”W	CAB10	12º43’48”S 038º30’02”W
SPR11	12º43’03”S 038º32’31”W	CAB11	12º43’50”S 038º30’07”W
SPR12	12º43’08”S 038º32’24”W	CAB12	12º43’54”S 038º30’16”W
SPR13	12º43’17”S 038º32’16”W	CAB13	12º43’56”S 038º30’20”W
SPR14	12º43’27”S 038º32’08”W	CAB14	12º43’57”S 038º30’26”W
SPR15	12º43’38”S 038º32’04”W	CAB15	12º43’58”S 038º30’29”W

Tabela 8 – Índices de diversidade dos microbiomas do Rio São Paulo e Praia do Caboto

Sample	**nº sing**	**nº seq**	**goods**	**ASV obs**	**Chao1**	**se.chao1**	**ACE**	**se.ACE**	**Shannon**	**Simpson**	**Inv Simpson**
SPR1	602	194335	99,69023	4555	5042,604	52,04944	4887,844	34,39159	6,307546	0,985819	70,51682
SPR2	664	215246	99,69152	4488	5046,67	56,84938	4900,975	34,55788	5,735728	0,9652633	28,78802
SPR3	495	84600	99,41489	3140	3474,057	39,2648	3430,23	28,1024	5,668844	0,9671825	30,47156
SPR4	724	352524	99,79462	5406	6060,315	63,95764	5875,193	37,84916	6,001827	0,9675002	30,76947
SPR5	539	80761	99,3326	3316	3715,424	44,98762	3631,687	28,83687	5,869991	0,9744894	39,19938
SPR6	444	46873	99,05276	2399	2726,82	40,68406	2663,811	23,94472	5,460406	0,9619162	26,25787
SPR7	426	55552	99,23315	2654	2946,016	36,997	2895,798	25,46665	6,106102	0,9850284	66,79296
SPR8	544	84075	99,35296	3275	3660,629	43,22454	3612,886	29,01132	6,149183	0,9843726	63,99005
SPR9	376	50438	99,25453	2161	2435,319	36,99543	2383,954	23,04779	5,257437	0,9545628	22,00838
SPR10	443	71141	99,37729	2664	2949,431	35,49028	2922,106	25,36984	5,215144	0,9598249	24,89103
SPR11	326	50258	99,35135	1816	2028,751	30,83639	2017,576	20,95903	4,475945	0,9182538	12,23299
SPR12	369	35829	98,97011	1873	2128,248	34,74806	2108,702	21,22856	5,060432	0,9434822	17,69353
SPR13	541	90689	99,40346	2972	3440,173	52,83976	3301,444	27,33465	5,033158	0,9253706	13,39954
SPR14	484	149404	99,67605	3516	3914,928	47,5204	3795,634	30,32795	6,011806	0,9836716	61,243
SPR15	453	87226	99,48066	2876	3219,55	42,22286	3135,977	26,88439	5,453623	0,9572234	23,37726
TOTAL				**47111**							
CAB1	735	283999	99,7412	5367	6110,099	72,59032	5803,926	37,63723	5,778972	0,9494875	19,79709
CAB2	895	330500	99,7292	6222	7133,31	80,71298	6821,152	40,72711	6,595472	0,9874935	79,95866
CAB3	550	45944	98,80289	2891	3373,348	54,02267	3228,808	26,6153	6,178179	0,9786847	46,91475
CAB4	792	268626	99,70517	5991	6772,137	73,3796	6482,433	39,73726	6,844306	0,9921754	12,780146
CAB5	885	338983	99,73892	6525	7346,786	72,75554	7089,872	41,49207	6,522858	0,9809008	52,35819
CAB6	868	502579	99,82729	7356	8207,308	76,35997	7906,382	43,16011	6,645424	0,9852949	68,0037
CAB7	556	156958	99,64577	4506	4927,557	46,76084	4789,757	33,93037	6,052605	0,9595756	24,73753
CAB8	653	279976	99,76677	4920	5475,817	57,05802	5294,721	35,91379	5,224416	0,9130569	11,50178
CAB10	574	120549	99,52385	4466	4842,318	41,08805	4756,736	33,31995	6,369798	0,9808004	52,08434
CAB11	727	254102	99,71389	5880	6467,753	57,08544	6270,05	39,0458	5,985874	0,9552592	22,35099
CAB12	607	164088	99,63008	5090	5515,743	45,17483	5392,738	35,94218	6,43409	0,9803238	50,82276
CAB13	678	209347	99,67614	5643	6202,763	56,33335	5990,036	38,13625	6,541823	0,981945	55,3864
CAB14	682	212271	99,67871	5752	6266,902	51,56282	6099,504	38,41649	6,4695	0,9778087	45,06267
CAB15	929	546219	99,82992	7390	8321,006	80,84018	7981,885	43,7185	5,925588	0,9468308	18,80788
TOTAL				**77999**							

Figura 16 – Curvas de rarefação dos metagenomas



Fonte: Próprio autor