Science of the Total Environment 740 (2020) 139556

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Oil leakage induces changes in microbiomes of deep-sea sediments of Campos Basin (Brazil)

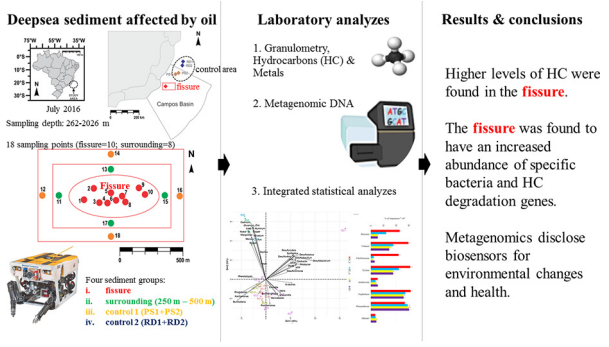
Luciana R. Appolinario [a], Diogo Tschoeke [a,b], Gabriela Calegario [a], Luiz Henrique Barbosa [a], Manuel A. Moreira [d], Ana Luiza S. Albuquerque [d], Cristiane C. Thompson [a], Fabiano L. Thompson [a,c,*]

[a] Laboratory of Microbiology, Instituto de Biologia, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[b] Center of Technology e Biomedical Engineer Program - COPPE, UFRJ, Rio de Janeiro, Brazil
[c] Center of Technology - CT2, SAGE-COPPE, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
[d] Programa de Pós-Graduação em Geoquímica, Universidade Federal Fluminense (UFF), Niterói, Brazil

HIGHLIGHTS

- Campos Basin sediments have higher hydrocarbon values compared to the control areas.
- *Geobacter*, *Pelobacter*, and SRB stood out as biosensors of oil pollution.
- Genes responsible for oil degradation are more abundant in oil impacted areas.
- The effects of oil contamination are restricted to the area around the bottom fissure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 April 2020
Received in revised form 16 May 2020
Accepted 17 May 2020
Available online 21 May 2020

Editor: Thomas Kevin V

Keywords:
Marine sediments
Metagenomics
Oil biodegradation
Campos basin
Microbial diversity

ABSTRACT

The Campos Basin (100,000 km^2) is located on the continental shelf of southeastern Brazil. Despite the significant oil and gas industrial activities underway in the Campos Basin, scarce information is available regarding the hydrocarbon contents and microbial communities in the deep-sea sediments. To gain new insights on these aspects, we first obtained deep-sea sediment samples with different degrees of oil exposure. We obtained samples from a seabed fissure ($N = 28$), surroundings (250 m to 500 m from the fissure; $N = 24$), and a control area ($N = 4$). We used shotgun metagenomics to characterize the taxonomic and metabolic diversity and analyzed biogeochemical parameters (metal and oil concentration) of all samples. The high levels of unresolved complex mixture of hydrocarbons in the fissure indicate a potentially recent petrogenic contribution in these sediments. The fissure area was found to have a higher abundance of hydrocarbonoclastic bacterial genera and hydrocarbon degradation genes. These bacteria may be used as biosensors of sediment contamination. The effects of oil contamination, mainly around the fissure, are less clear at 250 m and 500 m, suggesting that the surroundings may not have been heavily affected by the oil leakage. Our study demonstrates that metagenomics can disclose biosensors for environmental monitoring.

1. Introduction

Oil is released into the marine environment worldwide as a result of oil natural infiltrations into the seabed (Hazen et al., 2016). However, oil spills during exploration and production phases may have a greater

* Corresponding author at: Laboratory of Microbiology, Instituto de Biologia, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.
E-mail address: thompsoncristiane@gmail.com (F.L. Thompson).

https://doi.org/10.1016/j.scitotenv.2020.139556
0048-9697/

impact on marine systems (Berenshtein et al., 2020). Oil weathering and biodegradation occurs at a significantly slower rate in sediments than in the water column. In the Gulf of Mexico sediments, the half-life of crude oil hydrocarbons was 500 days for oil deposited (Bagby et al., 2017a). However, the unique biogeochemical features of sediment from different oil production basins possibly influence the pace of oil biodegradation and thus, it is not clear if all marine sediments respond in the same manner. It is relevant to analyze the effect of oil leakage on microbial communities affected by recent incidents in different geographic regions.

Although much of the hydrocarbons from sub-sea oil spills and natural seeps may rise to the surface, there are water-soluble components in oil as well as hydrocarbons that adhere to solid particulates that can settle in deep-sea sediments (Ramseur, 2010). For example, after the 1979 Ixtoc I oil spill in which over 3 million barrels of oil flowed into the Gulf of Mexico, it was estimated that 25% of the oil was transported to the seafloor (Jernelöv and Lindén, 1981). Studies in the Gulf of Mexico have shown that much of the oil spilled in the Macondo accident (about 4.9 million barrels spilled, Deep Water Horizon by BP in April of 2010) was deposited in the vicinity (<8 km) of the wellhead and contaminated an area of approximately 3200 km^2 around the wellhead (Valentine et al., 2014). After four years, approximately 74.8% of the aliphatic (with >29 carbons) and 24.9% of the aromatics (with >14 carbons) persisted in the samples because molecular mass and hydrocarbon structure influence biodegradation rates (Bagby et al., 2017b). Progressive degradation is slower with increasing molecular mass number of rings, and alkyl branches. Certain microbes, e.g. *Colwellia*, *Cycloclasticus*, *Roseobacter* and sulphate reducing bacteria, are considered markers of Macondo oil deposition in the Gulf of Mexico (Hamdan et al., 2018; Mason et al., 2014; Yang et al., 2016).

The Campos Basin is located on the continental shelf of southeastern Brazil and is the second largest oil production location in Brazil, with a total area of 100,000 km^2. Oil exploration began in 1977 and currently there are approximately 35 oil and gas producing fields in operation (ANP, *Boletim da Produção de Petróleo e Gás Natural*, Agosto 2019). It is a region of particular environmental relevance due to the diversity of pelagic and benthic species, including new deep-sea coral reefs (Almada and Bernardino, 2017; Yamashita et al., 2018). The heterogeneity of habitats in the Campos Basin results from its particular oceanographic features, such as the upwelling of cold and nutrient-rich waters and the presence of a complex set of muddy, sandy and bioclastic sediments (Muniz and Bosence, 2015). Despite the industrial activities underway in the Campos Basin, scarce information is available regarding the hydrocarbon contents and hydrocarbonoclastic microbial community diversity that thrives in these sediments.

An oil spill in the Campos Basin (20–24°S and 39–42°W), 120 km from the coast of Rio de Janeiro (Brazil), released ~3700 barrels of oil into the marine environment in the end of 2011 (ANP, 2012). In addition, during March of 2012, a new leakage was recorded and a sizeable seabed fissure was detected in the same area. Wells in the Campos Basin account for nearly 36% of national oil production (i.e., ≥1.2 million oil barrels per day) (ANP, 2019). Nevertheless, there is a lack of studies on the possible effects of this oil spill on the microbial metabolic repertoire and biogechemical features of affected sediments in the Campos Basin.

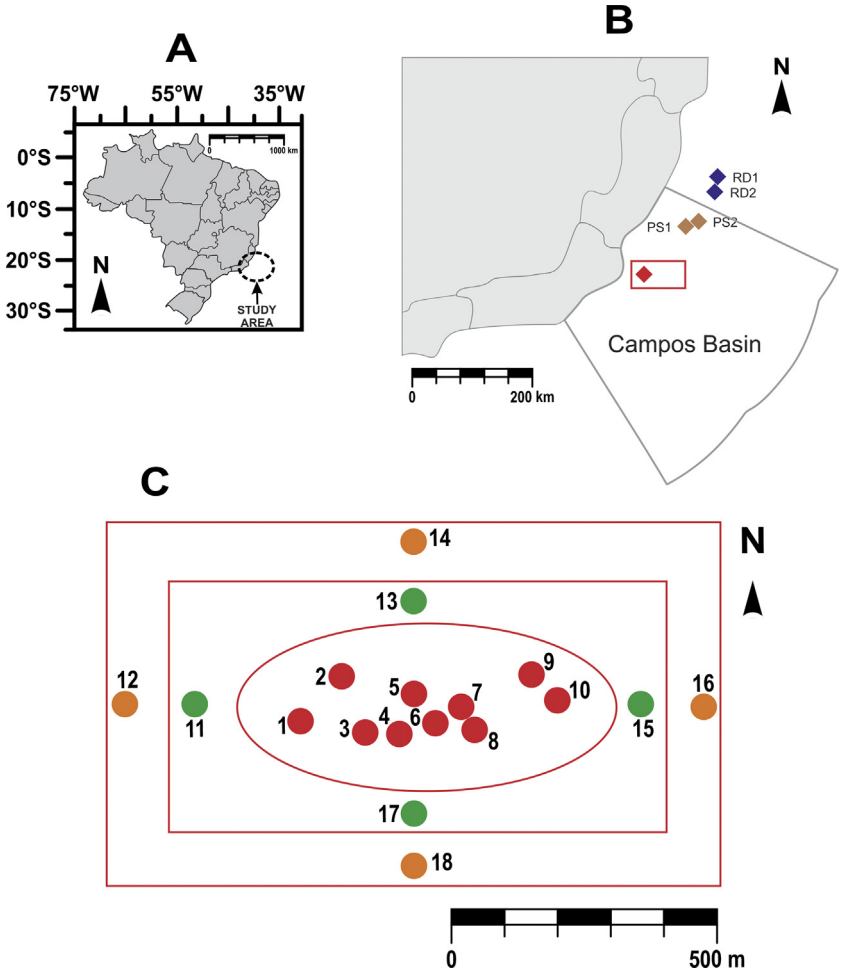


Fig. 1. Location of the study area. (A) Brazil. Campos Basin oil field-Rio de Janeiro and control points. (B) Campos Basin oil field and control points (PS and RD). (C) Points within the Campos Basin oil field. Points located in the fissure within the red ellipse. Smaller rectangle: Points located 250 m away from the fissure. Larger rectangle: Points located 500 m away from the fissure. Control locations included (PS1, PS2, RD1, and RD2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Sediment microbial communities may be involved in oil biodegradation, which is suggested in previous studies in Macondo and other oceanic regions (Babcock-Adams et al., 2017; Bacosa et al., 2018; Stout et al., 2017), however, no studies are available that investigate the deep-sea sediments of the South Atlantic Ocean.

The deep-sea sediment microbial diversity associated with an oil leakage event in Campos Basin was investigated in a broad geographic context (seabed fissure, surroundings, and control areas). Here, we test the hypothesis that oil leakage affects the microbial diversity of the different locations affected by the oil. Therefore, to characterize taxonomic and functional microbial diversity in sediments, we first obtained 56 deep-sea sediment samples from a seabed fissure ($N = 28$), surroundings (250 m–500 m distance from the fissure; $N = 24$), and control areas ($N = 4$). These control areas have not been impacted by oil spills, while the selected surrounding area around the fissure would have been affected if the oil plume would fall off as depositing particles. We then used shotgun metagenomics to characterize the microbial diversity (taxonomic and functional), analyzed physical-chemical parameters (metal and oil concentration), and evaluated the relationship between hydrocarbon microbial gene counts and oil concentration in the sediments. We also performed a comprehensive and integrated analysis to detect possible biosensors for oil pollution.

2. Materials and methods

2.1. Sediment sampling

Sediment samples were collected in the Campos Basin oil field (21°52′19.58″S/39° 49′44.86″W DATUM SIRGAS, 2000) at 1200 m depth over an area of a fissure and at a distance of 250 and 500 m during July of 2016 (see Fig. 1 and Table 1). Sampling was performed using a working-class ROV (Saab) system and a dedicated stainless steel core sampling device. This device was decontaminated after each sampling point. In total, three independent replicates from 18 points were obtained from the Campos Basin, except for point 7 for which only one replicate was obtained. Samples were stored in sterile polypropylene centrifuge tubes (50 mL) and immediately placed in liquid nitrogen (onboard) until further analysis at the Federal University of Rio de Janeiro (UFRJ).

Control sediment samples (PS1, PS2, RD1, and RD2) outside the oil field were also included (Table 1). These control samples were obtained >40 km north of the Campos Basin oil field in an area with no oil production activity or a history of oil spill. Comparisons were performed across the four groups: i. fissure (BC_F), ii. surrounding (comprised the points 250–500 m; BC_A), iii. control 1 (PS1 + PS2), and iv. control 2 (RD1 + RD2).

2.2. Measurements of hydrocarbons and metals

Concentrations of polycyclic aromatic hydrocarbons (PAH), unresolved complex mixture (UCM), resolved aliphatic hydrocarbons (HRP), total n-alkanes and total petroleum hydrocarbons (TPH) were measured using the U.S. Environmental Protection Agency (EPA) methods. To obtain the solids content, the EPA 3550C method was used. For which, the sample was mixed with a hydrous sodium sulfate to form a free-flowing powder. This was extracted using a solvent (once) and ultrasonic extraction. A portion of the extract was collected for cleanup and/or analysis. Total concentrations of metals, including aluminum, barium, cadmium, chromium, copper, iron, manganese, nickel, lead, vanadium, mercury, and zinc were analyzed as described previously (EPA 7474A, EPA 7473, EPA 1631E and EPA 7471E).

2.3. Metagenomics

DNA extraction and Illumina shotgun libraries were prepared as described previously (Appolinario et al., 2019). Quality control of generated sequence was performed with PRINSEQ (Schmieder and

Table 1
UTM Coordinates to each sample and measurements of nutrients organics and metals.

Sampling points	UTM coordinates (WGS84 datum)		Depth	Hydrocarbons (μg/kg^{-1})					Metals (mg/kg^{-1})														
	Latitude	Longitude	(m)	PAHS	UCM	HRF	n-alkanes	HTP	Al	Ba	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	V	Zn
BC1	414,140	7,579,063	1.169	42,288.51	1,380,260	236,790	5680	1,691,700	528.1	25.7	<0.365	10.2	3.01	5311.5	<0.015	66.5	2.65	2.48	15	16.9
BC2	414,206	7,579,132	1.172	146.25	2360	680	680	44,000	585.2	34.4	<0.402	10.9	3.49	5194.9	<0.016	105.8	3.59	2.73	14.2	18
BC3	414,227	7,579,064	1.173	11,814.28	180,610	23,040	3700	443,700	556.7	22.2	<0.386	9.35	2.97	4559	<0.015	60.7	3.24	2.47	12.6	15.6
BC4	414,266	7,579,061	1.175	73.16	7570	780	780	13,300	544.4	30.5	<0.377	9.76	2.86	4742.6	<0.015	101	3.45	2.46	13.1	15.6
BC5	414,297	7,579,113	1.177	46.02	<160	370	370	6000	553.2	26.5	<0.383	9.5	2.93	4659.2	<0.015	95.5	3.17	2.6	12.7	14.7
BC6	414,314	7,579,080	1.178	14,824.48	307,280	45,840	5570	641,200	530.4	20.1	<0.371	8.52	2.79	43344	<0.015	77	2.64	2.28	11.5	13.2
BC7	414,348	7,579,106	1.181	10,387.7	196,220	28,000	4400	434,600	538.6	27	<0.373	10.5	3.1	5220	<0.015	88.5	3.14	2.59	13.8	16.5
BC8	414,364	7,579,068	1.180	30.32	<160	260	260	1200	542	16.6	<0.382	4.88	1.64	3618.9	<0.015	69.9	2.59	1.73	6.7	8.87
BC9	414,461	7,579,144	1.185	45.22	<160	540	540	4900	601.3	105.3	<0.414	12	3.67	5613.3	<0.017	130.1	4.41	2.99	16.1	17.9
BC10	414,468	7,579,109	1.185	90.66	<160	480	480	7100	600.2	104	<0.414	12.8	3.88	5794.5	<0.017	132.4	4.25	3.31	16.7	19.7
BC11	413,882	7,579,058	1.156	149.22	<160	2040	2040	10,900	616.3	83.6	<0.424	11.4	3.3	5755.9	<0.017	94.8	3.74	3.24	18.1	17.8
BC12	413,630	7,579,060	1.143	149.81	<160	620	620	6200	610.5	50.8	<0.396	13.9	3.78	6009.8	<0.016	94.7	4.47	3.67	23.1	22.7
BC13	414,305	7,579,345	1.179	96.96	<160	360	360	4700	528.8	57.8	<0.372	9.08	2.74	4406.8	<0.015	100.3	3.22	2.46	12.2	14.2
BC14	414,305	7,579,605	1.181	119.52	3460	590	590	8000	593	38	<0.409	11.5	3.46	5670.2	<0.016	118.5	3.96	3.05	17.8	17
BC15	414,687	7,579,105	1.201	157.74	<160	630	630	11,900	550.3	47.5	<0.388	9.26	2.9	4611.6	<0.016	98.1	3.15	2.39	12.4	14.6
BC16	414,929	7,579,111	1.211	197.95	<160	430	430	8800	619.4	37	<0.436	12.3	3.68	5487.6	<0.017	115.3	4.22	2.89	15.4	19.3
BC17	414,305	7,578,828	1.175	142.43	<160	820	820	19,000	607.4	45.8	<0.421	11.9	3.37	5691.3	<0.017	106.1	4.03	2.81	16.5	19.1
BC18	414,304	7,578,576	1.175	98.09	<160	390	390	12,900	601.6	68.6	<0.416	7.51	2.21	3858.4	<0.017	63.5	2.31	1.79	9.3	10.7
PS1	7,614,673	445,128	875.5	22.51	<160	4.28	1.048	23.58	7713.8	103.1	<1.59	16.7	12.2	10,667.7	<0.016	219	6.3	7.19	25.3	26.6
PS2	7,623,587	466,852	2026.0	52.5	<160	2.87	0.869	17.97	11,229.3	140.5	<2.36	21	24.9	11,378.3	<0.024	278	6.9	7.47	32.8	34.2
RD1	7,862,533	567,227	262.6	19.32	<160	3.8	0.782	15.37	4196.9	8.08	<1.57	22.6	5.65	17,732.3	<0.016	138.2	6.87	5.81	57.1	33.3
RD2	7,787,334	552,299	1763.4	53.91	<160	2.95	0.917	19.86	11,041.6	152.4	<2.44	22.6	20.3	6201.7	<0.024	304.2	7.73	5.98	34.9	34.4

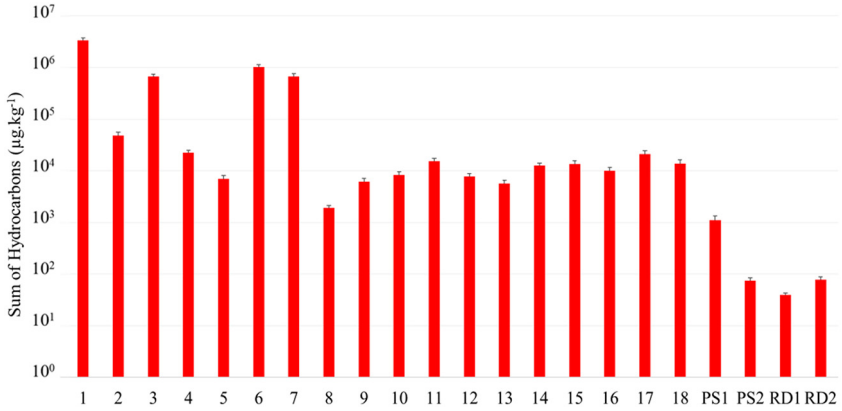


Fig. 2. Bar graph of the concentrations of sum of PAHS, UCM, HRF, n-alkanes, and TPH hydrocarbons found in the sediment in this study. Results are expressed with standard error. Values ($\mu g/kg^{-1}$) are mean (standard error; $n = 3$). Control locations included (PS1, PS2, RD1, and RD2).

Edwards, 2011) and paired-ends were merged with PEAR (Zhang et al., 2014) as previously described (Campeão et al., 2017). Annotation and functional analyses were carried out using the MG-RAST platform (Glass and Meyer, 2011). Protein sequences were predicted from assembled scaffolds using Prodigal (Hyatt et al., 2010). The NCBI nr database was used for functional identification, while the taxonomic identification was obtained by means of Diamond and an e-value cut-off of 10^5 (Buchfink et al., 2014). Assembled contigs were binned using the "superspecific" configuration of MetaBAT to obtain partial or complete microbial genomes (Kang et al., 2015). Genome quality was assessed by CheckM (Parks et al., 2015). Protein sequences related to monoaromatic hydrocarbons (benzene, toluene, ethylbenzene, and xylenes [collectively known as BTEX]), alkanes, and PAH-degrading genes were downloaded from Swiss-Prot (http://www.uniprot.org/uniprot) into a local database.

Metagenomic annotation (taxonomic and functional) and physicochemical parameters of the sediment samples were subjected to principal components analysis (PCA), Pearson correlation, and a Student's *t*-test (U de Mann-Whitney, Wilcoxon W were performed using the "vegan" package in R software, version 1.0.143) (Oksanen et al., 2017).

3. Results and discussion

3.1. Hydrocarbons (HC) and metal concentrations in sediments

Campos Basin sediments have higher Hydrocarbon [HC] values compared to the control area (Table 1). Campos Basin sampling points 1, 3, 6, and 7 had the highest HC concentration values, suggesting a possible recent source of oil leakage (Fig. 2). The sum of all polycyclic aromatic hydrocarbon (PAHs) values, unresolved complex mixture (UCM), hydrocarbons resolved fraction (HRF), n-alkanes and total petroleum hydrocarbons (TPH) was several orders of magnitude higher (3.35 g/kg^{-1}) in the fissure (BC1) than in the control area (39.27 $\mu g/kg^{-1}$; RD1) (Table 1). Six of the ten sampled points in the fissure (BC1, BC2, BC3, BC4, BC6 and BC7) had high levels of UCM (Table 1). The UCM was found to be significant in oils that survived degradation and is considered a marker for the presence of crude oil (Babcock-Adams et al., 2017; Gao et al., 2007; Harris et al., 2011). All analyzed metal elements were more abundant in the control areas (RD and PS), and Al and Fe were found to be even more abundant in control areas than the other metals. This may be a result of physical conditions, bioturbation and bio-irrigation, organic matter remineralization, and/or the Fe and Mn redox cycle (Burdige, 2006). The presence of oil around the oilfield sampling points may function as a barrier to prevent the exchange process between the water column and sediment, which would favor oil degradation and its release into the water column.

3.2. Taxonomic identification of metagenomic sequences

Approximately 39 million metagenomic sequences were annotated (Table 1S). The majority of the sequences belongs to *Bacteria* (~91%) and Archaea (~7.5%). Hydrocarbonoclastic archaeal genera (e.g. *Methanosarcina*) involved in hydrocarbon degradation were found (Fig. 1S). The phylum *Proteobacteria* corresponded to over 60% of the metagenomic sequences (Fig. 3), 61% in the fissure, while in the PS control

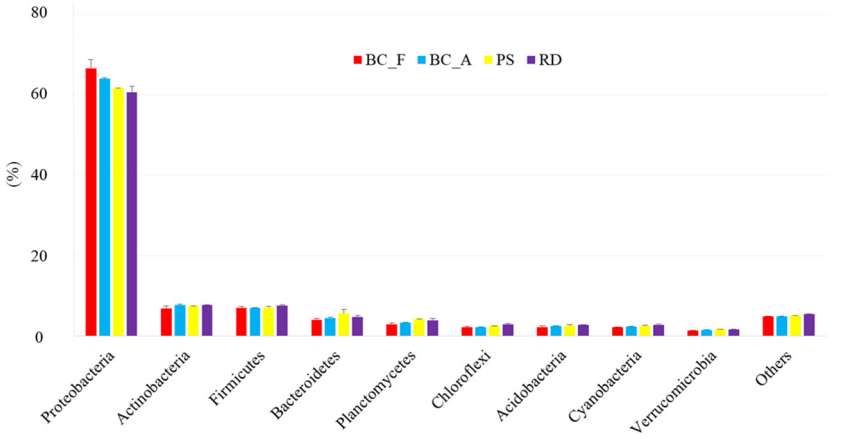


Fig. 3. Metagenomic sequences of phylum bacteria profile. Results are expressed as mean and standard error. BC_F (fissure, $n = 10$), BC_A (around, $n = 8$), PS (control 1, $n = 2$) and RD (control 2, $n = 2$).

achieve 54% (p-value = .0481) and at RD 53% (p = .0017). *Actinobacteria* and *Firmicutes* comprised <10% each. *Actinobacteria* in the fissure (6%) was lower than in the BC points of 250 m and 500 m BC_A (7.2%; p = .0855), as well the control areas PS (7.5%; p = .0191) and RD (7.6%; p = .027). *Geobacter* (3%) was also more abundant at metagenomes fissure. While response for 2.1% (p = 0.00058) and (, 2.3% (p = .07507) of the identified genera at PS and RD samples, respectively. *Pelobacter* genus (1.5%) were more abundant at fissure than in the control PS (0.61%; p-value = .03394) and RD (0.63%; p = .06405) samples (,), as well as *Desulfuromonas* genus had higher abundance at fissure (0.4%) than PS control (0.11%; p-value = .00399) and RD control (0.13%; p = .01334) samples (Fig. 4). The higher abundance of these bacteria in the fissure suggests their involvement in oil biodegradation and is in agreement with previous studies (Hamdan et al., 2018; Yang et al., 2016).

Pelobacter and *Geobacter* are found in marine sediments and oil reservoirs they can reduce Fe (III) (Lovley, 2017). *Geobacter* species are involved in the degradation of aromatic compounds in the Fe (III) reduction zone of petroleum-contaminated aquifers. Other hydrocarbonoclastic bacteria were also found, including sulfur and sulfate-reducing bacteria SRB (*Desulfovibrio*, *Desulfatibacillum*, *Desulfococcus*, *Desulfobacterium*, *Desulfobulbus*, *Desulfurivibrio*, and *Desulforomonas*). SRB were also found in metagenome assembled genomes (Table 2S). SRB can oxidize BTEX, while sulfate serves as the final electron acceptor. SRB can also promote anaerobic iron corrosion. Another relevant hydrocarbonoclastic bacteria found in the sediments in the present study was *Syntrophus*. Members of this genus can degrade alkanes and fatty acids in syntrophic association with methanogens (Jones et al., 2008). They are often critical components of the microbial community in oil affected sites. Typical oil-degrading bacteria found in the water column (e.g. *Alcanivorax*, *Alteromonas*, *Colwellia*, *Cycloclasticus*, *Marinobacter*, *Pseudoalteromonas*, and *Roseobacter*) were detected in Campos Basin sediment metagenomes in low abundance (<1%), suggesting that the oil plume formed after the oil spill may have not deposited in the study area.

3.3. Biosensors of oil contamination disclosed by metagenomics

The distribution of bacterial genera in the PCA suggests that some groups stood out as biosensors of oil pollution (Fig. 5). *Geobacter*, *Pelobacter*, and SRB are related to oil levels observed in the fissure, while other groups are related to levels of metals. The Pearson correlation coefficient analyzes corroborates this association pattern (Fig. 5). Previous studies have suggested that microbial communities respond quickly to environmental disturbances and carry an ecological memory even after the pollutant has been degraded, suggesting that certain microbes could serve as quantitative biosensors (Smith et al., 2015). Previous studies have reinforced this hypothesis of biosensors in controlled laboratory experiments whereby the biodegradation of oil was evaluated in seawater incubation experiments (Appolinario et al., 2019; Campeão et al., 2017). Hydrocarbonoclastic *Alcanivorax*, *Alteromonas*, *Colwellia*, *Marinobacter* and *Pseudomonas* grew immediately after the addition of oil into seawater. Several metabolic features stood out as biosensors, such as Sulfur, Fatty Acids, Lipids and Isoprenoids, Aromatic Compounds, and Iron Acquisition were related to oil levels in the sediment samples (Fig. 6).

3.4. Hydrocarbon degradation genes that increase in the fissure area

The expansion of hydrocarbonoclastic genes in the fissure (BC_F) is higher compared to the surrounding (250–500 m; BC_A) and control points (PS, RD) (Fig. 7). For example, in the fissure samples, the relative abundance of genes involved in benzene degradation increased approximately 1.9-fold relative to the surrounding area (250 m–500 m) and 3.9-fold relative to the control area (PS and RD). Genes involved in ethylbenzene degradation increased approximately 11-fold relative to surrounding samples and 22-fold relative to the control samples. The concomitant expansion of genes responsible for the degradation of hydrocarbons (BTEX, alkanes and PAHs) and microorganisms responding to these different fractions of oil biodegradation is consistent (Fig. 4). Despite the possible volatility and short-lived effects of BTEX on the contaminated sediment microbial communities, clear effects of these hydrocarbons were identified in the fissure area. These effects indicate a response of the microbial communities towards (and an enrichment of) the hydrocarbons gene pool to more efficient biodegrade hydrocarbons. Monoaromatic hydrocarbons (benzene, toluene, ethylbenzene, and xylenes, collectively known as BTEX) are of particular concern because of their high toxicity. BTEX compounds are the most water-soluble constituents of petroleum and have a relatively low sediment-water coefficient (Weelink et al., 2010). Polycyclic aromatic hydrocarbons (PAHs) contain two or more fused aromatic rings.

Among the expansion of hydrocarbonoclastic genes in the fissure, the most commonly found genes were within the BTEX (~55%) and naphthalene (~19%) biodegradation pathways (Fig. 7). These genes appear excellent biosensors for oil degradation in sediments. Although chemical, physicochemical, and thermal technologies are available for the remediation of impacted soils, microbial degradation is considered the leading natural degradation form of PAHs in sediments (Ghosal

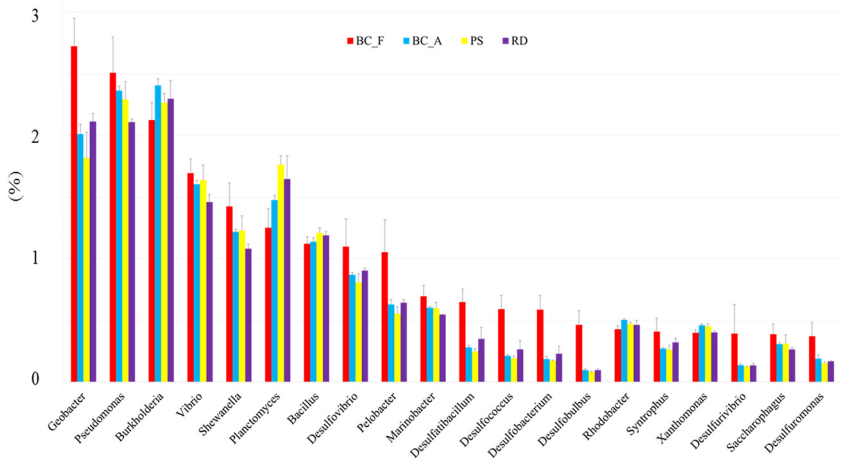


Fig. 4. Metagenomic sequences of hydrocarbonoclastic bacteria profile. Results are expressed as mean and standard error. BC_F (fissure, n = 10), BC_A (250 m + 500 m, n = 8), PS (control 1, n = 2) and RD (control 2, n = 2). The genera *Geobacter*, *Pseudomonas*, *Burkholderia*, *Vibrio*, *Shewanella*, *Planctomyces*, *Bacillus*, *Desulfovibrio*, *Pelobacter*, *Marinobacter*, *Desulfatibacillum*, *Desulfococcus*, *Desulfobacterium*, *Desulfobulbus*, *Rhodobacter*, *Syntrophus*, *Xanthomonas*, *Desulfurivibrio*, *Saccharophagus*, and *Desulforomonas* corresponded to 16–20% of the metagenomic sequences in the study area.

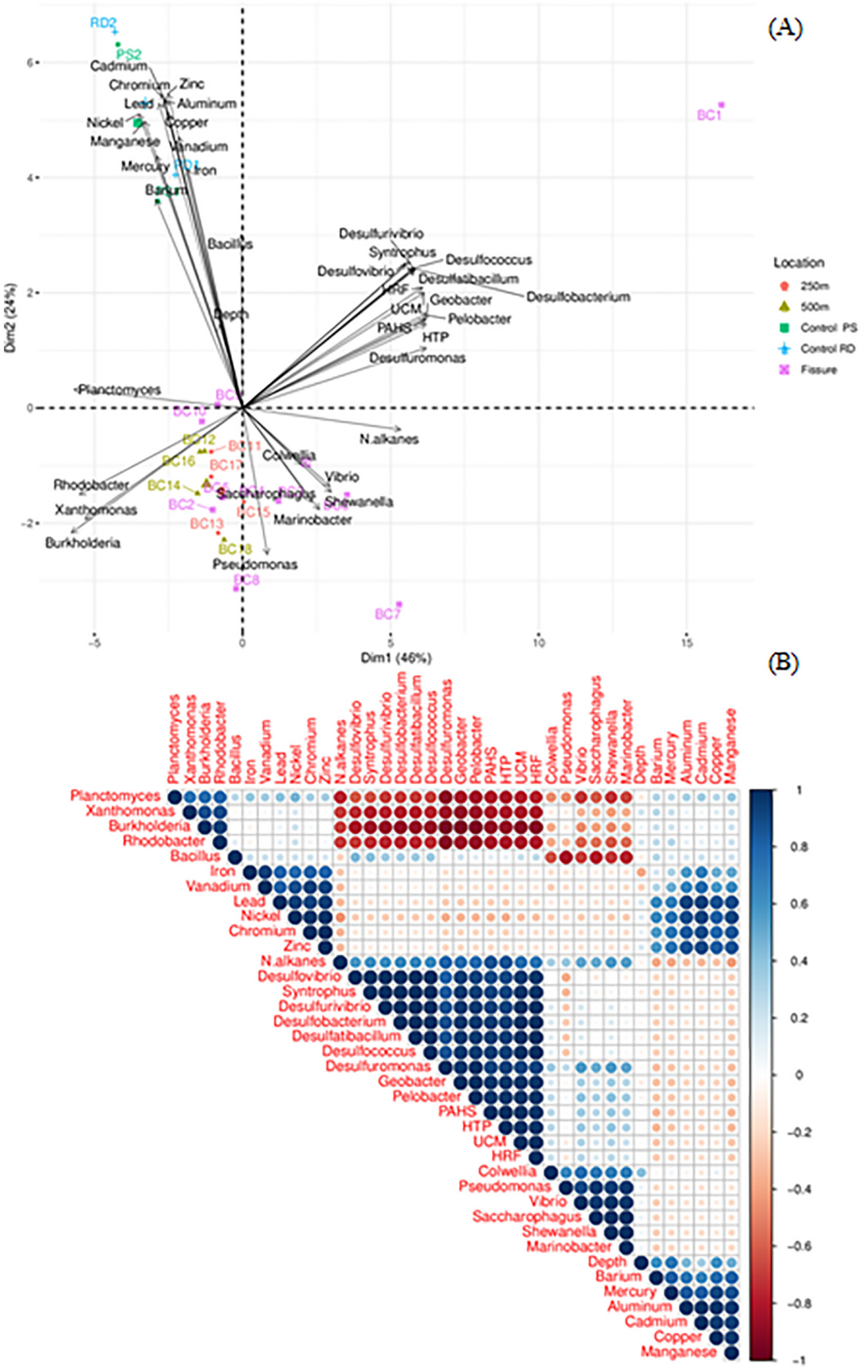


Fig. 5. (A) Principal components analysis (PCA) of sediment parameters. Biplot representing the correlation between metals, hydrocarbons, and the hydrocarbonoclastic bacterial taxa. (B) Pearson correlation matrix of metagenomic sequences of hydrocarbonoclastic bacteria, metals and hydrocarbons (HC) for all points. Red color indicates a negative correlation, and the blue color indicates a positive correlation. Circle size indicates the strength of the correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

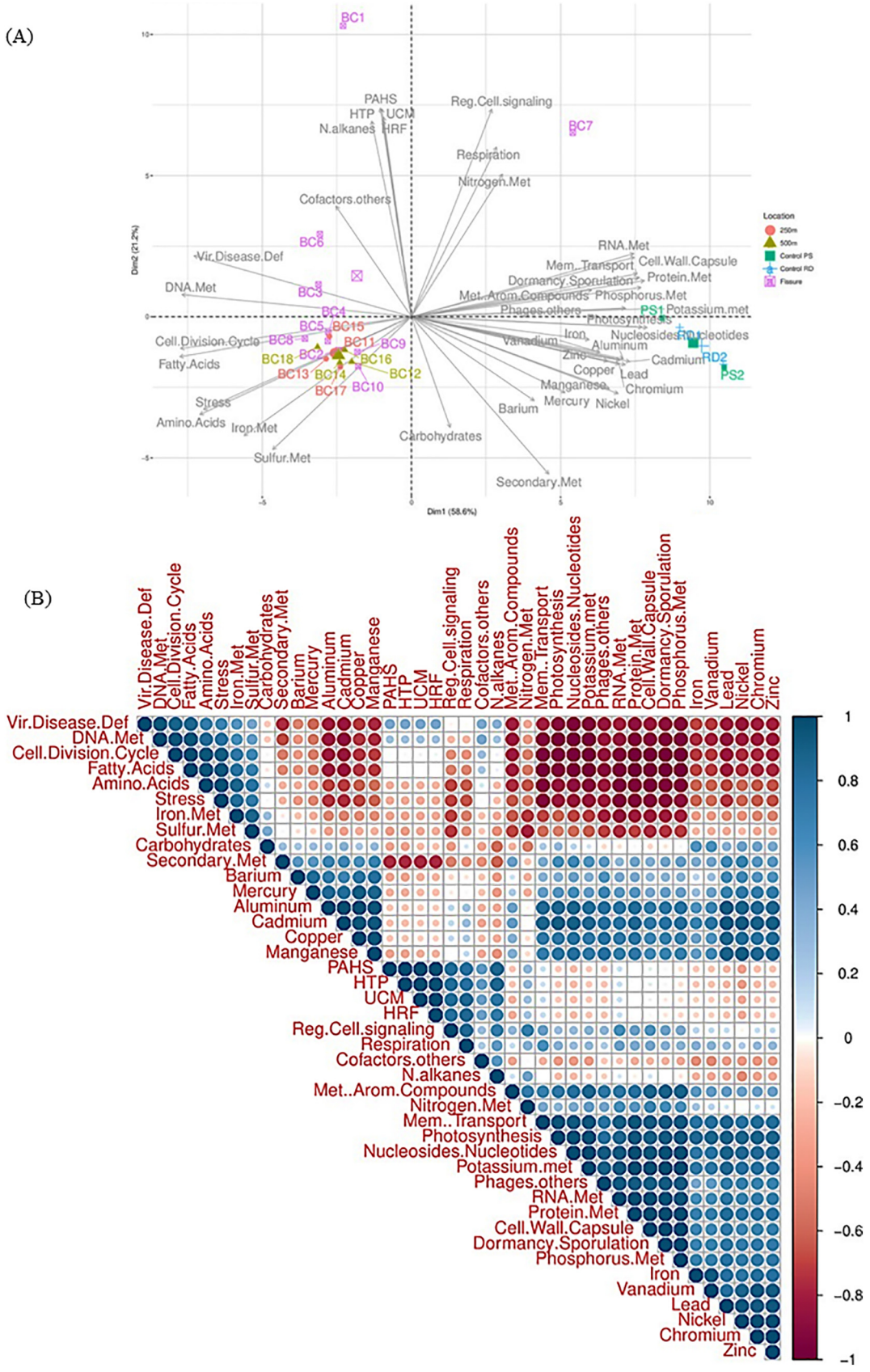


Fig. 6. Principal components analysis (PCA) of sediment parameters. Biplot representing the correlation between metals, hydrocarbons, and the functional annotation of metagenomes (according to MG-RAST, Seed level 1). (B) Pearson correlation matrix of metagenomic sequences (functional annotation), metals and hydrocarbons (HC) for all points. Red color indicates a negative correlation, and the blue color indicates a positive correlation. Circle size indicates the strength of the correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

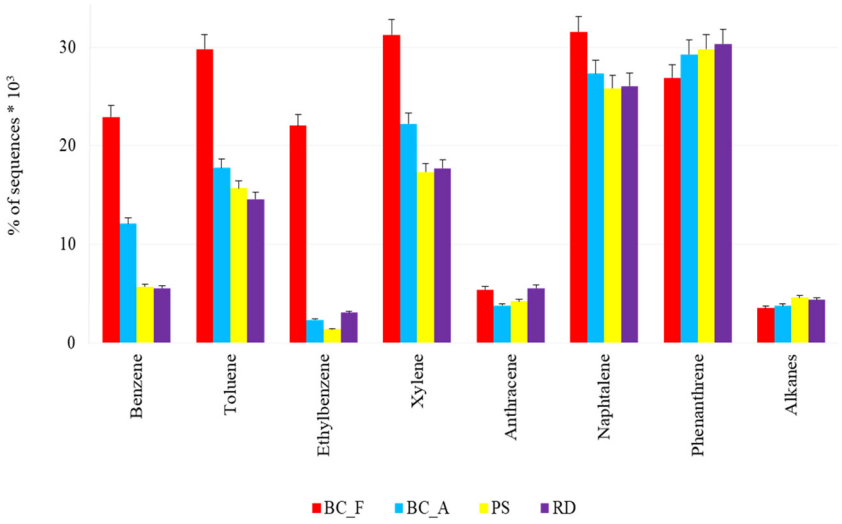


Fig. 7. Proportion (% of sequences × 103) of oil biodegradation related genes determined by metagenomic analyses for BTEX degradation, alkanes, and PAH degradation at Campos Basin. Results are expressed as the mean and standard error.

et al., 2016). The bacteria identified here are capable of degrading low molecular weight (LMW) or high molecular weight (HMW) PAHs under aerobic or anaerobic conditions (Mason et al., 2014). Oxidation of organic matter coupled with the reduction of Fe^{3+} ions in sediments requires the cooperation of a consortium of fermentative and Fe^{3+} reducing microorganisms. *Geobacter* and other Fe^{3+} reducing microorganisms metabolize fermentation products and organic compounds that fermentative microorganisms do not readily metabolize, oxidizing them into carbon dioxide with Fe^{3+} oxides serving as electron receptors (Lovley, 2017). Oil and sediment incubation experiments in the laboratory have also provided hints regarding microbial diversity and oil biodegradation kinetics (Potts et al., 2018). Analyses of oil biodegradation of sediments from the North Sea found a high level of *Pseudomonas* (after seven days incubation), which is related to BTEX biodegradation, and *Marinobacter* (after 21 days incubation), which is related to PAHs biodegradation.

4. Conclusions

The high levels of unresolved complex mixture of hydrocarbons in some fissure areas (BC_F points) indicate a possible recent petrogenic source in these sediments. The effects of oil contamination appear to be restricted to the proximal area around the fissure, and are not evident at 250 m and 500 m suggesting that the surroundings may not have been heavily affected by the oil leakage incident. The fissure area was found to have increased levels of specific bacterial genera and hydrocarbon degradation genes that may serve as relevant biosensors. Our study demonstrates that metagenomics is useful for monitoring environmental changes as suggested in previous studies (Francini-Filho et al., 2019). Monitoring programs may need to include such biosensors in their protocols in the future as early warning tools for the possible presence of spilled oil in sediments.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.139556.

Conflict of interest

None.

Declaration of competing interests

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

CRediT authorship contribution statement

Luciana R. Appolinario: Conceptualization, Methodology, Validation, Investigation. **Diogo Tschoeke:** Conceptualization, Validation, Formal analysis, Data curation, Writing - review & editing, Visualization. **Gabriela Calegario:** Methodology, Visualization. **Luiz Henrique Barbosa:** Resources, Visualization. **Manuel A. Moreira:** Formal analysis, Visualization. **Ana Luiza S. Albuquerque:** Writing - review & editing, Visualization. **Cristiane C. Thompson:** Writing - review & editing, Visualization. **Fabiano L. Thompson:** Conceptualization, Validation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgments

The authors thank the Brazilians funding agencies CNPq and FAPERJ for funding. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) - Finance Code 001, and PetroRio. The present study is part of the Ph.D. thesis of LRA.

References

Almada, G.V. de M.B., Bernardino, A.F., 2017. Conservation of deep-sea ecosystems within offshore oil fields on the Brazilian margin, SW Atlantic. Biol. Conserv. https://doi.org/10.1016/j.biocon.2016.12.026.

ANP, 2012. Investigação Do Incidente De Vazamento De Petróleo No Campo De Frade, Relatório Final. Available online at. http://www.anp.gov.br/exploracao-e-producao-de-oleo-e-gas/seguranca-operacional-e-meio-ambiente/comunicacao-e-investigacao-de-incidentes/campo-de-frade.

ANP, 2019. Boletim da Produção de Petróleo e Gás Natural – Circulação Externa. Available online at. http://www.anp.gov.br/arquivos/publicacoes/boletins-anp/producao/2019-07-boletim.pdf.

Appolinario, L.R., Tschoeke, D., Paixão, R.V.S., Venas, T., Calegario, G., Leomil, L., Silva, B.S., Thompson, C.C., Thompson, F.L., 2019. Metagenomics sheds light on the metabolic repertoire of oil-biodegrading microbes of the South Atlantic Ocean. Environ. Pollut. https://doi.org/10.1016/j.envpol.2019.03.007.

Babcock-Adams, L., Chanton, J.P., Joye, S.B., Medeiros, P.M., 2017. Hydrocarbon composition and concentrations in the Gulf of Mexico sediments in the 3 years following the Macondo well blowout. Environ. Pollut. 229, 329–338. https://doi.org/10.1016/j.envpol.2017.05.078.

Bacosa, H.P., Erdner, D.L., Rosenheim, B.E., Shetty, P., Seitz, K.W., Baker, B.J., Liu, Z., 2018. Hydrocarbon degradation and response of seafloor sediment bacterial community in the northern Gulf of Mexico to light Louisiana sweet crude oil. ISME J. https://doi.org/10.1038/s41396-018-0190-1.

Bagby, S.C., Reddy, C.M., Aeppli, C., Fisher, G.B., Valentine, D.L., 2017a. Persistence and biodegradation of oil at the ocean floor following Deepwater Horizon. Proc. Natl. Acad. Sci. 114, E9–E18.

Bagby, S.C., Reddy, C.M., Aeppli, C., Fisher, G.B., Valentine, D.L., 2017b. Persistence and biodegradation of oil at the ocean floor following *Deepwater Horizon*. Proc. Natl. Acad. Sci. 114, E9–E18. https://doi.org/10.1073/pnas.1610110114.

Berenshtein, I., Paris, C.B., Perlin, N., Alloy, M.M., Joye, S.B., Murawski, S., 2020. Invisible oil beyond the Deepwater Horizon satellite footprint. Sci. Adv. https://doi.org/10.1126/sciadv.aaw8863.

Buchfink, B., Xie, C., Huson, D.H., 2014. Fast and sensitive protein alignment using DIAMOND. Nat. Methods https://doi.org/10.1038/nmeth.3176.

Burdige, D.J, 2006. Geochemistry of Marine 324 Sediments. Princeton University Press, New Jersey Available online at http://press.princeton.edu/books/hardcover/9780691095066/geochemistry-of-marine-sediments.

Campeão, M.E., Reis, L., Leomil, L., de Oliveira, L., Otsuki, K., Gardinali, P., Pelz, O., Valle, R., Thompson, F.L., Thompson, C.C., 2017. The deep-sea microbial community from the Amazonian basin associated with oil degradation. Front. Microbiol. https://doi.org/10.3389/fmicb.2017.01019.

Francini-Filho, R.B., Cordeiro, M.C., Omachi, C.Y., Rocha, A.M., Bahiense, L., Garcia, G.D., Tschoeke, D., de Almeida, M.G., Rangel, T.P., De Oliveira, B.C.V., de Almeida, D.Q.R., Menezes, R., Mazzei, E.F., Joyeux, J.C., Rezende, C.E., Thompson, C.C., Thompson, F.L., 2019. Remote sensing, isotopic composition and metagenomics analyses revealed Doce River ore plume reached the southern Abrolhos Bank reefs. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.134038.

Gao, X., Chen, S., Xie, X., Long, A., Ma, F., 2007. Non-aromatic hydrocarbons in surface sediments near the Pearl River estuary in the South China Sea. Environ. Pollut. https://doi.org/10.1016/j.envpol.2006.11.001.

Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y., 2016. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Front. Microbiol. https://doi.org/10.3389/fmicb.2016.01369.

Glass, E.M., Meyer, F., 2011. The metagenomics RAST server: a public resource for the automatic phylogenetic and functional analysis of metagenomes. Handbook of Molecular Microbial Ecology I: Metagenomics and Complementary Approaches. https://doi.org/10.1002/9781118010518.ch37.

Hamdan, L.J., Salerno, J.L., Reed, A., Joye, S.B., Damour, M., 2018. The impact of the Deepwater Horizon blowout on historic shipwreck-associated sediment microbiomes in the northern Gulf of Mexico. Sci. Rep. https://doi.org/10.1038/s41598-018-27350-z.

Harris, K.A., Yunker, M.B., Dangerfield, N., Ross, P.S., 2011. Sediment-associated aliphatic and aromatic hydrocarbons in coastal British Columbia, Canada: concentrations, composition, and associated risks to protected sea otters. Environ. Pollut. https://doi.org/10.1016/j.envpol.2011.05.033.

Hazen, T.C., Prince, R.C., Mahmoudi, N., 2016. Marine oil biodegradation. Environ. Sci. Technol. 50, 2121–2129. https://doi.org/10.1021/acs.est.5b03333.

Hyatt, D., Chen, G.L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinforma. https://doi.org/10.1186/1471-2105-11-119.

Jernelöv, A., Lindén, O., 1981. Ixtoc I: A Case Study of the world's Largest Oil Spill. Allen Press.

Jones, D.M., Head, I.M., Gray, N.D., Adams, J.J., Rowan, A.K., Aitken, C.M., Bennett, B., Huang, H., Brown, A., Bowler, B.F.J., Oldenburg, T., Erdmann, M., Larter, S.R., 2008. Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. Nature 451, 176–180. https://doi.org/10.1038/nature06484.

Kang, D.D., Froula, J., Egan, R., Wang, Z., 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. PeerJ https://doi.org/10.7717/peerj.1165.

Lovley, D.R., 2017. Syntrophy goes electric: direct interspecies electron transfer. Annu. Rev. Microbiol. 71, 643–664. https://doi.org/10.1146/annurev-micro-030117-020420.

Mason, O.U., Scott, N.M., Gonzalez, A., Robbins-Pianka, A., Balum, J., Kimbrel, J., Bouskill, N.J., Prestat, E., Borglin, S., Joyner, D.C., Fortney, J.L., Jurelevicius, D., Stringfellow, W.T., Alvarez-Cohen, L., Hazen, T.C., Knight, R., Gilbert, J.A., Jansson, J.K., 2014. Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. ISME J. https://doi.org/10.1038/ismej.2013.254.

Muniz, M.C., Bosence, D.W.J., 2015. Pre-salt microbialites from the Campos Basin (offshoreBrazil): image log facies, facies model and cyclicity in lacustrine carbonates. In: Bosence, D.W.J., Gibbons, K.A., LeHeron, D.P., Morgan, W.A., Pritchard, T., Vining, B.A. (Eds.), Microbial Carbonates in Space and Time: Implications for Global Exploration and Production. 418. Geological Society, London, Special Publications, pp. 221–242. https://doi.org/10.1144/SP418.10.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2017. Community Ecology Package "Vegan". Version 2.4–3. R Packag. doi:https://doi.org/10.4135/9781412971874.n145.

Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. https://doi.org/10.1101/gr.186072.114.

Potts, L.D., Perez Calderon, L.J., Gontikaki, E., Keith, L., Gubry-Rangin, C., Anderson, J.A., Witte, U., 2018. Effect of spatial origin and hydrocarbon composition on bacterial consortia community structure and hydrocarbon biodegradation rates. FEMS Microbiol. Ecol. https://doi.org/10.1093/femsec/fiy127.

Ramseur, J.L., 2010. Deepwater Horizon Oil Spill: The Fate of the Oil. Congressional Research Service.

Schmieder, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27, 863–864. https://doi.org/10.1093/bioinformatics/btr026.

Smith, E., Thavamani, P., Ramadass, K., Naidu, R., Srivastava, P., Megharaj, M., 2015. Remediation trials for hydrocarbon-contaminated soils in arid environments: evaluation of bioslurry and biopiling techniques. Int. Biodeterior. Biodegrad. https://doi.org/10.1016/j.ibiod.2015.03.029.

Stout, S.A., Litman, E., Baker, G., Franks, J.S., 2017. Novel biological exposures following the Deepwater Horizon oil spill revealed by chemical fingerprinting. Oil Spill Environmental Forensics Case Studies. https://doi.org/10.1016/B978-0-12-804434-6.00033-1.

Valentine et al. , D.L., 2014. Propane respiration jump-starts microbial response to a deep oil spill. Sci. 330.208 208. doi:https://doi.org/10.1126/science.1196830.

Weelink, S.A.B., van Eekert, M.H.A., Stams, A.J.M., 2010. Degradation of BTEX by anaerobic bacteria: physiology and application. Rev. Environ. Sci. Biotechnol. https://doi.org/10.1007/s11157-010-9219-2.

Yamashita, C., Sousa, Mello e, de, S.H., Vicente, T.M., Martins, M.V., Nagai, R.H., Frontalini, F., Godoi, S.S., Napolitano, D., Burone, L., Carreira, R., Figueira, R.C.L., Taniguchi, N.K., Rezende, C.E. de, Koutsoukos, E.A.M., 2018. Environmental controls on the distribution of living (stained) benthic foraminifera on the continental slope in the Campos Basin area (SW Atlantic). J. Mar. Syst. https://doi.org/10.1016/j.jmarsys.2018.01.010.

Yang, T., Speare, K., McKay, L., MacGregor, B.J., Joye, S.B., Teske, A., 2016. Distinct bacterial communities in surficial seafloor sediments following the 2010 Deepwater Horizon blowout. Front. Microbiol. 7, 1–18. https://doi.org/10.3389/fmicb.2016.01384.

Zhang, J., Kobert, K., Flouri, T., Stamatakis, A., 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics https://doi.org/10.1093/bioinformatics/btt593.