



**HAL**  
open science

## Microbial indicators along a metallic contamination gradient in tropical coastal sediments

Vanessa Almeida Moreira, Cristiana Cravo-Laureau, Angelo Cezar Borges de Carvalho, Alice Baldy, Edison Dausacker Bidone, Elisamara Sabadini-Santos, Robert Duran

### ► To cite this version:

Vanessa Almeida Moreira, Cristiana Cravo-Laureau, Angelo Cezar Borges de Carvalho, Alice Baldy, Edison Dausacker Bidone, et al.. Microbial indicators along a metallic contamination gradient in tropical coastal sediments. *Journal of Hazardous Materials*, 2023, 443, pp.130244. 10.1016/j.jhazmat.2022.130244 . hal-03841345

**HAL Id: hal-03841345**

**<https://univ-pau.hal.science/hal-03841345>**

Submitted on 21 Feb 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Microbial indicators along a metallic contamination gradient in tropical coastal**  
2 **sediments**

3 Vanessa Almeida Moreira<sup>1,2</sup>, Cristiana Cravo-Laureau<sup>2</sup>, Angelo Cezar Borges de  
4 Carvalho<sup>1,2</sup>, Alice Baldy<sup>2</sup>, Edison Dausacker Bidone<sup>1</sup>, Elisamara Sabadini-Santos<sup>1</sup>,  
5 Robert Duran<sup>2\*</sup>

6 <sup>1</sup> Programa de Pós-Graduação em Geociências (Geoquímica), Instituto de Química,  
7 Universidade Federal Fluminense, Niterói, RJ 24020-150, Brazil.

8 <sup>2</sup> Universite de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Pau, France

9

10 Vanessa Almeida Moreira ORCID: 0000-0002-3963-3679

11 Cristiana Cravo-Laureau ORCID: 0000-0003-2011-813X

12 Angelo Cezar Borges de Carvalho ORCID: 0000-0001-8519-0365

13 Alice Baldy ORCID: 0000-0001-8945-468X

14 Edison Dausacker Bidone ORCID: 0000-0002-5771-5651

15 Elisamara Sabadini-Santos ORCID: 0000-0003-3783-4554

16 Robert Duran ORCID: 0000-0002-2351-8553

17

18 \*Corresponding author:

19 Robert Duran ORCID: 0000-0002-2351-8553

20 robert.duran@univ-pau.fr

21

## 22 **Abstract**

23 The structure and diversity of microbial community inhabiting coastal sediments reflect  
24 the exposition to contaminants. Aiming to assess the changes in the microbiota from  
25 Sepetiba Bay (SB, Brazil) sediments, correlations between the 16S rRNA gene data (V4-  
26 V5 region), metal contamination factors (CF), and the ecological risk classification  
27 provided by the Quality Ratio (QR) index were considered. The results show that  
28 microbial diversity differs significantly between the less (SB external sector) and the  
29 most (SB internal sector) polluted sectors. Also, differences in the microbial community  
30 structure regarding the ecological risk classifications validated the QR index as a reliable  
31 tool to report the SB chronic contamination. Microbial indicator genera resistant to metals  
32 (*Desulfatiglans*, *SEEP-SRBI*, *Spirochaeta 2*, among others) presented mainly anaerobic  
33 metabolisms. These genera are related to the sulfate reducing and methanogenic  
34 metabolisms probably participating in the natural attenuation processes but also  
35 associated with greenhouse gas emissions. In contrast, microbial indicator genera  
36 sensitive to metals (*Rubripirellula*, *Blastopirellula*, *Aquibacter*, among others) presented  
37 mainly aerobic metabolisms. It is suggested that future works should investigate the  
38 metabolic functions to evaluate the influence of metallic contaminants on microbial  
39 community inhabiting SB sediment.

40

## 41 **Environment implication**

42 The paper aims to decipher the impact of metal contamination on microbial communities  
43 in Sepetiba Bay (RJ-Brazil) sediments in order to assess the environmental quality status  
44 that we believe falls within the journal scope.

45 The ecological risk of sediments, based on Quality Ratio (QR) index, combined with  
46 metal content and 16S rRNA gene metabarcoding data, revealed that the most polluted  
47 sites own sulfate-reducing and methanogenic metabolisms, participating thus to  
48 greenhouse gas emissions. The microbial community changed according to metal content  
49 and QR status, allowing the identification of microbial taxa metal-tolerant/resistant and  
50 metal-sensitive, which represent bioindicator taxa for environmental management.

51

52

53 **Keywords:** Risk assessment, metal pollution, microbial ecology, coastal sediments,  
54 Quality Ratio index, co-occurrence network

55 **1 Introduction:**

56

57 Worldwide, the anthropogenic activities that lead to the economic and urban development  
58 around coastal areas result in the release of contaminants such as metals, that accumulate in  
59 sediments impacting the microbial community diversity, structure, and functional dynamics (Duran  
60 et al., 2015a,b; Pringault et al., 2008). Prokaryotic organisms (Bacteria and Archaea) are the most  
61 abundant component of the microbial community in the aquatic environment (Danovaro et al.,  
62 2010; Whitman et al., 1998). Because of their fast response to environmental disturbances and  
63 metabolic diversity (Flemming and Wingender, 2010; Harrison et al., 2007), they play a relevant  
64 role in the nutrient remineralization processes (Caruso et al., 2015; Dell'Anno et al., 2003), making  
65 them available to the entire trophic web, and also in the transformation/detoxification of  
66 contaminants (Chen et al., 2018; Nogales et al., 2011). Environmental changes caused by  
67 anthropogenic disturbances (e.g., metal pollution) could damage the ecosystem services provided by  
68 the microbial community (Birrer et al., 2019).

69 In pollution scenarios, microbial indicators could be used to provide information on the  
70 changes of pollutants over time and space (Hamza-Chaffai, 2014) because contaminants exert a  
71 selective pressure in the microbial community resulting in the alteration of their structure and  
72 composition (Ben Salem et al., 2019; DeLong et al., 2006; Duran et al., 2015; Flemming and  
73 Wingender, 2010). However, despite the valuable information they provide regarding the  
74 environmental health status (Astudillo-García et al., 2019), the development of microbial indicators  
75 as tools for monitoring environmental quality need further improvements (Caruso et al., 2015).

76 Since the 1960s, the Sepetiba Bay (SB, Rio de Janeiro – Brazil) is chronically contaminated  
77 by metals (mainly Zn and Cd) because of the Companhia Siderúrgica Mercantil Ingá SA activity.  
78 Now a day, SB receives metallic contaminants from industrial, harbor, and tourism activities as well  
79 as from urban expansion. The direct release of domestic effluent into the rivers of the SB watershed,  
80 covering a population of almost 8 million inhabitants is the major source of pollution (Barcellos et  
81 al., 1997; IBGE, 2011; Lacerda and Molisani, 2006; Monte et al., 2015). Therefore, careful  
82 monitoring of the SB environment quality with appropriate and accurate tools is urgently requested,  
83 once the Brazilian legislation proposes only *ex-situ* bioassays tool for monitoring dredged coastal  
84 sediments (CONAMA, 2012).

85 Recently, Nascimento et al. (2019) developed the Quality Ratio (QR) index as an alternative  
86 monitoring tool. The QR index, based on the integration of physical/geochemical variables listed as  
87 essential for monitoring the quality of dredged coastal sediment, as established in Brazilian  
88 legislation (CONAMA - Conselho Nacional do Meio Ambiente, 2012), and the *in situ* enzymatic  
89 biomarker response of the microbial community, allows classifying the potential ecological risk of

90 sediments for benthic biota. A cluster analysis considering the QR variables divided the SB samples  
91 into two groups (internal and external sectors), and the index showed that the SB sediments present  
92 low ( $QR \geq 10^{-1}$ ), moderate ( $10^{-2} \leq QR < 10^{-1}$ ), and high ( $QR \leq 10^{-3}$ ) risks according to their location  
93 (Moreira et al., 2021). The stations most exposed to human activities, showing the highest metallic  
94 contaminant levels, exhibited QR high risk. Also, the QR index attests that the benthic microbial  
95 community shows alterations in the energy metabolism (represented by the Esterase – EST - and  
96 Dehydrogenase – DHA - activities) when exposed to multiple contaminant, indicating that a shift in  
97 the microbial community diversity / structure might occur. The present study aimed to determine  
98 the microbial diversity and structure along a gradient of metallic contamination in SB sediments.  
99 We hypothesize that the variations in the community structure might represent the chronic exposure  
100 to metals identifying specific microbial taxa, which might represent potential bioindicators for  
101 metal contamination. Such bioindicators represent potential tools reporting the influence of metallic  
102 contaminants on microbial assemblages and on natural attenuation processes. Since we observed  
103 that the number of bacterial cells is not correlated with the degree of contamination of metals,  
104 defined as the sum of contamination factor of metals ( $\Sigma CF$ ) (Moreira et al., 2021), we believe that  
105 the microbial community inhabiting the stations with the lowest QR values (high risks for biota) are  
106 composed by taxa resistant to contamination, thus differing from the microbial taxa inhabiting the  
107 stations with high QR (low risk).

108

## 109 **2. Materials and Methods**

110

### 111 2.1 Site description

112 Sepetiba Bay is located between latitudes 22°55'00" S and 23°03'60" S and longitudes  
113 43°56'30" W and 43°36'20" W, 60 km distant from Rio de Janeiro city (Brazil). It is highly  
114 influenced by the tidal cycle (Signorini, 1980), ranging from 419 to 447 Km<sup>2</sup> with an average depth  
115 of 6 m, 6-day water renewal time, and a volume of  $2.56 \times 10^9$  m<sup>3</sup>, approximately (Barcellos et al.  
116 1997; Molisani et al. 2004; Smoak et al. 2012).

117 The São Francisco Channel is the main tributary of the 10 rivers that make up the drainage  
118 basin, being responsible for 86% of the freshwater inputs (Barcellos et al. 1997; Molisani et al.  
119 2004; Smoak et al. 2012). According to IBGE (2010), the 10 municipalities occupying the SB basin  
120 release sewage produced by more than 7.611.000 inhabitants into the SB tributary rivers with no  
121 proper treatment. In addition, the bay presents a history of chronic metal contamination (mainly Cd  
122 and Zn) from the activity of the former Campania Siderúrgica Mercantil Ingá SA. Since the  
123 beginning of its operation, more than 2 million tons of tailings were dumped in the Bay. The  
124 sampling sites SB11 and SB12 located at Saco do Engenho (Figure 1) were the most affected sites

125 due to their proximity to the tailings storage yard, which used to overflow during the rainy season  
126 (Barcellos et al. 1997; Lacerda et al. 1987; Molisani et al. 2004, 2006a; Monte et al. 2015; Ribeiro,  
127 Figueiredo, Santos, et al. 2013).

128 The area surrounding the bay also houses an industrial complex with industries belonging to  
129 the petrochemical, metallurgical, textile, and smelting sectors, and a harbor station with significant  
130 importance in the Brazilian economic scenario (IBGE, 2010). In addition, the territorial occupation  
131 around the bay is expanding rapidly, including residential demand, tourism, recreation and fishing,  
132 which results in an increased load of multiple pollutants in the SB.

133

## 134 2.2 Sampling

135 Surface sediment sampling was performed with a stainless steel Van Veen, in 26 stations  
136 distributed along the SB coastline (Figure 1). The localization of sampling stations aimed to study  
137 the contribution of continental drainage and the contamination gradient, both in the internal (eastern  
138 region - SB01 to SB12) and in the external sectors (western region - SB13 to SB26) of the bay  
139 (Figure 1). SB26 was considered because the site represents the former SB dredged sediment  
140 disposal area. Metal contents from the current oceanic disposal area for dredged sediments (Dump  
141 area, Figure 1) were used as ambient background since it corresponds to an unpolluted area because  
142 of the rapid dispersal of dredged sediments (Moreira et al., 2021) following the recommendation  
143 from (Gałuszka, 2007). Samples were collected with a sterile metal-free (10% HCl treated) plastic  
144 spatula, then fractionated, and stored according to the analysis protocols to be performed.

145 Aliquots for molecular analysis (V4-V5 region of 16S rRNA gene analysis) were stored in  
146 sterile Whirl-Pak<sup>®</sup> sample bags and kept cool until arrival at the laboratory where the samples were  
147 frozen at -20 °C, until DNA extraction. Aliquots used for determining the physical-chemical and  
148 geochemical parameters including total organic carbon (TOC), fine grain content, and metal content  
149 were also stored at -20 °C in metal-free containers. The aliquot samples for determining the esterase  
150 (EST) and dehydrogenase (DHA) activities were processed and stored as described in Moreira et al.  
151 (2021).

152

## 153 2.3 Quality Ratio (QR) index

154 The QR index integrates microbiological, physical-chemical and geochemical sedimentary  
155 parameters (Nascimento et al., 2019), aiming to classify sediments according to their potential  
156 ecological risks to the benthic microbiota. This index considers TOC, fine-grain content (% silt +  
157 clay) and the degree of contamination ( $\Sigma CF$ ) that are classic physical-chemical parameters

158 associated with contaminant retention. The quantification of these parameters is established by  
159 Brazilian legislation for the monitoring of dredged coastal sediments.

160 The quantified metals (Hg, Cd, As, Pb, Cr, Cu, and Zn) are those considered of high  
161 toxicological risk for the biota by the Brazilian legislation CONAMA (2012). In this study metal  
162 contents determined by Moreira et al. (2021) are used. The metals were quantified by ICP-MS  
163 (Agilent Technologies-7900) after acid extraction (3HNO<sub>3</sub> + 1HCl) following the US EPA 3051A  
164 (US EPA, 2007) as previously described (Moreira et al., 2021).

165 The metal contamination factors were calculated for each sample station as follows

$$166 \quad CF = \frac{C}{C_0} \quad (1)$$

167 Where C is the total metal content (mg/Kg) in the sediment sample, and C<sub>0</sub> is the  
168 background content (mg/Kg) of the metal (Dump site – Figure 1).

169 The degree of contamination (Cd) corresponds to the sum of the contamination factors of all  
170 quantified metals for a determined station

$$171 \quad Cd = \sum CF \quad (2)$$

172 Then, the above-mentioned physical/geochemical parameters integrate the Geochemical  
173 Term (GT)

$$174 \quad GT = \frac{TOC * \sum CF}{\text{fine grain content}} \quad (3)$$

175 Where TOC corresponds to total organic carbon (% w.w<sup>-1</sup>) and fine grain content is  
176 the percentage of fine grain (% silt + clay).

177 Then, the Microbial Term (MT) is determined as follow

$$178 \quad MT = \frac{DHA}{EST} \quad (4)$$

179 Where DHA (µgFDA.g<sup>-1</sup>) and EST (µgINT-F.g<sup>-1</sup>) represent the esterase and dehydrogenase  
180 activities respectively. The activities of EST and DHA enzymes are related to the process of energy  
181 production for the survival of the microbial cells.

182 Then, Quality Ratio (QR) is calculated as follows

$$183 \quad QR = \frac{MT}{GT} \quad (5)$$

184 The physical-chemical, geochemical and microbiological data from Moreira et al. (2021)  
185 were used to calculate the QR index. The methods for determining the DHA and EST activities are  
186 described in detail in Moreira et al. (2021). The SB sediments were classified into 3 classes of  
187 potential risk to biota according to QR index (Moreira et al., 2021) as:

188  $QR \geq 10^{-1}$  indicates low risk

189  $10^{-2} \leq QR < 10^{-1}$  indicates moderate risk

190 and  $QR \leq 10^{-3}$  indicates high risk

191

## 192 2.4 DNA extraction, PCR amplification and sequencing

193 The DNeasy® PowerSoil® Kit (QIAGEN) was used for DNA extraction following the  
194 manufacturer's protocol, using aliquots of 0.25-0.40g of wet sediment.

195 For each sample, the V4-V5 region of the 16S rRNA gene was amplified using a 25  $\mu$ L  
196 solution composed of 12.5  $\mu$ L of AmpliTaq® Gold 360 Master Mix (Applied Biosystems), 1  $\mu$ L of  
197 each primer 515F (5'-GTGYCAGCMGCCGCGGTA-3') (Parada et al., 2016) and 928R (5'-  
198 CCCCgycaattcmtttragt-3') (Wang and Qian, 2009), 2.0  $\mu$ L of DNA and 8.5  $\mu$ L of  
199 sterilized MilliQ water. PCR amplification was carried out in an Eppendorf 5333 Mastercycler  
200 Thermal Cycler thermocycler (Germany) as follows: initial DNA denaturing at 95°C for 10  
201 minutes, followed by 30 cycles of 30 seconds at 95°C, 30 seconds at 60°C, 40 seconds at 72°C and  
202 extension for 10 minutes at 72°C. The PCR products were run for 15 minutes at 150 V in 1.0%  
203 (w/v) agarose gel electrophoresis in 1 $\times$  Tris-Acetate-EDTA buffer and stained with ethidium  
204 bromide (0.2  $\mu$ g mL<sup>-1</sup>) as previously described (Bordenave et al., 2004). DNA bands were detected  
205 under UV light for checking the size of PCR products using DNA GeneRuler 1 kb as DNA  
206 molecular size marker.

207 Illumina sequencing was performed by the NED team (UMR1388 GenPhySE) and the GeT  
208 core facility (Toulouse, France), using Illumina MiSeq technology. The complete protocol is  
209 available on the website of the platform ([https://sites.google.com/site/olivierzembwebsite/16s-  
210 sequencing](https://sites.google.com/site/olivierzembwebsite/16s-sequencing)). QIIME 2 2019.4 (Quantitative Insights Into Microbial Ecology; Caporaso et al. 2010)  
211 pipeline was applied for demultiplexing, denoising (using dada2 package), and rarefying sequences.  
212 The taxonomy assignment (97% similarity) was performed comparing reference sequences,  
213 clustered in representative amplicon sequence variants (ASVs), to SILVA (v. 132) database of  
214 known 16S rRNA gene (Beccati et al., 2017; Quast et al., 2013).

215 The complete dataset was deposited in the NCBI Sequence Read Archive (SRA) database  
216 under SRA accession number SAMN20003651 to SAMN20003676, BioProject number  
217 PRJNA743207.

218

## 219 2.5 Data analysis

220 The community data (ASVs table) was transformed into relative abundances, and those  
221 greater than 1% were kept for the analysis. Statistical and community composition/diversity  
222 analysis were performed on R software (R Core Team, 2019), using specific packages. Since not all



223 data (physical, geochemical, enzymatic, and ASVs) presented normal distribution (Shapiro Wilk  
224 test,  $p < 0.05$ ), non-parametric analyzes were applied.

225 Alpha diversity indices (including species richness and Shannon index) of microbial  
226 community were calculated based on the ASVs table using Vegan package (Oksanen et al. 2019).  
227 For Spearman correlation, Mann-Whitney's U test, and Permutational Analysis of Variance  
228 (perMANOVA - Vegan package; Oksanen et al. 2019), the physical/geochemical data (TOC, fine  
229 grain content, metal contamination factor – CF - and the metallic degree of contamination -  $\Sigma$ CF),  
230 the microbiological parameters (EST and DHA) and the Shannon diversity index were standardized  
231 by ranging for variables with arbitrary zero, as proposed by Milligan and Cooper (1985). Canonical  
232 Correspondence Analysis (CCA), an ordination method where species distribution (microbial  
233 composition) is constrained by incorporating environmental variables (physical-chemical  
234 parameters), was performed for determining the correlation of microbial community composition  
235 (Phylum level) with physical-chemical and microbiological parameters. CCA uses two data  
236 matrices, one of species occurring in various samples and the other of the environmental  
237 characteristics of those samples. The relationships between the microbial species distributions and  
238 the physical-chemical parameters are thus an integral part of the ordination. CAA was performed  
239 using Vegan Package (Oksanen et al. 2019). The standardization of physical, geochemical, and  
240 microbiological variables were kept and community data was Hellinger transformed.

241 Venn Diagrams (VennDiagram package - Chenn 2018) were built to analyze the distribution  
242 of ASVs according to the habitat (SB sectors) and the ecological risk of sediment (QR  
243 classifications).

244 Heatmaps showing the phyla and class relative abundances distribution per station were  
245 performed using the pheatmap package (Kolde, 2019). The same package was used to perform  
246 cluster correlations based on the 50 most abundant genera, to determine the correlations (Spearman)  
247 between microbial genera and metal CF. The hierarchical clustering of correlations was based on  
248 Spearman correlation.

249 A Linear discriminant analysis effect size (LEfSe; Segata et al., 2011) was performed to  
250 detect bioindicator genera among the 50 most abundant, according to the habitat and the potential  
251 ecological risk of sediments. For this, the non-parametric Kruskal-Wallis sum-rank test was  
252 performed to detect taxa with significant differential abundance ( $p < 0.05$ ) considering contrasting  
253 conditions (e.g., internal and external sectors; high, moderate and low ecological risks). The  
254 biological consistency was investigated by performing a pairwise Wilcoxon test ( $p < 0.05$ ). A  
255 Linear Discriminant Analysis (LDA) threshold score of 2.0 and 1000 bootstrap interactions were  
256 applied.

257 In order to determine correlations between ASVs and metal contamination a clustered  
258 correlation (ClusCor) analysis was performed as described previously (Duran et al., 2015). Full  
259 description of ClusCor is provided by (Weinstein et al. 1997). A program for making clustered  
260 correlation is available (<http://discover.nci.nih.gov.gate1.inist.fr>).

261 To determine the ASV co-occurrence and mutual exclusion relationships, a network was  
262 built using a Cytoscape plugin CoNet 1.1.1 beta (Co-occurrence Network inference;  
263 <http://psbweb05.psb.ugent.be/conet/>; Faust et al., 2012). The input matrix consisted of ASVs > 1%  
264 data from all the sample stations and a metadata table consisting of metal contamination factors  
265 standardized by ranging for variables with arbitrary zero as already mentioned. The analysis was  
266 performed with Spearman correlation method and 0.05 p-value threshold. The co-occurrence  
267 network was visualized with Cytoscape 3.8.2 (Shannon et al., 2003).

268

### 269 **3 Results and Discussion**

#### 270 3.1 Ecological quality status assessed by Quality Ratio

271 A previous study (Moreira et al., 2021), comparing the stations on the SB sectors based on  
272 environmental variables, grouped the stations as follow: the internal sector (SB01 to SB12, Figure  
273 1) presents higher levels of fine-grain, TOC, and metal contents than the external sector (SB13 to  
274 SB26). Accordingly, the QR index classified the SB sediments into three levels of risk for the  
275 benthic biota (Supplementary material – Table S2): 2 stations with Low risk ( $QR \geq 10^{-1}$ ), 9 stations  
276 with Moderate risk ( $10^{-2} \leq QR < 10^{-1}$ ), and 15 stations with High risk ( $QR \leq 10^{-3}$ ). Higher  
277 ecological risks (i.e. the lowest QR values) in the internal sector stations are explained by the  
278 intense anthropogenic activities in the area, mainly from the domestic effluent input brought by the  
279 main tributary rivers. Also, the QR index indicated that SB11 and SB12 stations exhibited higher  
280 risk in comparison to the other stations located in the internal sector. Such observation is probably  
281 explained by the close location of stations SB11 and SB12 to a former mining site exploiting zinc  
282 ores in the 1960s, which resulted in metals contamination. SB26 presents moderate risk despite  
283 being the former SB dredged sediment deposition area. We hypothesize that stations with a high  
284 risk for biota (low QR) are predominantly colonized by microbial taxa resistant to metal  
285 contamination.

286

#### 287 3.2 Microbial community diversity along the contamination gradient

288 After trimming, the retained 188,782 sequences, with size  $376.6 \pm 3.5$  bp, were dispatched  
289 into 3,444 amplicon sequence variants (ASVs, Table 1), which were reduced to 518 ASVs  
290 following rarefaction applying a 1% abundance threshold.

291 The Venn diagram with the remaining 518 ASVs showed that both SB sectors shared 243  
292 ASVs (Figure 2). Considering (i) the chronic contamination of the study area (Monte et al., 2015;  
293 Moreira et al., 2021), (ii) the natural selection process responsible for the elimination of microbial  
294 taxa sensitive to contaminants at the beginning of the exposure period (Harrison et al., 2007), (iii)  
295 the rapid response of prokaryotes to variations in environmental conditions and their physiological  
296 diversity (Aylagas et al., 2017; Caruso et al., 2015), and (iv) their adaptability from the exchange  
297 (Flemming and Wingender, 2010; Harrison et al., 2007; Lewis, 2007) and expression of genes that  
298 confer adaptive advantages (Beelen and Doelman, 1997; Dick, 2018; Harrison et al., 2007) in  
299 polluted environments, it is assumed that more than 70% of the SB microbial taxa (ASVs shared  
300 between sectors + ASVs exclusive to the internal sector) is adapted to the pollution (Figure 2a). The  
301 effect of contaminants on microbial community structure is not only dependent on the concentration  
302 of contaminants, but also on their bioavailability according to environmental parameters controlling  
303 their accumulation in sediments (Burton, 2002; Gough, 2007; Løkke et al., 2013; Mumtaz et al.,  
304 2010), which can also directly influence microbial communities (Bai et al., 2012; Coclet et al.,  
305 2019; Yang et al., 2016). Thus, it is worth considering the physical and geochemical parameters  
306 such as the fine-grain and TOC contents - main metal-binding matrices (Di Toro et al., 1990; Marx  
307 and Kamber, 2010) to obtain a comprehensive view of the contaminant effects on microbial  
308 communities. Besides, several studies highlighted the need to integrate the biological response to  
309 contamination exposure (represented here by the EST and DHA enzyme activities) to the physical  
310 and geochemical parameters to assess the ecological quality status (Burton, 2002; Rosado et al.,  
311 2015). The observed higher number of microbial ASV richness in stations classified as moderate  
312 (408 ASVs) and high (491 ASVs) risks (Figure 2b) by the QR index (Moreira et al., 2021) in  
313 comparison to that observed in stations defined as low risk by QR index (285 ASVs) suggested that  
314 the microbial communities are adapted to the contamination level. Thus, the specific ASVs likely  
315 represent specialist ASVs adapted to the contamination level: 96 ASVs for the high risk (i.e. high  
316 contamination level), 19 ASVs for the moderate risk (i.e. moderate contamination level), and 1  
317 ASV for low risk (i.e. low contamination level).

318 The stations of the SB external sector, exhibiting lower contamination degree ( $\Sigma CF_{\text{mean}}$  SB  
319 external sector stations =  $49 \pm 6.8$ ; Table S1), had significantly higher (Mann Whitney's U test;  $w =$   
320  $143$ ,  $p = 0.002$ ) microbial ASV richness ( $S_{\text{mean}}$  external sector stations =  $159 \pm 10$ ; Table 1) and  
321 significantly higher (Mann Whitney's U test;  $w = 134$ ,  $p = 0.01$ ) Shannon's diversity index ( $H_{\text{mean}}$   
322 external sector stations =  $4.7 \pm 0.3$ ; Table 1) than the stations of the SB internal sector, exhibiting  
323 high contamination degree ( $\Sigma CF_{\text{mean}}$  SB internal sector stations =  $536 \pm 246$ ; Table S1). Despite the  
324 significant differences, it is noticeable that the range between the Shannon diversity index of the  
325 two sectors (Shannon diversity index range = Internal: 4.1 to 4.8, External: 3.9 to 5.0) is small,

326 which means that both sectors are diverse with the homogeneous distribution. However, it cannot be  
327 excluded that other environmental parameters such as salinity and dissolved oxygen, affect the ASV  
328 diversity (Beaulne et al., 2020; Ben Salem et al., 2019; Bourhane et al., 2022; Terrisse et al., 2017;  
329 Zhang et al., 2016). Indeed, it has been shown that the external sector at the proximity of the SB  
330 entrance is influenced by the ocean currents (Ribeiro et al., 2013). Conversely, in the internal sector,  
331 there is a reduction in water circulation speed resulting in the reduction of oxygenation (Ribeiro et  
332 al., 2013; Signorini, 1980) further favored by the high organic load from the continental drainage  
333 and the mangrove productivity (Carvalho et al., 2021, 2020). The QR index classified the internal  
334 sector as high risk ( $QR \leq 10^{-3}$ ) corresponding to significantly (Mann Whitney's U test;  $w = 8$ ,  $p =$   
335  $0.0001$ ) higher contamination degree ( $\Sigma CF_{\text{mean}}$  SB internal sector stations =  $536 \pm 246$ ; Table S1)  
336 and significantly (Mann Whitney's U test;  $w = 16$ ,  $p = 0.0005$ ) higher geochemical term ( $GT_{\text{mean}}$  SB  
337 internal sector =  $9989 \pm 3442$ ; Table S2). Despite these differences, both sectors showed similar  
338 (Mann Whitney's U test;  $w = 52$ ,  $p = 0.1$ ) microbial terms ( $MT_{\text{mean}}$  SB internal sector =  $3.78 \pm 1.3$ ;  
339  $MT_{\text{mean}}$  SB external sector =  $5.2 \pm 1.7$ ; Table S2), suggesting that the microbial activities were not  
340 affected by the presence of contaminants in the SB internal sector, probably because the microbial  
341 communities were adapted to cope with contaminants.

342 The lower microbial diversity found in the most polluted sector is consistent with previous  
343 reports (Bruneel et al., 2006; Li et al., 2020). However, the microbial diversity in the contaminated  
344 SB internal sector remains high compared with other contaminated sites (Chen et al., 2018; Paissé  
345 et al., 2008; Stauffert et al., 2013; Wang et al., 2007), which is in agreement with the microbial  
346 diversity levels observed in chronically impacted environments. In a chronic contamination  
347 scenario, the microbial diversity decreases at the beginning of the pollutant exposure period;  
348 consequently, the microbial community becomes as diverse as a microbial community from the  
349 non-impacted environment over time (Gillan et al., 2005; Jeanbille et al., 2016b; Mossa et al., 2017;  
350 Paissé et al., 2008), which corresponds to the microbial adaptation to the pollutant also known as  
351 legacy effect (Jurburg et al., 2017). It is likely that in the initial phase of exposure, there is a  
352 selection of pollutant-resistant microorganisms (Bouskill et al., 2010), which can be subsequently  
353 amplified by mechanisms exchanging genetic materials (e.g., horizontal gene transfer) (Brochier-  
354 Armanet and Moreira, 2015; Lewis, 2007), resulting thus to the stability of the microbial  
355 community (Harrison et al., 2007; Nascimento et al., 2020; Waite et al., 2016).

356 CCA analysis, explaining almost 40% of the microbial community distribution, corroborated  
357 the differences observed between the microbial communities from each SB sector (Figure 3). The  
358 microbial communities from the internal sector were correlated with metal CF, TOC, fine-grain  
359 content, and EST activity, while the microbial communities from the external sector were linked  
360 with DHA activity. It is important to highlight that high levels of fine-grain and TOC (nutrient

361 supply) favor the accumulation of metals (high  $\Sigma$ CF) (Förstner and Wittmann, 1981). The stress  
362 provoked by metal accumulation in sediment leads to an increase in EST activity that, consequently,  
363 would make the metals bioavailable after the breakdown of organic matter (Nascimento et al., 2019;  
364 Waite et al., 2016). When absorbed by microbial cells, metals would compromise DHA activity  
365 (Coppolecchia et al., 2011; Sabadini-Santos et al., 2014; Waite et al., 2016), which would result in  
366 an imbalance between the enzymatic biomarkers. This imbalance designates the increased energy  
367 demand of the microbial community, represented by the low MT (MT = DHA / EST) when exposed  
368 to multiple chronic contaminants (Moreira et al., 2021; Nascimento et al., 2019).

369

### 370 3.2.1 Microbial community structure in response to chronic exposure to metals

371

372 The 518 ASVs were classified into 23 phyla and 48 classes. Proteobacteria (53.5%) was the  
373 most abundant phylum (Figure 4a), with a predominance of the Gammaproteobacteria (29%),  
374 Deltaproteobacteria (21%), and Alphaproteobacteria (3.4%) classes. The other representative phyla  
375 were Bacteroidetes, Firmicutes, Acidobacteria, and Chloroflexi (17.0%, 9%, 7.6%, and 5.8%,  
376 respectively).

377 The predominance of Proteobacteria, more specifically of the Gammaproteobacteria and  
378 Deltaproteobacteria classes, suggests that they play an important role in biogeochemical cycles in  
379 the sediments (Ben Salem et al., 2019). ASVs affiliated to Gammaproteobacteria present the highest  
380 relative abundances in stations located in the internal sector (Figure 4b), which is consistent with  
381 the fact that they are linked with sediments contaminated by metals (Bouskill et al. 2010). The  
382 positive correlation between the abundance of Gammaproteobacteria ASVs and the Cd and Zn  
383 contents (Spearman;  $r > 0.39$ ,  $p < 0.05$  - Figure 4c) further supports the hypothesis that  
384 Gammaproteobacteria can tolerate the presence of metals.

385 Microbial communities composition from SB proved to be significantly different  
386 (Permanova analysis) when comparing: (i) internal and external sectors ( $p = 0.001$ ) and (ii) the QR  
387 index classifications ( $p = 0.02$ ). It is important to note that microorganisms involved in the sulfur  
388 cycle were found more abundant in the internal sector (Figure 4b), especially at the stations close to  
389 the mouth of the main tributary rivers (SB06 to SB11) with high organic load from domestic  
390 effluent, which represents a reduced environment (Carvalho et al., 2021, 2020, n.d.; Paissé et al.,  
391 2008).

392 Following the reducing nature of sediments from the internal sector, Thermoplasmata class,  
393 related to methanogenic activities (Ma et al., 2016) exclusively detected in this sector (absent in  
394 SB06, SB08, SB10, and SB11), presents positive correlations with all metal CF ( $r > 0.44$ ,  $p < 0.05$ ;

395 Figure 4c). Similarly, ASVs affiliated to Thermococci were found only at SB06 and SB07, with the  
396 latter presenting 19 times greater abundance (Figure 4b). These microorganisms are  
397 hyperthermophilic heterotrophs, strictly anaerobic, and related to reducing environments rich in  
398 organic matter with high concentrations of sulfur, which is a limiting factor for their growth (Teske,  
399 2009; Zillig and Reysenbach, 2015). In the present study, Thermococci proves to be resistant to  
400 metallic contaminants due to its significant ( $p < 0.05$ ) positive correlation with Cr, Cu, and Hg  
401 (Spearman;  $r = 0.42, 0.42, \text{ and } 0.47$ , respectively - Figure 4c).

402 Also, ASVs affiliated to *Thermodesulfovibrionia* and *Dehalococcoidia* presented higher  
403 abundances in the internal sector (Figure 4b) and significant ( $p < 0.05$ ) positive correlation with  
404 some metal CF (Spearman;  $r > 0.39$  - Figure 4c). *Thermodesulfovibrionia* is known for the  
405 reduction of sulfate and other sulfur compounds (Tamburini et al., 2020) that have been found  
406 abundant in coastal sediments with a high contamination degree by metals and polycyclic aromatic  
407 hydrocarbons (PAHs) (Tangherlini et al., 2020). *Dehalococcoidia* is an organohalide respiring  
408 bacteria, playing a role in detoxifying anoxic contaminated sediments with polychlorinated  
409 biphenyls (PCBs) through reductive dechlorination activities (Maturro et al., 2016; Nuzzo et al.,  
410 2017). In the present study, *Dehalococcoidia* is present in 9 stations from the internal sector (except  
411 SB01, SB06, and SB10 – Figure 4b), with average relative abundance an order of magnitude higher  
412 than that found in the external sector, where they were found in only 5 stations (SB21 > SB22 >  
413 SB15 > SB16 > SB13). Such observation suggests that *Dehalococcoidia* present selective  
414 advantages for living in environments chronically impacted by multiple contaminants such as SB,  
415 where metallic contaminants are indicators of the pollutants' mixture (Carvalho et al., 2021, 2020;  
416 Moreira et al., 2021).

417 In decreasing order, the stations SB06, SB11, and SB07 also present the highest relative  
418 abundances of ASVs affiliated to *Anaerolinea* (Figure 4b), which was consistent with the results  
419 shown in Figure 4c – presenting strong correlations with metals (Spearman;  $r > 0.39, p < 0.05$ ,  
420 except for Cr) – and with previous reports showing that members of *Anaerolinea* were found  
421 abundant in river sediments, soils, and coastal sediments contaminated with metals (Meng et al.,  
422 2019; Sun et al., 2013; Yin et al., 2015). In contrast, *Nitrososphaeria* was present in all the stations  
423 of the external sector (except SB15 and SB18) and at the SB10 station in the internal sector (Figure  
424 4b). This observation was in accordance with reports showing that members of the *Nitrososphaeria*  
425 have been found more abundant in less contaminated sites (Tangherlini et al. 2020). The negative  
426 correlation between *Nitrososphaeria* and the metals (Spearman;  $r < -0.39, p < 0.05$ ) in the present  
427 study (Figure 4c) indicates their sensitivity to metallic contamination.

428 The enrichment of methanogenic and sulfate-reducing organisms - as detected in the SB  
429 anoxic polluted sediments from the internal sector stations, Figure 4b - could represent the taxa

430 exhibiting function involved in the natural attenuation of contaminants, as suggested by Li et al.  
431 (2019). Their high abundances reflect the impact of contamination on the structure of microbial  
432 communities. Also, energy metabolism specifically linked to methane metabolism is pointed out in  
433 the literature as typical in metal-contaminated environments (Bergsveinson et al., 2020; Coclet et  
434 al., 2021; Guo et al., 2018). Recently, predicted metabolic pathways from 16S rRNA gene revealed  
435 methane metabolism as an indicator of high metallic pollution in the stations of SB internal sector  
436 (Moreira et al., 2022). Studies considering sedimentary microbial community functional data show  
437 an increase in the relative proportion of genes involved in methanogenesis, which are correlated  
438 with eutrophic, anoxic, and metal-polluted sediments (Coclet et al., 2021; Li et al., 2019). Research  
439 conducted in the study area shows that the content and quality of organic matter are not the main  
440 factors that influence methanogenesis (Marinho et al., 2012). Recently, it has been shown that  
441 microbial communities inhabiting pristine (non-contaminated) sediments exhibit metabolic potential  
442 involved in greenhouse gases consumption (Li et al., 2019). In contrast, it has been shown that the  
443 production of greenhouse gases in marine coastal areas is correlated with urban waste load (Burgos  
444 et al., 2015), particularly including metals (Brown et al., 2022). The presence of adapted  
445 microorganisms to highly polluted anoxic sediments (as those mainly inhabiting the SB internal  
446 sector) have the capacity to increase the greenhouse gases production (Wang et al., 2022). This is  
447 due to the activation of adaptive mechanisms for coordinating intracellular toxic-metal homeostasis  
448 (Coclet et al., 2021; Prabhakaran et al., 2016) such as metal immobilization. Thus, sulfate reduction  
449 is reported as one of the anaerobic metabolism that promotes metal immobilization, resulting in  
450 CO<sub>2</sub> emission (Ayangbenro et al., 2018). Additionally, the comparison of metagenomes from  
451 contaminated sediment to that of pristine sediment revealed that the presence of contaminants,  
452 particularly heavy metals, increased methanogenesis and sulfate reduction capabilities while  
453 reducing the capacity of nitrogen fixation (Li et al., 2019). In contrast, the pristine site harbored  
454 higher consumption of methane limiting the amount of greenhouse gases (Li et al., 2019).

455

### 456 3.2.2 Microbial taxa specific to QR risk level and habitat specific

457

458 Linear discriminant analysis effect size (LEfSe), performed with the 50 most abundant  
459 genera, revealed the ASVs showing significant different abundance according to the habitat  
460 (internal vs. external sectors, Figure 5a) and to the QR risk level of sediments (high vs. moderate vs.  
461 low risks, Figure 5b). This observation was consistent with previous reports showing habitat  
462 (Jeanbille et al., 2016a,b; Ben Salem et al., 2019), and/or contamination specificities (Bourhane et  
463 al., 2022; Hafez et al., 2022) for microbial taxa. Such ASVs differentially abundant to a specific

464 condition are considered as bioindicators (Segata et al., 2011), which can be useful to develop tools  
465 for monitoring contamination (Aylagas et al., 2017; Bourhane et al., 2022; Hamza-Chaffai, 2014).

466 Among the genera found specifically more abundant in the metal contaminated internal  
467 sector by LEfSe, the Uncultured *Thiotrichaceae* and *SCGC-AB-539-J10* genera, known for their  
468 anaerobic metabolism (Sharrar et al., 2017; Wasmund et al., 2014), were also found specifically  
469 more abundant in stations classified as high QR risk (Figure 5b). Such observation further supports  
470 the role of anaerobic microorganisms in metal contaminated sites (Liu et al., 2019; Zárata et al.,  
471 2021). Consistently, the specific presence of the *Desulfatiglans*, *SEEP-SRB1*, *Candidatus Thiobos*,  
472 *Spirochaeta 2*, uncultured (Class: *Anaerolineae*), and Uncultured *Calditrichaceae* genera known for  
473 their anaerobic metabolisms (Kublanov et al., 2017; Miyazaki et al., 2014; Petro et al., 2019; Suzuki  
474 et al., 2014; Yamada et al., 2006) also characterizes the internal sector.

475 In contrast, the genera found specifically more abundant in the external sector (low metal  
476 content) by LEfSe are known for their aerobic metabolism, such as Subgroup 10 (*Acidobacteria*),  
477 *Actibacter*, *Exiguobacterium*, *Blastopirellula*, *Paeniclostridium*, *Romboustia* genera (Kim et al.,  
478 2008; Kristensen et al., 2021; Tedesco et al., 2021). Consistently, these genera were also found  
479 specifically more abundant in stations classified as low and moderate QR risks (Figure 5b).

480

### 481 3.2.3 Influence of metallic contaminants on microbial assemblages

482

483 A cluster correlation analysis was carried out to determine the interactions between the 50  
484 most abundant genera and metallic contaminants (Figure 6). Following the LEfSe analysis, the  
485 identified bioindicators for the internal sector/high ecological risk - such as *SCGC-AB-539-J10* and  
486 the uncultured *Thiotrichaceae* - presented a significant positive correlation (Spearman;  $r > 0.39$ ;  $p <$   
487  $0.05$ ) with five or more metals. Thus, the microbial community in the internal sector presents a high  
488 abundance of taxa resistant to contaminants (Figure 4b). In contrast, bioindicators for the external  
489 sector/low and moderate ecological risk - such as *Actibacter*, *Candidatus Nitrosopumilus*,  
490 *Thermoanaerobaculaceae Subgroup 10*, uncultured *Cyclobacteriaceae*, and uncultured  
491 *Sandaracinaceae* - presented significant negative correlation (Spearman;  $r < -0.39$ ;  $p < 0.05$ ) with at  
492 least 4 metals. Thus, the microbial community in the external sector presents a high abundance of  
493 taxa sensitive to contaminants (Figure 4b).

494 The co-occurrence network analysis shows four sub-networks (Figure 7). But the  
495 interactions between ASVs and metals were evidenced only in the main network (Figure 7a).  
496 Among the 97 ASVs that showed significant non-random interactions (Spearman;  $r > 0.6$ ,  $p <$



497 0.003) in the network, 39 ASVs were directly linked with metals (Figure 7b), while the other 58  
498 ASVs showed indirect correlations (Figure 7a).

499 Among the ASVs directly correlated with metal contamination (Figure 7b – connected by  
500 green edges), 8 ASVs (6 gray ellipses representing the Proteobacteria phylum, belonging to  
501 Gammaproteobacteria class; and 2 white hexagons representing the Bacteroidetes phylum,  
502 belonging to Bacteroidia class) are directly positively linked (Spearman;  $r > 0.6$ ,  $p < 0.003$ ) to metal  
503 contamination, which indicates that they are probably resistant to metallic contamination. Among  
504 them, the abundance of 3 ASVs was specifically correlated with Hg contamination level, (ASV-3  
505 and ASV-609, both Gammaproteobacteria class, and ASV-1659, Bacteroidia class). The abundance  
506 of the other 5 ASVs presented a significant positive correlation with the contamination level of at  
507 least 2 metals.

508 From these 8 ASVs which abundance was found to be positively and directly linked to metal  
509 content, 3 belong to the genera that were identified as bioindicators for the metallic contamination  
510 of the internal sector, and for the high ecological risk stations (Figure 5a): ASV-483 (uncultured  
511 Thiotrichaceae) and ASV-1321 (Robiginitalea) (Figure 6).

512 The 31 ASVs, presenting abundances with significant negative correlations (exclusions -  
513 Spearman;  $r < -0.6$ ,  $p < 0.003$ ) with metal contents (Figure 7b – connected by red edges), represent  
514 taxa probably sensitive to contaminants influencing other ASVs by mutual exclusion (Figure 7a –  
515 red edges). These 31 ASVs belong to the Proteobacteria (grey ellipses; Gammaproteobacteria,  
516 Deltaproteobacteria, and Alphaproteobacteria classes), Bacteroidetes (white hexagons; Bacteroidia  
517 and Ignavibacteria classes), Firmicutes (white rectangle; Bacilli class), Actinobacteria (blue  
518 hexagon; Acidimicrobia class), Acidobacteria (white ellipse; Blastocatellia class - Subgroup 4), and  
519 Planctomycetes (blue ellipse; Phycisphaerae class) phyla. Among them, 7 ASVs – ASV-755  
520 (Ulvibacter), ASV-1398 (uncultured Cyclobacteriaceae), ASV-2097 (Methyloceanibacter), ASV-  
521 2191 (Eudoraea), ASV-2949 (Desulfobulbus), ASV-3252 (Exiguobacterium), and ASV-3419  
522 (Actibacter) – were among the top 50 genera. Except for ASV-755, all the other 6 ASVs were  
523 revealed, by LEfSe analysis, as bioindicators for the SB external sector and for low ecological risk  
524 (ASVs 1398, 3252, and 3419) (Figure 5). The 7 ASVs were affiliated to genera showing  
525 abundances with negative or no correlation with metallic contaminants (Figure 6).

526 The ASVs linked to ASVs directly correlated to metals (Figure 7a, green edges) are likely to  
527 constitute a transformation network involving cooperation, probably using metal compound  
528 transformation intermediates (Nogales et al., 2011). Bacteria resistant to metallic contamination  
529 have homeostatic mechanisms that make them able to eliminate the metals that have been  
530 excessively absorbed by cells, through efflux pumps (Nies, 1995). They also produce sulfur-rich  
531 molecules (thiol group) capable of sequestering metal cations and immobilizing them from the

532 formation of complex molecules; also some of these organisms are capable of reducing metal ions  
533 to a less toxic oxidation state (Nies, 1999).

534

#### 535 **4 Conclusions:**

536

537 The V4-V5 hypervariable region of the 16S rRNA gene sequencing analysis revealed that  
538 microbial diversity was significantly different between Sepetiba Bay sectors. Such significant  
539 difference in microbial community structure between both sectors was consistent with the  
540 classifications of ecological risk according to QR index, validating the QR index as a reliable tool  
541 representative of the chronic contamination of the studied area.

542 Bioindicators, taxa significantly more abundant under a specific condition, identified for the  
543 SB internal sector represent the selective pressure induced by metallic contaminants. They mainly  
544 present anaerobic metabolism, which is consistent with the internal sector characteristics, being  
545 anoxic and receiving high organic input from terrestrial drainage. Moreover, anaerobic  
546 bioindicators in the most polluted sector are affiliated to taxa resistant to metals. Particularly, the  
547 high abundance of sulfate-reducing taxa in the internal sector participates probably to the natural  
548 attenuation function involved in mitigating the metal contamination effects by producing sulfide, a  
549 metal-ligand that makes metal unavailable to the biota. But, the anaerobic processes provided by the  
550 sulfate-reducing metabolisms could also contribute to greenhouse gas emissions. In contrast, the  
551 identified bioindicators of the SB external sector belong to microbial taxa sensitive to multiple  
552 contaminants with mainly aerobic metabolism, which is consistent with the environmental  
553 conditions prevailing in the SB external sector.

554 In order to provide detailed information on the microbial communities, it is suggested that  
555 future works investigate the metabolic functions affected by the chronic exposition to metallic  
556 contamination of SB to gain information on the influence of contaminants on microbial metabolism.

557

558

#### 559 **Acknowledgments:**

560

561 The study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível  
562 Superior – Brazil (CAPES/Finance Code 001), PRINT-UFF-FEEDBACKS Project  
563 (CAPES/Finance Code 88887.310301/2018-00). We acknowledge the financial support from the  
564 European programme ERANET-MED through the AQUASALT (NMED-0003-01) project, the  
565 French Research Agency (ANR) through the ANR-17-NMED-0003-01.

567 **References:**

568

- 569 Astudillo-García, C., Hermans, S.M., Stevenson, B., Buckley, H.L., Lear, G., 2019. Microbial  
570 assemblages and bioindicators as proxies for ecosystem health status: potential and limitations.  
571 *Appl. Microbiol. Biotechnol.* 103, 6407–6421. <https://doi.org/10.1007/s00253-019-09963-0>
- 572 Ayangbenro, A.S., Olanrewaju, O.S., Babalola, O.O., 2018. Sulfate-reducing bacteria as an  
573 effective tool for sustainable acid mine bioremediation. *Front. Microbiol.* 9, 1986.  
574 <https://doi.org/10.3389/FMICB.2018.01986/BIBTEX>
- 575 Aylagas, E., Borja, Á., Tangherlini, M., Dell’Anno, A., Corinaldesi, C., Michell, C.T., Irigoien, X.,  
576 Danovaro, R., Rodríguez-Ezpeleta, N., 2017. A bacterial community-based index to assess the  
577 ecological status of estuarine and coastal environments. *Mar. Pollut. Bull.* 114, 679–688.  
578 <https://doi.org/10.1016/j.marpolbul.2016.10.050>
- 579 Bai, Y., Shi, Q., Wen, D., Li, Z., Jefferson, W.A., Feng, C., Tang, X., 2012. Bacterial communities  
580 in the sediments of Dianchi Lake, a partitioned eutrophic waterbody in China. *PLoS One* 7,  
581 e37796–e37796. <https://doi.org/10.1371/JOURNAL.PONE.0037796>
- 582 Barcellos, C., De Lacerda, L.D., Ceradini, S., 1997. Sediment origin and budget in Sepetiba Bay  
583 (Brazil) - an approach based on multielemental analysis. *Environ. Geol.* 32, 203–209.  
584 <https://doi.org/10.1007/s002540050208>
- 585 Beaulne, J.S., Mishra, S.R., Suar, M., Panda, A.N., Rastogi, G., Pattnaik, A.K., Raina, V., Vogel,  
586 T.M., 2020. Spatial analysis of bacteria in brackish lake sediment. *Int. J. Sediment Res.* 35,  
587 227–236. <https://doi.org/10.1016/j.ijsrc.2020.01.003>
- 588 Beccati, A., Gerken, J., Quast, C., Yilmaz, P., Glöckner, F.O., 2017. SILVA tree viewer: Interactive  
589 web browsing of the SILVA phylogenetic guide trees. *BMC Bioinformatics* 18.  
590 <https://doi.org/10.1186/s12859-017-1841-3>
- 591 Beelen, P. V., Doelman, P., 1997. Significance and application of microbial toxicity tests in  
592 assessing ecotoxicological risks of contaminants in soil and sediment. *Chemosphere* 34, 455–  
593 499.
- 594 Ben Salem, F., Ben Said, O., Cravo-Laureau, C., Mahmoudi, E., Bru, N., Monperrus, M., Duran,  
595 R., 2019. Bacterial community assemblages in sediments under high anthropogenic pressure at  
596 Ichkeul Lake/Bizerte Lagoon hydrological system, Tunisia. *Environ. Pollut.* 252, 644–656.  
597 <https://doi.org/10.1016/j.envpol.2019.05.146>
- 598 Bergsveinson, J., Roy, J., Maynard, C., Sanschagrín, S., Freeman, C.N., Swerhone, G.D.W., Dynes,  
599 J.J., Tremblay, J., Greer, C.W., Korber, D.R., Lawrence, J.R., 2020. Metatranscriptomic  
600 insights into the response of river biofilm communities to ionic and nano-zinc oxide exposures.  
601 *Front. Microbiol.* 11, 15. <https://doi.org/10.3389/fmicb.2020.00267>
- 602 Birrer, S.C., Dafforn, K.A., Sun, M.Y., Williams, R.B.H., Potts, J., Scanes, P., Kelaher, B.P.,  
603 Simpson, S.L., Kjelleberg, S., Swarup, S., Steinberg, P., Johnston, E.L., 2019. Using meta-  
604 omics of contaminated sediments to monitor changes in pathways relevant to climate  
605 regulation. *Environ. Microbiol.* 21, 389–401. <https://doi.org/10.1111/1462-2920.14470>
- 606 Bordenave, S., Fourçans, A., Blanchard, S., Goñi, M. S., Caumette, P., Duran, R., 2004. Structure  
607 and functional analyses of bacterial communities changes in microbial mats following  
608 petroleum exposure. *Ophelia*, 58, 195-203. doi:10.1080/00785236.2004.10410227
- 609 Bourhane, Z., Lanzén, A., Cagnon, C., Ben Said, O., Mahmoudi, E., Coulon, F., Atai, E., Borja, A.,

- 610 Cravo-Laureau, C., Duran, R., 2022. Microbial diversity alteration reveals biomarkers of  
611 contamination in soil-river-lake continuum. *J. Hazard. Mater.* 421, 126789.  
612 <https://doi.org/10.1016/J.JHAZMAT.2021.126789>
- 613 Bouskill, N.J., Barker-Finkel, J., Galloway, T.S., Handy, R.D., Ford, T.E., 2010. Temporal bacterial  
614 diversity associated with metal-contaminated river sediments. *Ecotoxicology* 19, 317–328.  
615 <https://doi.org/10.1007/s10646-009-0414-2>
- 616 Brochier-Armanet, C., Moreira, D., 2015. Horizontal gene transfer in microbial ecosystems, in:  
617 Bertrans, J.-C., Caumette, P., Lebaron, P., Matheron, R., Normand, P., Sime-Ngando, T.  
618 (Eds.), *Environmental Microbiology: Fundamentals and Applications*. Springer Netherlands,  
619 pp. 445–481. [https://doi.org/10.1007/978-94-017-9118-2\\_12](https://doi.org/10.1007/978-94-017-9118-2_12)
- 620 Brown, A.M., Bass, A.M., Pickard, A.E., 2022. Anthropogenic-estuarine interactions cause  
621 disproportionate greenhouse gas production: A review of the evidence base. *Marine Poll. Bul.*  
622 174, 113240. doi:10.1016/j.marpolbul.2021.113240
- 623 Bruneel, O., Duran, R., Casiot, C., Elbaz-Poulichet, F., Personné, J.C., 2006. Diversity of  
624 microorganisms in Fe-As-rich acid mine drainage waters of Carnoulès, France. *Appl. Environ.*  
625 *Microbiol.* 72, 551–556. <https://doi.org/10.1128/AEM.72.1.551-556.2006>
- 626 Burgos, M., Sierra, A., Ortega, T., Forja, J.M., 2015. Anthropogenic effects on greenhouse gas  
627 (CH<sub>4</sub> and N<sub>2</sub>O) emissions in the guadalete river estuary (SW Spain). *Sci. Total Environ.* 503-  
628 504, 179-189. doi:10.1016/j.scitotenv.2014.06.038
- 629 Burton, G.A., 2002. Sediment quality criteria in use around the world. *Limnology* 3, 65–75.  
630 <https://doi.org/10.1007/s102010200008>
- 631 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer,  
632 N., Pêa, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig,  
633 J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J.,  
634 Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J.,  
635 Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat.*  
636 *Methods*. <https://doi.org/10.1038/nmeth.f.303>
- 637 Caruso, G., La Ferla, R., Azzaro, M., Zoppini, A., Marino, G., Petoichi, T., Corinaldesi, C.,  
638 Leonardi, M., Zaccone, R., Fonda, S., Caroppo, C., Monticelli, L., Azzaro, F., Decembrini, F.,  
639 Maimone, G., Cavallo, R., Stabili, L., Todorova, N., Karamfilov, V., Rastelli, E., Cappello, S.,  
640 Acquaviva, M.I., Narracci, M., De Angelis, R., Del Negro, P., Latini, M., Danovaro, R., 2015.  
641 Microbial assemblages for environmental quality assessment: Knowledge, gaps and usefulness  
642 in the European marine strategy framework directive. *Crit. Rev. Microbiol.* 42.  
643 <https://doi.org/10.3109/1040841X.2015.1087380>
- 644 Carvalho, A.C.B., Moreira, V. de A., Vicente, M. de C., Bernardes, M.C., Bidone, E.D., Sabadini-  
645 Santos, E., 2021. Evolution of the sources contribution of aliphatic hydrocarbons and their fate  
646 in Sepetiba Bay, Rio De Janeiro, Brazil. *Estuar. Coast. Shelf Sci.* 261, 107548.  
647 <https://doi.org/10.1016/J.ECSS.2021.107548>
- 648 Carvalho, A.C.B., Moreira, V. de A., Vicente, M. de C., Bidone, E.D., Bernardes, M.C., Sabadini-  
649 Santos, E., 2022. Sterols and PAHs as indicators of pollution and sources of organic matter at  
650 Southeast Brazilian Bay. *Marine Poll. Bul.* 181, 113899.  
651 <https://doi.org/10.1016/j.marpolbul.2022.113899>.
- 652 Carvalho, A.C.B., Silva, L.J., Dick, D.P., Moreira, V. de A., Vicente, M. de C., Andrade, A.F.O. de,  
653 Bidone, E.D., Sabadini-Santos, E., 2020. Análise espectroscópica da matéria orgânica no  
654 sedimento superficial da Baía de Sepetiba, Rio de Janeiro, Brasil. *Quim. Nova* 43, 552–557.  
655 <https://doi.org/10.21577/0100-4042.20170518>
- 656 Chen, Y., Jiang, Y., Huang, H., Mou, L., Ru, J., Zhao, J., Xiao, S., 2018. Long-term and high-

- 657 concentration heavy-metal contamination strongly influences the microbiome and functional  
658 genes in Yellow River sediments. *Sci. Total Environ.* 637–638, 1400–1412.  
659 <https://doi.org/10.1016/j.scitotenv.2018.05.109>
- 660 Chenn, H., 2018. Generate High-Resolution Venn and Euler Plots. VennDiagram package. R  
661 Packag.
- 662 Coclet, C., Garnier, C., D’Onofrio, S., Durrieu, G., Pasero, E., Le Poupon, C., Omanović, D.,  
663 Mullot, J.U., Misson, B., Briand, J.F., 2021. Trace metal contamination impacts predicted  
664 functions more than structure of marine prokaryotic biofilm communities in an anthropized  
665 coastal area. *Front. Microbiol.* 12, 16. <https://doi.org/10.3389/fmicb.2021.589948>
- 666 Coclet, C., Garnier, C., Durrieu, G., Omanović, D., D’Onofrio, S., Le Poupon, C., Mullot, J.U.,  
667 Briand, J.F., Misson, B., 2019. Changes in bacterioplankton communities resulting from direct  
668 and indirect interactions with trace metal gradients in an urbanized marine coastal area. *Front.*  
669 *Microbiol.* 10, 257. <https://doi.org/https://doi.org/10.3389/fmicb.2019.00257>
- 670 CONAMA - Conselho Nacional do Meio Ambiente, 2012. Resolução nº 454, de 01 de novembro de  
671 2012. *Diário Of. da União* 17. <https://doi.org/10.1073/pnas.0703993104>
- 672 Coppolecchia, D., Puglisi, E., Vasileiadis, S., Suciú, N., Hamon, R., Maria Beone, G., Trevisan, M.,  
673 2011. Relative sensitivity of different soil biological properties to zinc. *Soil Biol. Biochem.* 43,  
674 1798–1807. <https://doi.org/10.1016/j.soilbio.2010.06.018>
- 675 Danovaro, R., Company, J.B., Corinaldesi, C., D’Onghia, G., Galil, B., Gambi, C., Gooday, A.J.,  
676 Lampadariou, N., Luna, G.M., Morigi, C., Olu, K., Polymenakou, P., Ramirez-Llodra, E.,  
677 Sabbatini, A., Sardá, F., Sibuet, M., Tselepidis, A., 2010. Deep-sea biodiversity in the  
678 Mediterranean Sea: The known, the unknown, and the unknowable. *PLoS One* 5, 25.  
679 <https://doi.org/10.1371/journal.pone.0011832>
- 680 Dell’Anno, A., Mei, M.L., Ianni, C., Danovaro, R., 2003. Impact of bioavailable heavy metals on  
681 bacterial activities in coastal marine sediments. *World J. Microbiol. Biotechnol.* 19, 93–100.  
682 <https://doi.org/10.1023/A:1022581632116>
- 683 DeLong, E.F., Preston, C.M., Mincer, T., Rich, V., Hallam, S.J., Frigaard, N., Martinez, A.,  
684 Sullivan, M.B., Edwards, R., Brito, B.R., Chisholm, S.W., Karl, D.M., 2006. Community  
685 genomics among stratified microbial assemblages in the ocean’s interior. *Science* 311, 496–  
686 503. <https://doi.org/10.1126/science.1120250>
- 687 Di Toro, D.M., Mahony, J.D., Hansen, D.J., Scott, K.J., Hicks, M.B., Mayr, S.M., Redmond, M.S.,  
688 1990. Toxicity of cadmium in sediments: The role of acid volatile sulfide. *Environ. Toxicol.*  
689 *Chem.* 9, 1487–1502. <https://doi.org/10.1002/etc.5620091208>
- 690 Dick, G.J., 2018. *Genomic approaches in Earth and environmental sciences*, 1st ed. Wiley-  
691 Blackwell, Oxford.
- 692 Duran, R., Bielen, A., Paradžik, T., Gassie, C., Pustijanac, E., Cagnon, C., Hamer, B., Vujaklija, D.,  
693 2015a. Exploring Actinobacteria assemblages in coastal marine sediments under contrasted  
694 Human influences in the West Istria Sea, Croatia. *Environ. Sci. Pollut. Res.* 22, 15215–15229.  
695 <https://doi.org/10.1007/s11356-015-4240-1>
- 696 Duran, R., Cuny, P., Bonin, P., Cravo-Laureau, C., 2015b. Microbial ecology of hydrocarbon-  
697 polluted coastal sediments. *Environ. Sci. Pollut. Res.* 22, 15195–15199. doi:10.1007/s11356-  
698 015-5373-y
- 699 Faust, K., Sathirapongsasuti, J.F., Izard, J., Segata, N., Gevers, D., Raes, J., Huttenhower, C., 2012.  
700 Microbial co-occurrence relationships in the Human Microbiome. *PLoS Comput. Biol.* 8, 17.  
701 <https://doi.org/10.1371/journal.pcbi.1002606>
- 702 Flemming, H.C., Wingender, J., 2010. The biofilm matrix. *Nat. Rev. Microbiol.* 8, 623–633.

- 703 <https://doi.org/10.1038/nrmicro2415>
- 704 Förstner, U., Wittmann, G.T.W., 1981. Metal pollution in the aquatic environment, 2nd ed, Metal  
705 pollution in the aquatic environment. Springer Verlag. [https://doi.org/10.1007/978-3-642-](https://doi.org/10.1007/978-3-642-69385-4_1)  
706 [69385-4\\_1](https://doi.org/10.1007/978-3-642-69385-4_1)
- 707 Gałuszka, A., 2007. A review of geochemical background concepts and an example using data from  
708 Poland. *Environ. Geol.* 52, 861–870. <https://doi.org/10.1007/s00254-006-0528-2>
- 709 Gillan, D.C., Danis, B., Pernet, P., Joly, G., Dubois, P., 2005. Structure of sediment-associated  
710 microbial communities along a heavy-metal contamination gradient in the marine  
711 environment. *Appl. Environ. Microbiol.* 71, 679–690. [https://doi.org/10.1128/AEM.71.2.679-](https://doi.org/10.1128/AEM.71.2.679-690.2005)  
712 [690.2005](https://doi.org/10.1128/AEM.71.2.679-690.2005)
- 713 Guo, X.-P., Yang, Y., Niu, Z.-S., Lu, D.-P., Zhu, C.-H., Feng, J.-N., Wu, J.-Y., Chen, Y.-R., Tou,  
714 F.-Y., Liu, M., Hou, L., Yang, : Y, 2018. Characteristics of microbial community indicate  
715 anthropogenic impact on the sediments along the Yangtze Estuary and its coastal area, China.  
716 *Sci. Total Environ.* 648, 306–314. <https://doi.org/10.1016/j.scitotenv.2018.08.162>
- 717 Hafez, T., Ortiz-Zarragoitia, M., Cagnon, C., Cravo-Laureau, C., Duran, R., 2022. Legacy and  
718 dispersant influence microbial community dynamics in cold seawater contaminated by crude  
719 oil water accommodated fractions. *Environ. Res.* 212, 113467.  
720 [doi:10.1016/j.envres.2022.113467](https://doi.org/10.1016/j.envres.2022.113467)
- 721 Hamza-Chaffai, A., 2014. Usefulness of bioindicators and biomarkers in pollution biomonitoring.  
722 *Int. J. Biotechnol. Wellness Ind.* 3, 19–26. <https://doi.org/10.6000/1927-3037.2014.03.01.4>
- 723 Harrison, J.J., Ceri, H., Turner, R.J., 2007. Multimetal resistance and tolerance in microbial  
724 biofilms. *Nat. Rev. Microbiol.* 5, 928–938. <https://doi.org/10.1038/nrmicro1774>
- 725 IBGE, 2011. Atlas geográfico das zonas costeiras e oceânicas do Brasil.
- 726 IBGE - Instituto Brasileiro de Geografia e Estatística, 2010. Censo demográfico – características  
727 gerais da população. Resultados da amostra [WWW Document]. Inst. Bras. Geogr. e  
728 Estatística. URL <http://censo2010.ibge.gov.br/resultados.html>
- 729 Jeanbille, M., Gury, J., Duran, R., Tronczynski, J., Agogué, H., Saïd, O. Ben, Ghiglione, J.F.,  
730 Auguet, J.C., 2016a. Response of core microbial consortia to chronic hydrocarbon  
731 contaminations in coastal sediment habitats. *Front. Microbiol.* 7. 1637.  
732 [doi:10.3389/fmicb.2016.01637](https://doi.org/10.3389/fmicb.2016.01637)
- 733 Jeanbille, M., Gury, J., Duran, R., Tronczynski, J., Ghiglione, J.F., Agogué, H., Saïd, O. Ben, Taïb,  
734 N., Debroas, D., Garnier, C., Auguet, J.C., 2016b. Chronic polyaromatic hydrocarbon (PAH)  
735 contamination is a marginal driver for community diversity and prokaryotic predicted  
736 functioning in coastal sediments. *Front. Microbiol.* 7. 1303.  
737 <https://doi.org/10.3389/fmicb.2016.01303>
- 738 Jurburg, S.D., Nunes, I., Brejnrod, A., Jacquioud, S., Priemé, A., Sørensen, S.J., Van Elsas, J.D.,  
739 Salles, J.F., 2017. Legacy effects on the recovery of soil bacterial communities from extreme  
740 temperature perturbation. *Front. Microbiol.* 8, 13. <https://doi.org/10.3389/fmicb.2017.01832>
- 741 Kim, J., Kim, K., Hahm, Y., Kim, B., Chun, J., Cha, C. 2008. *Actibacter sediminis* gen. nov., sp.  
742 nov. , a marine bacterium of the family flavobacteriaceae isolated from tidal flat sediment. *Int.*  
743 *J. Syst. Evol. Microbiol.* 58, 139-143. [doi:10.1099/ijs.0.65346-0](https://doi.org/10.1099/ijs.0.65346-0)
- 744 Kolde, R., 2019. pheatmap: Pretty Heatmaps.
- 745 Kristensen, J.M., Singleton, C., Clegg, L., Petriglieri, F., Nielsen, P.H., 2021. High diversity and  
746 functional potential of undescribed Acidobacteriota” in Danish wastewater treatment plants.  
747 *Front. Microbiol.* 12, 643950. [doi:10.3389/fmicb.2021.643950](https://doi.org/10.3389/fmicb.2021.643950)

- 748 Kublanov, I. V., Sigalova, O.M., Gavrilov, S.N., Lebedinsky, A.V., Rinke, C., Kovaleva, O.,  
749 Chernyh, N.A., Ivanova, N., Daum, C., Reddy, T.B., Klenk, H.P., Spring, S., Göker, M., Reva,  
750 O.N., Miroshnichenko, M.L., Kyrpides, N.C., Woyke, T., Gelfand, M.S., Bonch-  
751 Osmolovskaya, E.A., 2017. Genomic analysis of *Caldithrix abyssi*, the thermophilic anaerobic  
752 bacterium of the novel bacterial phylum *Calditrichaeota*. *Front. Microbiol.* 8, 195.  
753 doi:10.3389/fmicb.2017.00195
- 754 Lacerda, L.D., Molisani, M.M., 2006. Three decades of Cd and Zn contamination in Sepetiba Bay ,  
755 SE Brazil : Evidence from the mangrove oyster *Crassostrea rhizophorae* 52, 2004–2007.  
756 <https://doi.org/10.1016/j.marpolbul.2006.04.006>
- 757 Lacerda, L.D., Pfeiffer, W.C., Fiszman, M., 1987. Heavy metal distribution, availability and fate in  
758 Sepetiba Bay, S.E. Brazil. *Sci. Total Environ.* 65, 163–173. [https://doi.org/10.1016/0048-](https://doi.org/10.1016/0048-9697(87)90169-0)  
759 [9697\(87\)90169-0](https://doi.org/10.1016/0048-9697(87)90169-0)
- 760 Lewis, K., 2007. Persister cells, dormancy and infectious disease. *Nat. Rev. Microbiol.*  
761 <https://doi.org/10.1038/nrmicro1557>
- 762 Li, C., Quan, Q., Gan, Y., Dong, J., Fang, J., Wang, L., Liu, J., 2020. Effects of heavy metals on  
763 microbial communities in sediments and establishment of bioindicators based on microbial  
764 taxa and function for environmental monitoring and management. *Sci. Total Environ.* 749, 9.  
765 <https://doi.org/10.1016/j.scitotenv.2020.141555>
- 766 Li, Y., Zheng, L., Zhang, Y., Liu, H., Jing, H., 2019. Comparative metagenomics study reveals  
767 pollution induced changes of microbial genes in mangrove sediments. *Sci. Rep.* 9, 1–11.  
768 <https://doi.org/10.1038/s41598-019-42260-4>
- 769 Liu, J. L., Yao, J., Wang, F., Min, N., Gu, J. -, Li, Z.F., Sunahara, G., Duran, R., Solevic-Knudsen,  
770 T., Hudson-Edwards, K.A., Alakangas, L., 2019. Bacterial diversity in typical abandoned  
771 multi-contaminated nonferrous metal(loid) tailings during natural attenuation. *Environmental*  
772 *Pollution*, 247, 98-107. doi:10.1016/j.envpol.2018.12.045
- 773 Ma, Y., Liu, F., Kong, Z., Yin, J., Kou, W., Wu, L., Ge, G., 2016. The distribution pattern of  
774 sediment archaea community of the poyang lake, the largest freshwater lake in China. *Archaea*  
775 2016, 12. <https://doi.org/10.1155/2016/9278929>
- 776 Marinho, C.C., Campos, E.A., Guimarães, J.R.D., Esteves, F.A., 2012. Effect of sediment  
777 composition on methane concentration and production in the transition zone of a mangrove  
778 (Sepetiba Bay, Rio de Janeiro, Brazil). *Brazilian J. Biol.* 72, 429–436.  
779 <https://doi.org/10.1590/S1519-69842012000300003>
- 780 Marx, S.K., Kamber, B.S., 2010. Trace-element systematics of sediments in the Murray-Darling  
781 Basin, Australia: Sediment provenance and palaeoclimate implications of fine scale chemical  
782 heterogeneity. *Appl. Geochemistry* 25, 1221–1237.  
783 <https://doi.org/10.1016/j.apgeochem.2010.05.007>
- 784 Maturro, B., Ubaldi, C., Rossetti, S., 2016. Microbiome dynamics of a polychlorobiphenyl (PCB)  
785 historically contaminated marine sediment under conditions promoting reductive  
786 dechlorination. *Front. Microbiol.* 7, 14. <https://doi.org/10.3389/fmicb.2016.01502>
- 787 Meng, D., Li, J., Liu, T., Liu, Y., Yan, M., Hu, J., Li, X., Liu, X., Liang, Y., Liu, H., Yin, H., 2019.  
788 Effects of redox potential on soil cadmium solubility: Insight into microbial community. *J.*  
789 *Environ. Sci.* 75, 224–232. <https://doi.org/10.1016/j.jes.2018.03.032>
- 790 Milligan, G.W., Cooper, M.C., 1985. An examination of procedures for determining the number of  
791 clusters in a data set. *Psychometrika*. <https://doi.org/10.1007/BF02294245>
- 792 Miyazaki, M., Sakai, S., Yamanaka, Y., Saito, Y., Takai, K., Imachi, H., 2014. *Spirochaeta*  
793 *psychrophila* sp. nov., a psychrophilic spirochaete isolated from subseafloor sediment, and

- 794 emended description of the genus spirochaeta. *Int. J. Syst. Evol. Microbiol.* 64, 2798-2804.  
795 doi:10.1099/ijs.0.062463-0
- 796 Molisani, M.M., Kjerfve, B., Silva, A.P., Lacerda, L.D., 2006. Water discharge and sediment load  
797 to Sepetiba Bay from an anthropogenically-altered drainage basin, SE Brazil. *J. Hydrol.* 331,  
798 425–433. <https://doi.org/10.1016/j.jhydrol.2006.05.038>
- 799 Molisani, M.M., Marins, R. V., Machado, W., Paraquetti, H.H.M., Bidone, E.D., Lacerda, L.D.,  
800 2004. Environmental changes in Sepetiba Bay, SE Brazil. *Reg. Environ. Chang.* 4, 17–27.  
801 <https://doi.org/10.1007/s10113-003-0060-9>
- 802 Monte, C.N., Rodrigues, A.P.C., Cordeiro, R.C., Freire, A.S., Santelli, R.E., Machado, W., 2015.  
803 Changes in Cd and Zn bioavailability upon an experimental resuspension of highly  
804 contaminated coastal sediments from a tropical estuary. *Sustain. Water Resour. Manag.* 1,  
805 335–342. <https://doi.org/10.1007/s40899-015-0034-3>
- 806 Moreira, V.A., Carvalho, A.C.B. de, Fontana, L.F., Bidone, E.D., Sabadini-Santos, E., 2021.  
807 Applying enzymatic biomarkers of the in situ microbial community to assess the sediment risk  
808 from Sepetiba Bay (Brazil). *Mar. Pollut. Bull.* 169, 11.  
809 <https://doi.org/10.1016/j.marpolbul.2021.112547>
- 810 Moreira, V.A., Cravo-Laureau, C., Borges de Carvalho, A.C., Baldy, A., Bidone, E.D., Sabadini-  
811 Santos, E., Duran, R., 2022. Microbial community metabolic alterations and resistance to  
812 metals and antibiotics driven by chronic exposition to multiple pollutants in a highly impacted  
813 tropical coastal bay. *Chemosphere* 307, 135928.  
814 <https://doi.org/10.1016/J.CHEMOSPHERE.2022.135928>
- 815 Mossa, A.W., Dickinson, M.J., West, H.M., Young, S.D., Crout, N.M.J., 2017. The response of soil  
816 microbial diversity and abundance to long-term application of biosolids. *Environ. Pollut.* 224,  
817 16–25. <https://doi.org/10.1016/j.envpol.2017.02.056>
- 818 Nascimento, J.R., Easson, C.G., Jurelevicius, D. de A., Lopez, J. V., Bidone, E.D., Sabadini-Santos,  
819 E., 2020. Microbial community shift under exposure of dredged sediments from a eutrophic  
820 bay. *Environ. Monit. Assess.* 192, 1–16. <https://doi.org/10.1007/s10661-020-08507-8>
- 821 Nascimento, J.R., Silveira, A.E.F., Bidone, E.D., Sabadini-Santos, E., 2019. Microbial community  
822 activity in response to multiple contaminant exposure: a feasible tool for sediment quality  
823 assessment. *Environ. Monit. Assess.* 191. <https://doi.org/10.1007/s10661-019-7532-y>
- 824 Nies, D.H., 1999. Microbial heavy-metal resistance. *Appl Microbiol Biotechnol* 730–750.
- 825 Nies, D.H., 1995. The cobalt, zinc, and cadmium efflux system CzcABC from *Alcaligenes*  
826 *eutrophus* functions as a cation-proton antiporter in *Escherichia coli*. *J. Bacteriol.* 2707–2712.  
827 <https://doi.org/10.1128/jb.177.10.2707-2712.1995>
- 828 Nogales, B., Lanfranconi, M.P., Piña-Villalonga, J.M., Bosch, R., 2011. Anthropogenic  
829 perturbations in marine microbial communities. *FEMS Microbiol. Rev.* 35, 275–298.  
830 <https://doi.org/10.1111/j.1574-6976.2010.00248.x>
- 831 Nuzzo, A., Negroni, A., Zanaroli, G., Fava, F., 2017. Identification of two organohalide-respiring  
832 *Dehalococcoidia* associated to different dechlorination activities in PCB-impacted marine  
833 sediments. *Microb. Cell Fact.* 16, 13. <https://doi.org/10.1186/s12934-017-0743-4>
- 834 Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R.,  
835 O'hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Maintainer,  
836 H.W., 2019. *Vegan: Community Ecology Package*. R package version 2.5-6.
- 837 Paissé, S., Coulon, F., Goñi-Urriza, M., Peperzak, L., McGenity, T.J., Duran, R., 2008. Structure of  
838 bacterial communities along a hydrocarbon contamination gradient in a coastal sediment.  
839 *FEMS Microbiol. Ecol.* 66, 295–305. <https://doi.org/10.1111/j.1574-6941.2008.00589.x>



- 840 Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: Assessing small subunit  
841 rRNA primers for marine microbiomes with mock communities, time series and global field  
842 samples. *Environ. Microbiol.* 18, 1403–1414. <https://doi.org/10.1111/1462-2920.13023>
- 843 Petro, C., Jochum, L. M., Schreiber, L., Marshall, I.P.G., Schramm, A., Kjeldsen, K.U., 2019.  
844 Single-cell amplified genomes of two uncultivated members of the deltaproteobacterial SEEP-  
845 SRB1 clade, isolated from marine sediment. *Mar. Genom.* 46, 66-69.  
846 [doi:10.1016/j.margen.2019.01.004](https://doi.org/10.1016/j.margen.2019.01.004)
- 847 Prabhakaran, P., Ashraf, M.A., Aqma, W.S., 2016. Microbial stress response to heavy metals in the  
848 environment. *RSC Adv.* 6, 109862–109877. <https://doi.org/10.1039/c6ra10966g>
- 849 Pringault, O., Duran, R., Jacquet, S., & Torr ton, J., 2008. Temporal variations of microbial activity  
850 and diversity in marine tropical sediments (New Caledonia Lagoon). *Microbial Ecol.* 55, 247-  
851 258. [doi:10.1007/s00248-007-9272-8](https://doi.org/10.1007/s00248-007-9272-8)
- 852 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Gl ckner, F.O.,  
853 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-  
854 based tools. *Nucleic Acids Res.* 41, 590–596. <https://doi.org/10.1093/nar/gks1219>
- 855 R Core Team, 2019. R: A Language and Environment for Statistical Computing. Vienna, Austria.
- 856 Ribeiro, A.P., Figueiredo, A.M.G., Santos, J.O. dos, Dantas, E., Cotrim, M.E.B., Cesar Lopes  
857 Figueira, R., V. Silva Filho, E., Cesar Wasserman, J., 2013. Combined SEM/AVS and  
858 attenuation of concentration models for the assessment of bioavailability and mobility of  
859 metals in sediments of Sepetiba Bay (SE Brazil). *Mar. Pollut. Bull.* 68, 55–63.  
860 <https://doi.org/10.1016/j.marpolbul.2012.12.023>
- 861 Rosado, D., Usero, J., Morillo, J., 2015. Application of a new integrated sediment quality  
862 assessment method to Huelva estuary and its littoral of influence (Southwestern Spain). *Mar.*  
863 *Pollut. Bull.* 98, 106–114. <https://doi.org/10.1016/j.marpolbul.2015.07.008>
- 864 Sabadini-Santos, E., Da Silva, T.S., Lopes-Rosa, T.D., Mendonça-Filho, J.G., Santelli, R.E.,  
865 Crapez, M.A.C., 2014. Microbial activities and bioavailable concentrations of Cu, Zn, and Pb  
866 in Sediments from a tropic and eutrothicated bay. *Water. Air. Soil Pollut.* 225, 1949.  
867 <https://doi.org/10.1007/s11270-014-1949-2>
- 868 Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C.,  
869 2011. Metagenomic biomarker discovery and explanation. *Genome Biol.* 12, R60.  
870 [doi.org/10.1186/gb-2011-12-6-r60](https://doi.org/10.1186/gb-2011-12-6-r60)
- 871 Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski,  
872 B., Ideker, T., 2003. Cytoscape: A software Environment for integrated models of  
873 biomolecular interaction networks. *Genome Res.* 2498–2504.  
874 <https://doi.org/10.1101/gr.1239303>
- 875 Sharrar, A. M., Flood, B. E., Bailey, J. V., Jones, D. S., Biddanda, B. A., Ruberg, S. A., Marcus, D.  
876 N., Dick, G. J., 2017. Novel large sulfur bacteria in the metagenomes of groundwater-fed  
877 chemosynthetic microbial mats in the lake huron basin. *Front. Microbiol.* 8, 791.  
878 [doi:10.3389/fmicb.2017.00791](https://doi.org/10.3389/fmicb.2017.00791)
- 879 Signorini, S.R., 1980. A study of the circulation in bay of Ilha Grande and bay of Sepetiba - Part II:  
880 An assessment to the tidally and wind-driven circulation using a finite element numerical  
881 model. *Bolm Inst. Ocean.* 29, 57–68.
- 882 Smoak, J.M., Sanders, C.J., Patchineelam, S.R., Moore, W.S., 2012. Radium mass balance and  
883 submarine groundwater discharge in Sepetiba Bay, Rio de Janeiro State, Brazil. *J. South Am.*  
884 *Earth Sci.* 39, 44–51. <https://doi.org/10.1016/j.jsames.2012.07.004>
- 885 Stauffert, M., Cravo-Laureau, C., J z quel, R., Barantal, S., Cuny, P., Gilbert, F., Cagnon, C.,

- 886 Militon, C., Amouroux, D., Mahdaoui, F., Bouyssiere, B., Stora, G., Merlin, F.X., Duran, R.,  
887 2013. Impact of oil on bacterial community structure in bioturbated Ssediments. PLoS One 8,  
888 16. <https://doi.org/10.1371/journal.pone.0065347>
- 889 Sun, M.Y., Dafforn, K.A., Johnston, E.L., Brown, M. V., 2013. Core sediment bacteria drive  
890 community response to anthropogenic contamination over multiple environmental gradients.  
891 Environ. Microbiol. 15, 2517–2531. <https://doi.org/10.1111/1462-2920.12133>
- 892 Suzuki, D., Li, Z., Cui, X., Zhang, C., Katayama, A., 2014. Reclassification of *Desulfobacterium*  
893 *anilini* as *Desulfatiglans anilini* comb. nov. within *Desulfatiglans* gen. nov., and description of  
894 a 4-chlorophenol-degrading sulfate-reducing bacterium, *Desulfatiglans parachlorophenolica*  
895 sp. nov. Int. J. Syst. Evol. Microbiol. 64, 3081–3086. doi: 10.1099/ijs.0.064360-0
- 896 Tamburini, E., Doni, L., Lussu, R., Meloni, F., Cappai, G., Carucci, A., Casalone, E., Mastromei,  
897 G., Vitali, F., 2020. Impacts of anthropogenic pollutants on benthic prokaryotic communities  
898 in Mediterranean touristic ports. Front. Microbiol. 11, 1234.  
899 <https://doi.org/10.3389/fmicb.2020.01234>
- 900 Tanagerlini, M., Corinaldesi, C., Rastelli, E., Musco, L., Armiento, G., Danovaro, R., Dell’Anno,  
901 A., 2020. Chemical contamination can promote turnover diversity of benthic prokaryotic  
902 assemblages: The case study of the Bagnoli–Coroglio bay (southern Tyrrhenian Sea). Mar.  
903 Environ. Res. 160, 11. <https://doi.org/10.1016/j.marenvres.2020.105040>
- 904 Tedesco, P., Palma Esposito, F., Masino, A., Vitale, G.A., Tortorella, E., Poli, A., Nicolaus, B., van  
905 Zyl, L., Trindade, M., de Pascale, D., 2021. Isolation and characterization of strain  
906 *Exiguobacterium* sp. Krl4, a producer of bioactive secondary metabolites from a tibetan  
907 glacier. Microorganisms, 9, 890 doi:10.3390/microorganisms9050890
- 908 Terrisse, F., Cravo-Laureau, C., Noël, C., Cagnon, C., Dumbrell, A.J., McGenity, T.J., Duran, R.,  
909 2017. Variation of oxygenation conditions on a hydrocarbonoclastic microbial community  
910 reveals Alcanivorax and Cycloclasticus ecotypes. Front. Microbiol. 8, 1–15.  
911 <https://doi.org/10.3389/fmicb.2017.01549>
- 912 Waite, C.C. da C., da Silva, G.O.A., Bitencourt, J.A.P., Sabadini-Santos, E., Crapez, M.A.C., 2016.  
913 Copper and lead removal from aqueous solutions by bacterial consortia acting as biosorbents.  
914 Mar. Pollut. Bull. 109, 386–392. <https://doi.org/10.1016/j.marpolbul.2016.05.044>
- 915 Wang, X., Yu, L., Liu, T., He, Y., Wu, S., Chen, H., Yuan, X., Wang, J., Li, X., Li, H., Que, Z.,  
916 Qing, Z., Zhou, T., 2022. Methane and nitrous oxide concentrations and fluxes from heavily  
917 polluted urban streams: comprehensive influence of pollution and restoration. Env. Pol. 313,  
918 120098. <https://doi.org/10.1016/j.envpol.2022.120098>.
- 919 Wang, Y., Qian, P.-Y., 2009. Conservative fragments in bacterial 16S rRNA genes and primer  
920 design for 16S Ribosomal DNA amplicons in metagenomic studies. PLoS One 4, e7401.  
921 <https://doi.org/10.1371/journal.pone.0007401>
- 922 Wang, Y., Shi, J., Wang, H., Lin, Q., Chen, X., Chen, Y., 2007. The influence of soil heavy metals  
923 pollution on soil microbial biomass, enzyme activity, and community composition near a  
924 copper smelter. Ecotoxicol. Environ. Saf. 67, 75–81.  
925 <https://doi.org/10.1016/j.ecoenv.2006.03.007>
- 926 Wasmund, K., Schreiber, L., Lloyd, K.G., Petersen, D.G., Schramm, A., Stepanauskas, R.,  
927 Jørgensen, B.B., Adrian, L., 2014. Genome sequencing of a single cell of the widely  
928 distributed marine subsurface dehalococcoidia, phylum chloroflexi. ISME Journal, 8, 383–397.  
929 doi:10.1038/ismej.2013.143
- 930 Whitman, W.B., Coleman, D.C., Wiebe, W.J., 1998. Prokaryotes: The unseen majority. Proc. Natl.  
931 Acad. Sci. U. S. A. 95, 6578–6583. <https://doi.org/10.1073/pnas.95.12.6578>

- 932 Yamada, T., Sekiguchi, Y., Hanada, S., Imachi, H., Ohashi, A., Harada, H., Kamagata, Y., 2006.  
933 *Anaerolinea thermolimosa* sp. nov., *levilinea saccharolytica* gen. nov., sp. nov. and *leptolinea*  
934 *tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes  
935 anaerolineae classis nov. and caldilineae classis nov. in the bacterial phylum chloroflexi. Int. J.  
936 Syst. Evol. Microbiol. 56, 1331-1340. doi:10.1099/ijms.0.64169-0
- 937 Yang, J., Ma, L., Jiang, H., Wu, G., Dong, H., 2016. Salinity shapes microbial diversity and  
938 community structure in surface sediments of the Qinghai-Tibetan Lakes. Sci. Reports 2016 61  
939 6, 1–6. <https://doi.org/10.1038/srep25078>
- 940 Yin, H., Niu, J., Ren, Y., Cong, J., Zhang, Xiaoxia, Fan, F., Xiao, Y., Zhang, Xian, Deng, J., Xie,  
941 M., He, Z., Zhou, J., Liang, Y., Liu, X., 2015. An integrated insight into the response of  
942 sedimentary microbial communities to heavy metal contamination. Sci. Rep. 5, 12.  
943 <https://doi.org/10.1038/srep14266>
- 944 Zárate, A., Dorador, C., Valdés, J., Molina, V., Icaza, G., Pacheco, A. S., Castillo, A., 2021.  
945 Benthic microbial diversity trends in response to heavy metals in an oxygen-deficient  
946 eutrophic bay of the Humboldt current system offshore the Atacama Desert. Env. Pol. 286,  
947 117281. doi:10.1016/j.envpol.2021.117281
- 948 Zhang, Y., Chen, L., Sun, R., Dai, T., Tian, J., Zheng, W., Wen, D., 2016. Temporal and spatial  
949 changes of microbial community in an industrial effluent receiving area in Hangzhou Bay. J.  
950 Environ. Sci. (China) 44, 57–68. <https://doi.org/10.1016/j.jes.2015.11.023>
- 951
- 952

953 **Figure legends**

954 **Figure 1 Location of sampling station in Sepetiba Bay (Rio de Janeiro – Brazil).**

955 **Figure 2 Comparison of microbial communities inhabiting SB sediments.** The comparison was  
956 performed according to (a) sectors and (b) ecological risk classifications provided by the QR  
957 index (Moreira et al., 2021). The Venn diagram shows the shared and the exclusive ASVs  
958 between either SB sectors or QR index classification.

959 **Figure 3 Comparison of microbial community structures in SB sediments.** The canonical  
960 correspondence analysis (CCA) shows the correlations between microbial communities (at  
961 Phylum level - represented by white diamonds) with the physical/geochemical - fine grain  
962 content ( $< 63\mu\text{m}$ ), TOC, and metal CF – and the microbiological - EST and DHA activities -  
963 parameters. Internal sector stations are represented by red triangles, while external sector  
964 stations are represented by blue triangles.

965 **Figure 4 Comparison of microbial community composition in the different stations of SB.** The  
966 bar plot (a) shows the phyla composition and their relative abundances (%) in the different  
967 stations. The Heatmap (b) shows the class composition and their relative abundances (%) in  
968 the different stations. The cluster correlation (c) shows the relationship between prokaryotic  
969 classes and the metal CF, based on Spearman correlations ( $r < -0.39$  or  $r > 0.39$  are significant,  
970  $p < 0.05$ ).

971 **Figure 5 Comparison of microbial communities from the SB stations.** Linear discriminant analysis  
972 effect size (LEfSe) shows the significantly different abundances of prokaryotic genera  
973 according to (a) SB sectors, and (b) the ecological risk classifications provided by the QR  
974 index application (Moreira et al., 2021).

975 **Figure 6 Cluster correlation between microbial ASVs and pollutants.** The analysis was performed  
976 with the relative abundances (%) of the most 50 abundant genera and metal CF  
977 (Supplementary material - Table S1). Heatmap and clustering were based on Spearman  
978 correlations, in which  $r < -0.39$  or  $r > 0.39$  are significant ( $p < 0.05$ ). \* Significant positive  
979 correlations ( $p < 0.05$ ).

980 **Figure 7 Co-occurrence network between ASV abundances and metal contamination factors**  
981 **(CF).** The network was inferred from ASVs relative abundances and CF (Supplementary  
982 material – Table S1) applying Spearman correlations (straight lines), p-value threshold 0.05.  
983 The positive correlations are indicated in green lines and mutual exclusions in red lines. Metal  
984 contamination factors are indicated by yellow diamonds, while the different geometric shapes  
985 represent ASVs sorted by phylum.

986 **Supplementary material – Table S1** Granulometric characteristics, total organic carbon (TOC),  
987 Metal concentrations and Contamination Factor (CF) for sediments at sampling sites (Moreira  
988 et al., 2021).

989 **Supplementary material – Table S2** Microbial parameters and calculated geochemical term (GT),  
990 microbial term (MT), Quality Ratio index (QR), threshold values and estimated sediment risk  
991 (Moreira et al., 2021).

992

993 **Table 1 Sequencing and microbial community data analysis** - Number of sequences before and  
 994 after demultiplexing; observed richness (S), alpha diversity (Shannon diversity index), and QR  
 995 index classification for each station.

	<b>Sample names</b>	<b>Input sequences</b>	<b>Trimmed sequences</b>	<b>Species Richness (S)</b>	<b>Shannon (H)</b>	<b>QR*</b>
996						
997	<b>SB01</b>	24354	3701	100	4.4	4.10 <sup>-05</sup>
	<b>SB02</b>	25785	4375	124	4.6	1.10 <sup>-04</sup>
998	<b>SB03</b>	26583	6800	156	4.8	3.10 <sup>-04</sup>
	<b>SB04</b>	25419	4921	138	4.7	1.10 <sup>-04</sup>
999	<b>SB05</b>	16952	2708	82	4.2	2.10 <sup>-04</sup>
	<b>SB06</b>	22266	5117	102	4.2	1.10 <sup>-04</sup>
1000	<b>SB07</b>	23979	5921	80	4.1	3.10 <sup>-03</sup>
	<b>SB08</b>	22967	6835	111	4.3	1.10 <sup>-01</sup>
1001	<b>SB09</b>	28696	4051	107	4.5	3.10 <sup>-03</sup>
	<b>SB10</b>	26469	6179	110	4.3	1.10 <sup>-02</sup>
1002	<b>SB11</b>	27844	7803	111	4.3	1.10 <sup>-04</sup>
	<b>SB12</b>	29637	4674	126	4.6	1.10 <sup>-04</sup>
1003	<b>SB13</b>	25209	8198	136	4.7	3.10 <sup>-02</sup>
	<b>SB14</b>	26544	7504	156	4.7	1.10 <sup>-02</sup>
1004	<b>SB15</b>	22612	6338	128	4.6	1.10 <sup>-03</sup>
	<b>SB16</b>	31310	12678	195	5.0	4.10 <sup>-03</sup>
1005	<b>SB17</b>	43498	12700	220	4.9	1.10 <sup>-01</sup>
	<b>SB18</b>	31447	10636	210	5.0	2.10 <sup>-03</sup>
1006	<b>SB19</b>	30169	11350	202	5.0	1.10 <sup>-02</sup>
	<b>SB20</b>	22576	7833	126	4.6	1.10 <sup>-02</sup>
1007	<b>SB21</b>	22995	8142	166	4.6	4.10 <sup>-03</sup>
	<b>SB22</b>	23412	7500	176	4.9	3.10 <sup>-02</sup>
1008	<b>SB23</b>	30491	9464	108	4.3	4.10 <sup>-02</sup>
	<b>SB24</b>	25470	9316	92	3.9	4.10 <sup>-03</sup>
1009	<b>SB25</b>	26372	6432	165	4.8	1.10 <sup>-02</sup>
	<b>SB26</b>	25014	7606	144	4.7	1.10 <sup>-02</sup>
1010	<b>Total</b>	<b>688070</b>	<b>188782</b>			

1011 \*  
 1012 \*QR are from Moreira et al. (2021)

1013