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Christophe Leboulanger, L. Kolanou Biluka, A.R. Nzigou, V. Djuidje Kenmogne, J.L.M. Happi, F.E. Ngohang, A.S. Eleng, N.O.Z. Abaga, Marc Bouvy

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1 **Urban inputs of fecal bacteria to the coastal zone of Libreville, Gabon, Central**  
2 **Western Africa.**

3 Christophe LEBOULANGER <sup>1\*</sup>, Lévie KOLANOU BILUKA <sup>2,3</sup>, Aimé-Roger NZIGOU<sup>2</sup>, Véronique DJUIDJE  
4 KENMOGNE<sup>2,3</sup>, Johann Ludovic Martial HAPPI<sup>2</sup>, Franck Estimé NGOHANG<sup>3</sup>, Aminata Spanian ELENG<sup>4</sup>,  
5 Norbert ONDO ZUE ABAGA<sup>2</sup>, Marc BOUVY<sup>1</sup>

6 1. MARBEC, Université Montpellier, CNRS, Ifremer, IRD, Sète, France  
7 2. Université des Sciences et Techniques de Masuku, Franceville, Gabon  
8 3. Ecole Normale Supérieure, Libreville, Gabon  
9 4. Institut de Recherche Agronomique et Forestière, CENAREST, Libreville, Gabon

10 \* corresponding author: [christophe.leboulanger@ird.fr](mailto:christophe.leboulanger@ird.fr)

11 **Abstract**

12 Libreville, the largest city in Gabon, adversely impacts the Komo Estuary and the Akanda National  
13 Park aquatic ecosystems through discharge of domestic and industrial waste. Fecal Indicator Bacteria  
14 (FIB: *Escherichia coli* and fecal streptococci) were enumerated using culture-based methods in water  
15 from 40 sites between 2017 and 2019 including coastal outlets, mangrove channels, open bays and  
16 littoral rivers. Contamination levels were high in discharge waters from small urban rivers in Libreville  
17 agglomeration, frequently exceeding international safety guidelines, whereas FIB concentrations  
18 decreased downstream from the city in main mangrove channels. Littoral forest rivers were  
19 significantly impacted by fecal contamination despite the absence of settlements in the watersheds.  
20 Protected areas are not effective in avoiding FIB contamination, indicating inefficient waste  
21 management. Dedicated management policies should be implemented to reduce both the sanitary  
22 concern and global pollution, poorly assessed in a context of demographic increase in tropical littoral  
23 zones.

24 **Keywords**

25 Fecal indicator bacteria, Marine protected area, Cultivability, Tropical coastal ecosystems, Coastal  
26 pollution

27 Coastal marine ecosystems are submitted to increasing anthropogenic impact worldwide (Lotze et  
28 al., 2006), including disposal of solid waste and discharge of treated and untreated wastewater, from  
29 various sources and activities. These sources of pressure are among the main drivers that pose a  
30 global threat (Halpern et al., 2008) to highly valuable resources and benefits specifically provided by  
31 coastal ecosystems (Barbier et al., 2011). Although prominent for public policies and management,  
32 scientific knowledge about the extent and nature of anthropogenic impact is not equally available on  
33 a global scale. Southern ecosystems, particularly in low to middle-income countries, are scarcely  
34 studied, especially in Africa and Asia (Garcia Rodrigues et al., 2017; Lüthi et al., 2020). These  
35 countries are highly sensitive to pollution-driven issues in littoral and coastal zones, with problematic  
36 lags between increasing demography and sustainable development goals. For many sub-Saharan  
37 countries, sewage discharge is pointed out as a significant concern to further human development  
38 and well-being, and for the conservation of coastal marine ecosystem functions and uses (Bouvy et  
39 al., 2008; Lewis, 2016). Among pollutants of concern, potentially infectious microorganisms  
40 stemming from fecal contamination pose a threat to human health by direct exposure, mainly  
41 through recreational, cultural or professional contact with contaminated waters, or through food  
42 with soiled fish and shellfish. Uncontrolled population growth in the littoral zone, worsened by the  
43 absence of sewage treatment, leads to increased risk of waterborne diseases, which are a major  
44 handicap to sustainable development in the tropics, and especially in Africa (Bastaraud et al., 2020;  
45 Rochelle-Newall et al., 2015). To support local policies and advise public decision making, knowledge  
46 about actual contamination levels and pathogenic risks is often lacking and needs to be urgently  
47 documented by validated methods (Wu et al., 2011; Lüthi et al., 2020).

48 With an estimated census of ca. 2.1 M inhabitants for a total area of 276,000 km<sup>2</sup> (United Nations,  
49 2020), Gabon is a relatively scarcely populated country. By contrast, Gabon is now the most  
50 urbanized country of continental Africa, with an estimate of 89% of the population currently  
51 established in cities, expected to top 95% by 2050 (United Nations, 2019). The hinterland is gradually  
52 depopulating as the coastal cities of Port Gentil and mostly Libreville and its suburbs face  
53 demographic increase. Libreville is the political, administrative and economic center, with an  
54 estimated agglomeration population of around 800,000. The conurbation is located in the Estuary  
55 province of Gabon, in the southern part of the Gulf of Guinea close to the equatorial line, on the  
56 estuary of Komo River, arisen from the southwestern part of the Woleu-Ntem plateau in Equatorial  
57 Guinea.

58 Three protected areas that constitute the *Arc d'Émeraude* (translates into "Emerald Ark", named for  
59 the shape of the green forest belt around the city) closely surround the urban zone: a coastal tropical  
60 forest (*Arboretum Raponda Walker*) and two National Parks, Pongara NP to the south, and Akanda

61 NP to the northeast (Walters et al., 2016). Both NPs are characterized by an extended mangrove  
62 ecosystem, highlighted for its outstanding biomass and diversity in Gabon (Simard et al., 2019),  
63 which spreads from the Mondah Estuary up to the city suburbs in Akanda. The Akanda National Park  
64 lies east from Libreville around the Mondah bay, mostly consisting of mangrove forests and channels,  
65 harboring traditional fisheries (Cardiec et al., 2020).

66 Submitted to a typical equatorial climate with rainy seasons in February-May and October-November  
67 (Camberlin et al. 2019), Gabon possesses a still overlooked biodiversity, and littoral protected zones  
68 in the capital's area are declining despite management policies are in place (Walters et al., 2016;  
69 Goussard and Ducrocq, 2017; Simard et al., 2019), challenging the balance between conservation and  
70 development. Marine protected areas (MPAs) have been created, comprising almost 23% of the  
71 Gabonese Exclusive Economic Zone, and littoral parks protect mangroves and beaches that give  
72 shelter to endangered marine mammals (African manatees) or chelonians (leatherback turtles).  
73 Efficient conditions for the successful implementation of these conservation tools are nevertheless  
74 currently not fully established. Particularly, public sanitation and waste management do not match  
75 the spreading of the city and increase in population over the last decades, resulting in the  
76 uncontrolled release of waste in the littoral zone, along with associated significant threats to the  
77 coastal ecosystems and to human health (Moubélé and Mbonda, 2017). Despite the improvement of  
78 sanitary conditions within the population, Gabon still suffers from a significant incidence of diarrheic  
79 diseases (Troeger et al., 2017). Viral and bacterial pathogens are for example frequently involved in  
80 the spreading of diseases in infant populations (Koko et al., 2013), which can significantly be  
81 accounted for by fecal contamination of food and environment. Local regulations of water quality  
82 rely on international guidelines (World Health Organization, 2018) and no adaptation to the national  
83 context was implemented to date.

84 The context of Libreville agglomeration, surrounded by the protected areas of the *Arc d'Émeraude*,  
85 makes coastal contamination especially worrying: local populations are exposed during recreational  
86 activities on the urban and peri-urban beaches, and through consumption of local seafood.  
87 Assessment of actual levels of contamination by fecal indicator bacteria (FIB) is therefore mandatory  
88 for (i) documenting the current extent and nature of FIB contamination in the littoral zone, (ii)  
89 providing a baseline for the population and decision makers, and (iii) allowing to implement efficient  
90 monitoring of the coastal zone of Gabon, which will further support the success of future waste  
91 management and conservation policies. In order to establish a first step in this process, an  
92 assessment of FIB contamination in coastal rivers, estuaries and marine waters of the *Arc*  
93 *d'Émeraude* area was performed using culture-based methods, proven to be suitable to document  
94 pathogen occurrence in water systems (Wu et al., 2011).

95 Forty locations were sampled in the study area (Fig. 1, Supplementary Table S1) between April 2017  
96 and April 2019, including estuarine surface water, coastal river outlets, mangrove channels, sandy  
97 beaches, and forest rivers, spreading from dense urban areas to fully protected ecosystems  
98 (mangrove system of Akanda National Park). Coastal rivers and outlets were sampled aiming at the  
99 first rainy season of the year, typically from February to May, to avoid drying of the smaller water  
100 bodies and flash floods which are more likely during the second rainy season in October-November  
101 (Camberlin et al. 2019). Ntsini channel was sampled at four dates to cover most of the yearly climate  
102 variability in the area. All field-sampling sessions were arranged by local agencies staff and boat  
103 availability to cope with current regulations.

104 Water samples were taken immediately below the surface using a 2 L horizontal Niskin bottle  
105 previously cleaned with 0.1 M HCl, and thoroughly rinsed with mineral water between each  
106 sampling. Measurements of pH, temperature and conductivity on surface water were conducted  
107 using an YSI 600 multiparameter probe. Calibration for conductivity and pH sensors was performed  
108 in the field immediately before each deployment, using fresh standard solutions. All water samples  
109 were taken in the morning, stored in polycarbonate bottles at ambient temperature in a dark cool  
110 box and processed immediately upon return to the laboratory. Samples from river outlets were  
111 taken as close as possible to the estuary shoreline, whereas mangrove channel locations were  
112 sampled twice in the same day at rising and ebb tides.

113 Two types of fecal indicator bacteria (FIB) were detected and enumerated using culture-based  
114 assays, with commercially available kits comprising sterile gridded membranes and ready-to-use  
115 dehydrated selective medium ("Nutrient Pad Sets, Sartorius, Gottingen, Germany). Two different  
116 selective medium and incubation conditions were applied to enumerate fecal streptococci (FS) and  
117 *Escherichia coli* (EC). AZIDE kits (ref. 14051) incubated at 37°C for 48 h for FS enumeration are based  
118 on standard medium (Slanetz and Bartley, 1957), with added sodium azide as gram-negative bacteria  
119 inhibitor and triphenyltetrazolium chloride as stain for growing colonies (red-brown). ENDO kits (ref.  
120 14053) incubated at 44°C for 24h for EC enumeration are based on the lactose fermenting ability of  
121 coliforms, with sodium sulfite and fuschin addition to inhibit gram-positive bacteria. Each water  
122 sample was filtrated onto a squared sterile 47 mm polycarbonate membrane, rinsed with 10 mL  
123 sterile mineral water to remove salts, and deposited on the pre-wetted medium pad in a plastic petri  
124 dish, according to the recommendations of the manufacturer. To account for the expected range of  
125 FIB densities in the samples, three volumes (1, 10 and 100 mL) were processed in duplicate for each  
126 water sample. Blank controls were made by processing accordingly 100 mL of mineral water (Andza  
127 brand, <https://sobraga.net/eaux/>). After incubation, petri dishes were examined under bright light,  
128 and relevant colonies (dark brown for FS, dark purple with greenish metallic glint for EC) were

129 counted on each filter. Results are expressed as colony forming units (CFU) per 100 mL, according to  
130 the most useful expression of water contamination levels.

131 Chemical analysis was performed on raw water subsamples (50 mL) passed through 0.2  $\mu\text{m}$  porosity  
132 polycarbonate syringe filters to remove particulate matter. Dissolved ammonium ( $\text{NH}_4^+$ ) and soluble  
133 reactive phosphorus (SRP, equivalent to bioavailable orthophosphate  $\text{PO}_4^{3-}$ ) were determined using  
134 spectrophotometric kits for seawater (Spectroquant, Merck), according to the recommendations of  
135 the manufacturer. Calibration curves were carried out for each sample processing session, using  
136 reagent grade (Sigma Aldrich) ammonium chloride ( $\text{NH}_4^+$  analysis) or potassium phosphate (SRP  
137 analysis) diluted in double distilled water. Six concentrations and a reagent blank, each in triplicate,  
138 were used for each chemical, within the specified range of application of the corresponding kit.

139 FIB densities were expressed as the mean CFU/100 mL  $\pm$  SD; in the most polluted locations, colonies  
140 were counted on half or a quarter of the filter. If the dilution was not appropriate for accurate and  
141 distinct counting of each colony on the filter membrane after incubation, values were extrapolated  
142 and considered arbitrarily to be above the highest validated count during the whole study, namely 10  
143 000 CFU/100 mL.

144 Dissolved nutrient calibration curves (photometric readings of standard samples) and FIB  
145 relationships were drawn by linear regression using Past 3.0 freeware for OS X (Hammer et al., 2001),  
146 and slope and intercept values were considered to calculate nutrient concentrations from  
147 absorbance readings.

148 The relationships between pH, salinity, concentrations of ammonium and soluble reactive  
149 phosphorus on the one hand, and abundances of *Escherichia coli* and fecal streptococci on the other  
150 hand, were studied using multivariate analysis. All data (x) were  $\log(x + 1)$  transformed and a  
151 centered Principal Component Analysis (PCA) was performed using SigmaPlot 10 software to identify  
152 the major sources of temporal and spatial variability in the fecal indicator bacteria (FIB) in Komo  
153 estuary. Log-transformed ( $\log(x+1)$ ) FIB values in Ntsini channel were tested for significant influence  
154 of tide period (rising vs. ebb) on measured densities, using a repeated analysis of variance (Statistica  
155 v.7, Statsoft, France), with *post hoc* Scheffé test.

156 A gradient in waste pressure was clear from the visible accumulation of macro-waste in the urban  
157 outlets, increasing in sites near the city center, whereas mangrove roots acted as traps for floating  
158 debris even in the most remote protected areas of Akanda National Park. Upper forest rivers were  
159 obviously under low human pressure, but solid and liquid waste was collected in their lower part  
160 before reaching the seashore. Water contamination by FIB varied accordingly, depending on the  
161 hydrosystem considered and discussed in this study.

162 On the littoral of Komo estuary in Libreville, increasing concentrations of both *Escherichia coli* and  
163 fecal streptococci were recorded in the most urban outlets (Fig. 2), with consistent differences in CFU

164 densities depending on the sampling month. Maximal EC densities were above the performance  
165 range of the method in four of the sampling sites located in the center of Libreville city in June 2018,  
166 and one in March 2018 (Fig. 2A). A similar pattern was observed for FS numbers, with two samples  
167 out of the measuring range at one site and on two dates (Batavea outlet, Fig. 2B). Lower FIB  
168 densities, mostly represented by FS, characterized the locations distant from the city center.

169 A weak but significant relation ( $r^2 = 0.40789$ ;  $p = 0.0002$ ) was established between logarithmic  
170 concentrations of EC and FS (Fig. 3). Contamination level was generally higher for *E. coli* at the three  
171 most central urban outlets (Awondo, Arambo and Batavea), whereas fecal streptococci were  
172 recorded at higher densities in the more suburban (Otende, Sablière) and industrial (Awoungou,  
173 located in the industrial settlement of Owendo harbor) sites. The PCA was performed on the  
174 independent data set (6 descriptors, 10 sites, 4 sampling dates). The first two eigenvalues of the PCA  
175 analysis accounted for 88.7% of the total variability. The analysis, therefore, only considered these  
176 two first axes to highlight the relationship between descriptors along a spatial and temporal  
177 distribution. On both axes, the two FIBs were opposed to salinity (Fig. 4). The two nutrient  
178 concentrations ( $\text{NH}_4^+$  and  $\text{PO}_4^{2-}$ ) were also linked to bacterial abundances, while pH did not explain  
179 any relation with the other variables. On the first axis, the analysis clearly differentiated the sampling  
180 locations, with the identification of two groups. On the right, a group of locations (ARB, BTV, AWD,  
181 ALM), identified as the most urban outlets (“Center” group), was clearly linked to FIB abundances,  
182 while the other sites, in scattered habitats and industrial areas (“Suburbs” group on the left), were  
183 characterized by low values of FIB concentrations and amounts of nutrient (Fig. 4). These results  
184 support the prominent influence of sampling locations over sampling dates, most likely linked to  
185 population densities in the respective watersheds.

186 Out of the forty locations sampled, only six complied with the bathing water guidelines of the World  
187 Health Organization and the European Union applying to marine and transitional waters for “good  
188 quality”, set to 200 and 500 CFU/100 mL (95-percentile of the geometric mean) for fecal streptococci  
189 and *E. coli* respectively (European Parliament 2006, World Health Organization 2018). FIB  
190 contamination in beach waters along the littoral of Libreville is therefore worrying since recreational  
191 bathing by urban populations is usual practice, especially during weekends.

192 Regarding the FIB contamination in the mangrove protected area in Akanda National Park, seven  
193 sites were sampled on the Ntsini channel, representing ca. 27 km of watercourse from Ambowé,  
194 close to an urban fishing pier, to Nendé East, at the mouth of the Mondah Bay (Fig. 1). Samples were  
195 taken at four dates between April and November 2017, during both rising and ebb tides of the same  
196 day. A clear decreasing pattern from up- to downstream was observed for both categories of FIB (Fig.  
197 5), consistent with an important contamination source from urbanized areas with subsequent

198 transport, mortality decay and dilution to the open coastal waters. In 48 out of 56 samples analyzed,  
199 *E. coli* densities (Fig. 5A and 5B) were lower than fecal streptococci counts (Fig. 5C and 5D) by a  
200 factor up to 10 (maximal counts of  $655\pm99$  and  $6666\pm1100$  for EC and FS respectively), while rising  
201 waters (Fig. 5A and 5C) appeared often more contaminated than ebb waters (Fig. 5B and 5D).  
202 Contamination of surface waters during rising tide was significantly higher (ANOVA,  $p < 0.001$ ) for EC  
203 at ABW, TMG, MKO and NDE locations, and for FS at all locations except MKO. This tide-dependent  
204 pattern was consistent with the leaching of contaminated sediments by inflowing waters. Discharged  
205 sewage during low tides was retained on emerged sediment banks acting as a buffer zone, later  
206 released during flow and further increasing the overall densities of FIB in the mangrove channel  
207 surface water.

208 Survival of FIB in tropical estuarine waters is not favored, since salinity, temperature and sunlight  
209 rapidly lower bacterial cultivability (Korajkic et al., 2019). In the Senegal Estuary for example,  $T_{90}$   
210 (time to decrease the FIB density by 90%) was reported below one day for *E. coli* strains (Troussellier  
211 et al., 2004), whereas fecal streptococci are reputedly more persistent in aquatic environments. FIB  
212 tracers, resulting in significant contamination that exceeded the quality levels for recreational  
213 waters, therefore confirm the extent of sewage discharge into the upper mangrove fringes. Despite  
214 or because of the absence of any leisure infrastructures, local inhabitants are used to bathing in the  
215 mangrove upper creeks, a risky activity regarding sanitary hazards and the lack of regulation. The fact  
216 that FIB levels recorded in the most distant locations were often detectable is likely to unveil a  
217 diffuse but significant human pressure even in the highly regulated area of Akanda National Park.

218 Beaches and upstream rivers were sampled in April 2018 and April 2019 in water bodies surrounding  
219 the settlement of Cap Estérias, including forest streams of the Arboretum Raponda Walker, a  
220 protected littoral forest. Out of 29 samples, only one was devoid of measurable FIB contamination  
221 (Table 1), sampled on a rocky shore at high tide.

222 By contrast with the other sampling sites studied in urban littorals and mangrove channels,  
223 contamination by *Escherichia coli* was extremely rare, with the detection of EC in only 9 samples  
224 within the area. Nevertheless, a significant occurrence of FIB and especially fecal streptococci was  
225 reported in the protected area of Arboretum Raponda Walker, despite a high level of regulation. It is  
226 worth noticing that the most contaminated sample, with EC and FS evaluated at 600 and 11000 CFU  
227 / 100 mL respectively, was taken from Idakogo river, in a touristic forest circuit. The lower parts of  
228 Abagna and Obagna rivers were also significantly contaminated by FIB, the first one crossing the  
229 main village, the second one draining poultry farms and scattered habitats. Regarding the coastal  
230 zone, significant contamination exceeding safety levels was measured on beaches and rocky shores,  
231 putting recreational and fishing activities at risk: Cap Estérias is a renowned leisure place for city

232 dwellers and for the existence of an emblematic fishery for the razor clam, *Solen guinensis*, highly  
233 valued locally as seafood (Cardiec et al., 2020). As noticed for the urban zone of Libreville, sewage  
234 management is absent and will need to be considered in a near future to reduce pollution and safety  
235 hazards, as recommended by Lüthi et al. (2020).

236 This study provides the first evaluation of fecal pollution in the coastal zone of Gabon, focusing on  
237 the most densely populated region of Libreville. The diagnosis is worrying, with numerous outlets,  
238 rivers and beaches contaminated by sewage discharge, resulting in significant concentrations of FIB  
239 in coastal waters. Exceeding by far the safety guidelines for recreational waters (European  
240 Parliament, 2006; World Health Organization, 2018) in most of the samples, the study area deserves  
241 further monitoring and risk assessment. The reported pattern suggests a threat to coastal marine  
242 waters and to the production of local fisheries through potential hazardous pathogens (Padovan et  
243 al. 2020), since FIB are closely linked to waterborne microbial diseases (Djuikom et al., 2006;  
244 Bastaraud et al., 2020). Recreational activities are particularly exposed, being practiced by a young  
245 population that is not adequately informed about the risks and, furthermore, inherently fragile in the  
246 face of intestinal diseases (Bouvy et al., 2010; Koko et al., 2013).

247 Direct comparison of the current status of Libreville coastal ecosystems regarding FIB contamination  
248 to other subtropical and tropical locations is difficult since studied markers and detection methods  
249 often differ. In the Senegal Estuary, Bouvy et al. (2010) reported the importance of both direct  
250 (fishermen community settlements and city hospital) and indirect (upper freshwater reservoirs)  
251 contributions to estuarine and coastal contamination by *E. coli* and fecal streptococci, enumerated  
252 by culture-based methods, with 20% of samples not complying to international guidelines. A worse  
253 condition was reported using the same approach in the Hann Bay (Dakar, Senegal), suffering from  
254 direct sludge releases, where coastal open sea was highly contaminated by FIB, with most samples  
255 exceeding quality guidelines (Bouvy et al., 2008). A comparable survey from seashore waters in  
256 Zanzibar (Tanzania) reported the occurrence of fecal streptococci exceeding guidelines in a touristic  
257 area (Moynihan et al., 2012), together with highlighting the differences in marine pollution  
258 perception by local and foreign populations. The temporal variability in FIB densities reported during  
259 this study has also been highlighted, for example, in a Hawaiian estuary (Wiegner et al., 2017) where  
260 fecal streptococci contamination increased during storm events. Seasonal changes in rain intensity  
261 were also proven important, especially in tropical regions submitted to monsoon events  
262 (Khandeparker et al., 2015).

263 Bacterial enumeration by culture-based methods does not consist in a direct assessment of health  
264 risk. For example, only a few strains of *E. coli* are known to be toxic to humans, and their detection  
265 relies on specific methods such as gene targeting (Snoussi et al., 2011; Walker et al., 2013). Detection  
266 and enumeration of FIB are often limited tools in forecasting microbially-associated risks in coastal

267 waters and are still being debated regarding the accuracy of the different methods (Wu et al., 2011;  
268 Vukić Lušić et al., 2013; Padovan et al., 2020). Nevertheless, cultivation procedures have proven  
269 useful in addressing wastewater-related pollution issues for decades, relying on robust methods that  
270 require affordable equipment and consumables, easy-to-learn technical skills, and which benefit  
271 from an internationally validated framework for sample processing and results interpretation. As  
272 such, FIB detection using selective growth media could be widely implemented as a first-step  
273 monitoring tool in developing countries (Condé-Lamparelli et al., 2015; Krepsky et al. 2021). Together  
274 with direct counts in coastal waters, more synoptic approaches could be later considered in order to  
275 overcome the high temporal variability of FIB in surface waters, such as the detection of fecal sterols  
276 in surficial sediment (Isobe et al., 2002; Muniz et al., 2015; Frena et al., 2019). To improve  
277 significance of FIB enumeration in the specific frame of human health risk management, robust and  
278 univocal determination of potential pathogens could be attained by using locally adapted  
279 methodologies. For example, the availability of isolated strains could serve as positive control in  
280 culture-based and genomic detection (Padovan et al. 2020) together with ongoing epidemiological  
281 surveys of waterborne diseases.

282 National parks and marine protected areas are keystone elements of the 21st Century conservation  
283 policy in Gabon, expected to contribute to the forecasted sustainable development of the country  
284 (Goussard and Ducrocq, 2017). On a global scale, Marine Protected Areas represent a significant  
285 hope for reducing human pressures on the oceans and making their use sustainable (Duarte et al.,  
286 2020). Nevertheless, conservation objectives and preservation of artisanal and patrimonial activities  
287 are weakened in the case of the *Arc d'Émeraude* area, mostly through the increasing demographic  
288 pressure which largely exceeds land use planning, resulting in solid and liquid waste spreading to the  
289 coastal ecosystem. Uncontrolled peri-urban extension around Libreville directly threatens  
290 conservation purposes, reducing the buffer zone and putting in direct contact the suburbs and the  
291 protected areas (Goussart and Ducrocq, 2017). Waste management should be raised as a priority  
292 objective for stakeholders in order to reduce pollution pressure, with foremost benefits for both  
293 human and environmental health. In such a context, long-term monitoring of waste indicator  
294 microorganisms will contribute to increasing the safety of citizens and allow managers to assess the  
295 effectiveness of mitigation and regulation layouts in a context of urban sanitation (Wu et al., 2011;  
296 Lüthi et al., 2020). FIB determination can be an appropriate measure of global pollution in marine  
297 coastal environments (Lyimo, 2008), accounting for the multiple dissolved and particulate  
298 contaminants carried together.

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440 **Figures captions**

441 **Figure 1.** Location of the sampling sites (red circles) in the Libreville area (Estuary province, Gabon).  
442 A.R.W.: Arboretum Raponda Walker (protected littoral forest); A.N.P.: Akanda National Park; P.N.P.:  
443 Pongara National Park. Sampling locations are indicated by red circles.

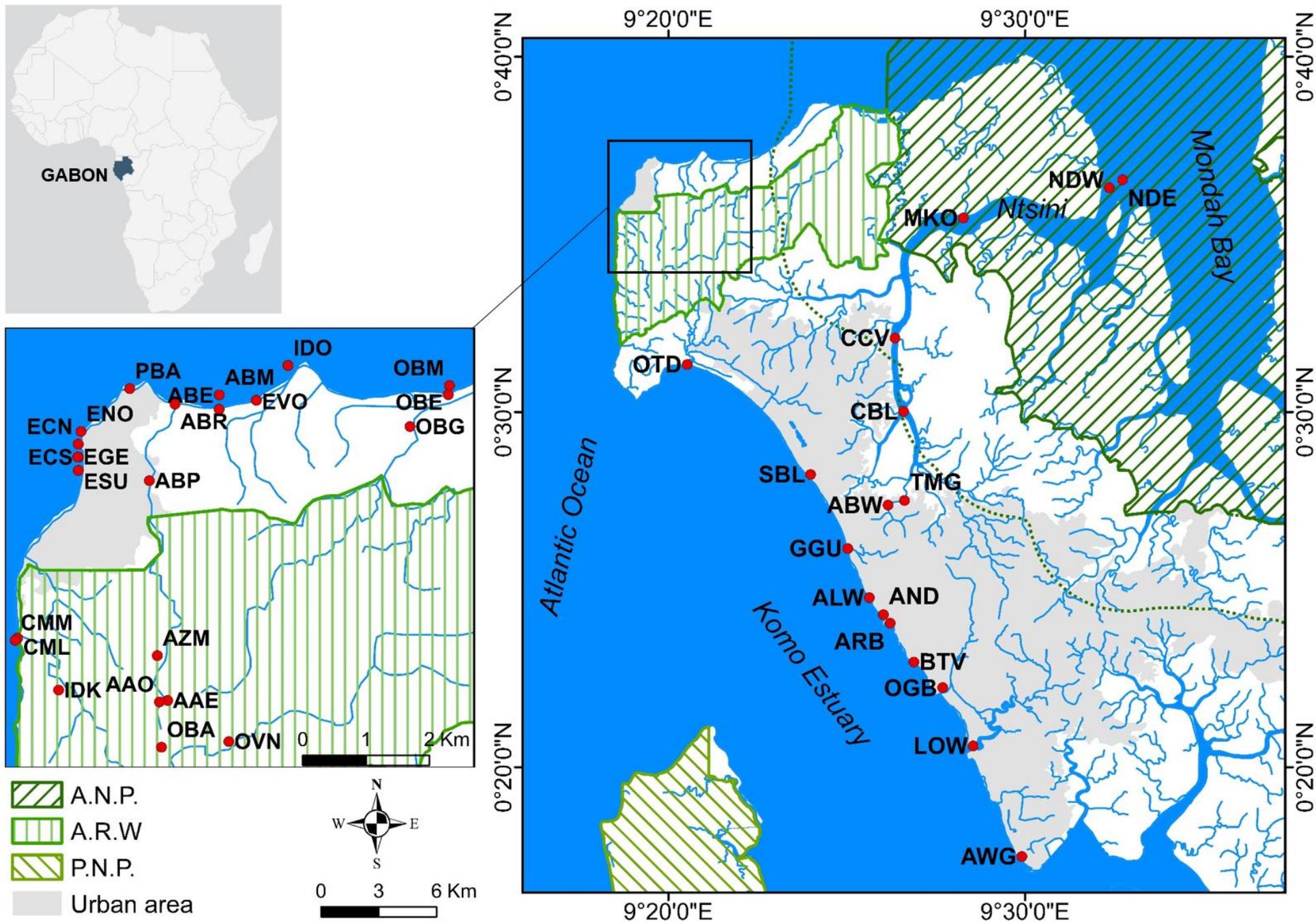
444 **Figure 2.** Concentrations of FIB *Escherichia coli* (A) and fecal streptococci (B) expressed as CFU/100  
445 mL in the ten littoral outlets of the Komo estuary sampled within the Libreville area, depending on  
446 the sampling month (March-June, 2018). Error bars are given except for samples exceeding the  
447 counting capacity of the method, set at 10,000 CFU / 100 mL.

448 **Figure 3.** Relation between *Escherichia coli* (EC) and fecal streptococci (FS) densities (expressed as  
449 log[CFU/100 mL]) in the littoral outlets of the Komo estuary within the Libreville area during the  
450 present survey (March-June, 2018). Regression ( $y = 0.38737 x + 2.0063$ ) is marked by the red line,  
451 95% confidence intervals (bootstrapped, N = 1999) by blue curves. Dashed line indicates 1:1 relation,  
452 vertical and horizontal solid lines indicate European Union bathing water guidelines for information,  
453 green and orange area being below and above quality criteria for both FIB types respectively.

454 **Figure 4.** Principal component analysis (PCA) on the two first axes, based on environmental  
455 parameters and FIB concentrations at the outlet of Libreville coastal rivers in the Komo Estuary.  
456 Eigenvalues for each axis of the PCA are reported. Abbreviations for sampled locations are listed in  
457 Table 1, sampling dates in 2018 are identified by month numbers (3: March; 4: April; 5: May; 6: June).  
458 STREP: abundance of fecal streptococci; COLI: abundance of *Escherichia coli*;  $\text{NH}_4^+$ : ammonium  
459 concentration;  $\text{PO}_4^{3-}$ : orthophosphate concentration. Two groups of locations can be defined  
460 (Suburbs and Center).

461 **Figure 5.** Concentrations of FIB *Escherichia coli* (A, B) and fecal streptococci (C, D) expressed as  
462 CFU/100 mL in the Ntsini channel from the suburbs to Akanda National Park depending on sampling  
463 date, at rising (left panels A and C) and ebb (right panels C and D) tides. Locations are ordered from  
464 the most urban zone (left, Ambowé) to the central protected area (right, Nendé East). The range  
465 differences in y axes for rising and ebb data are highlighted by the blue dashed lines.

Fig. 1 MPB-D-21-00288\_R1



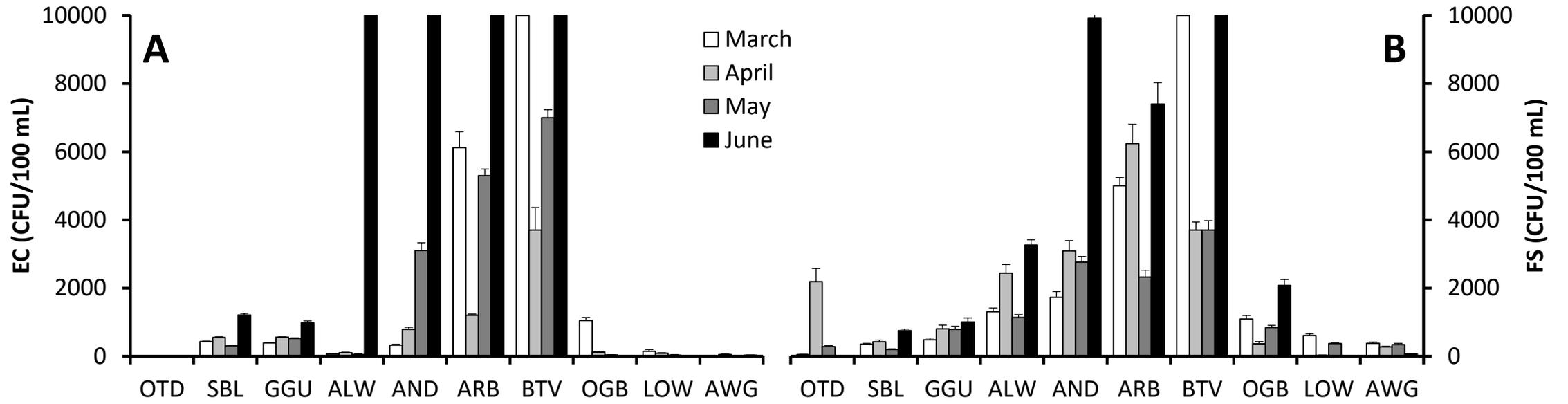


Fig. 2 MPB-D-21-00288\_R1

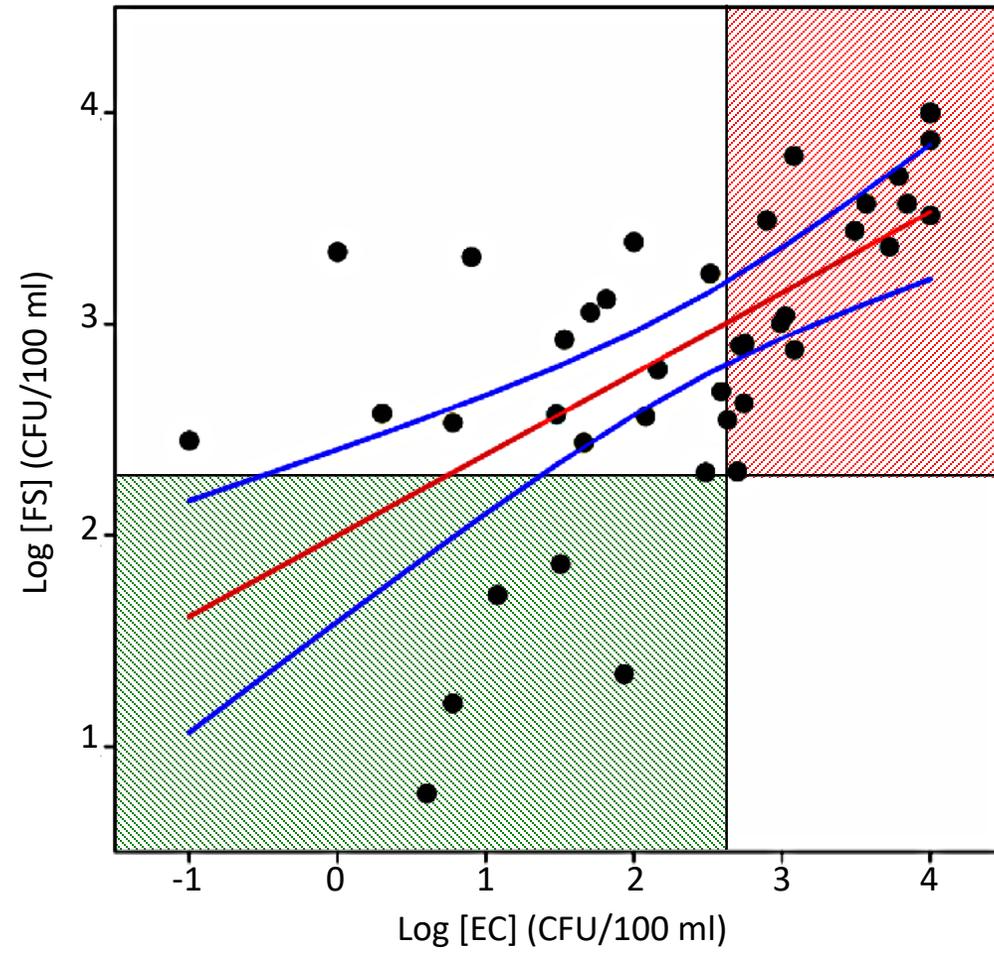


Fig. 3 MPB-D-21-00288\_R1

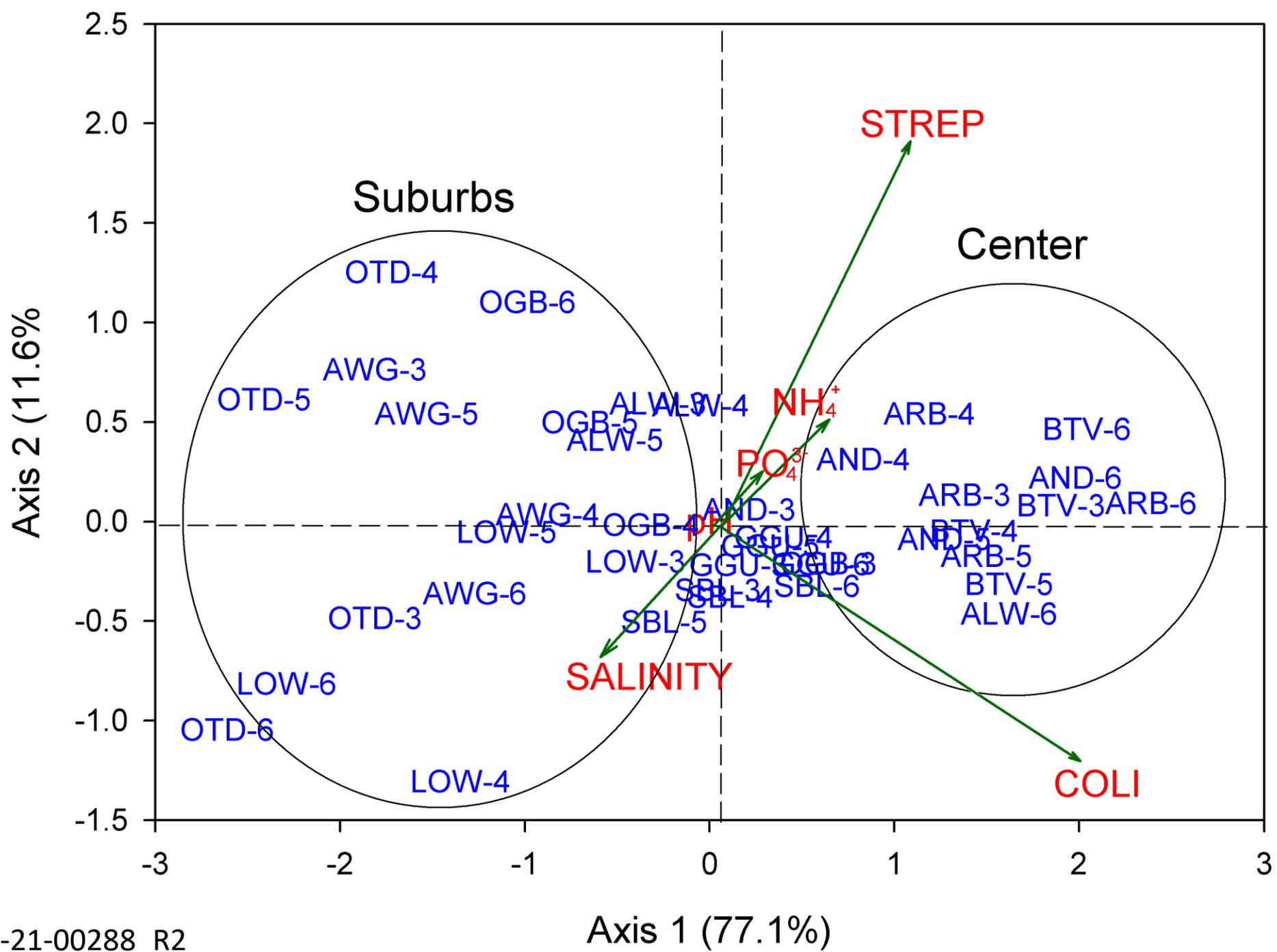


Fig. 4 MPB-D-21-00288\_R2

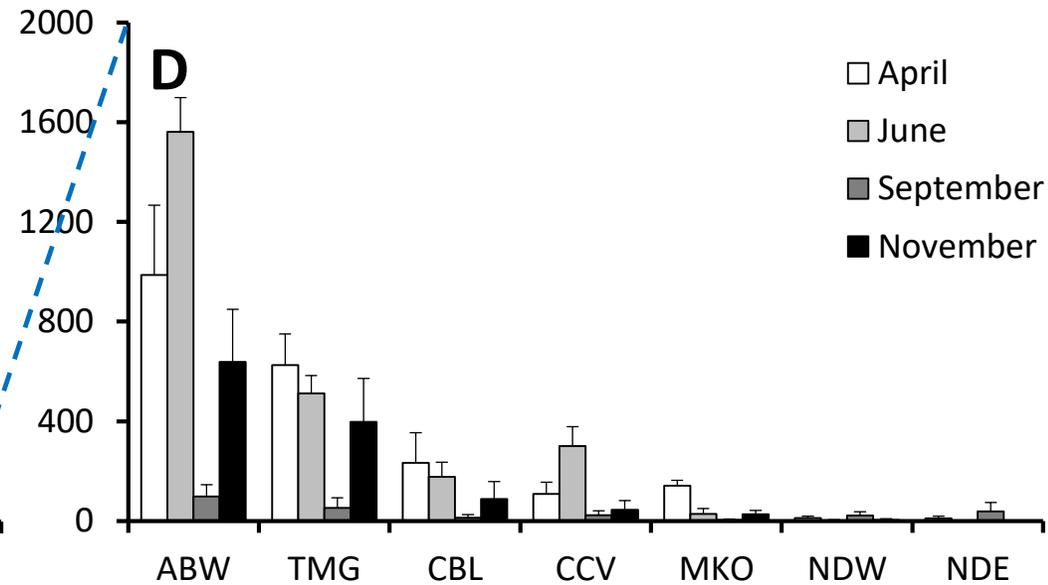
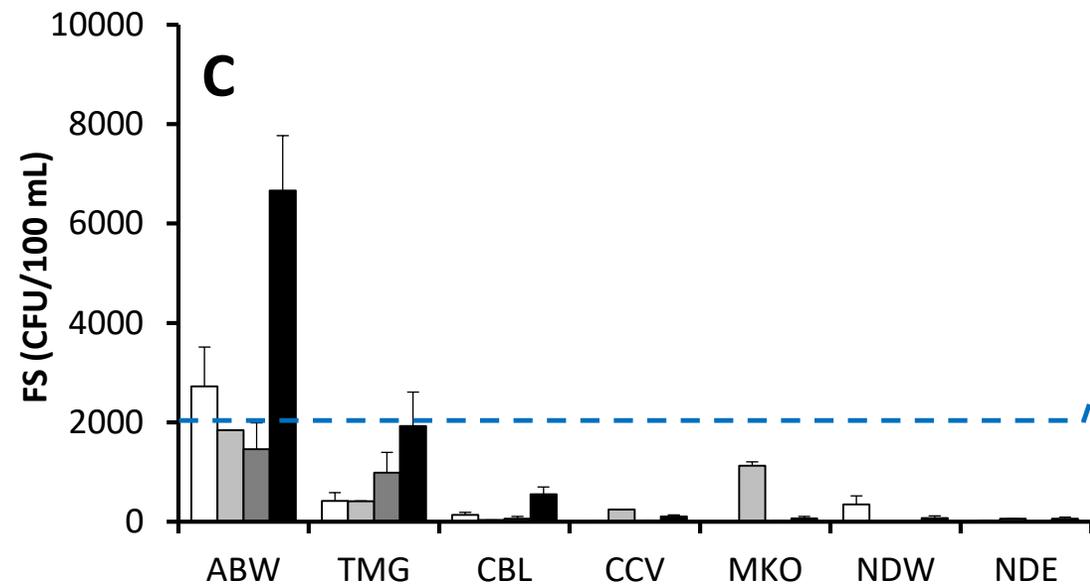
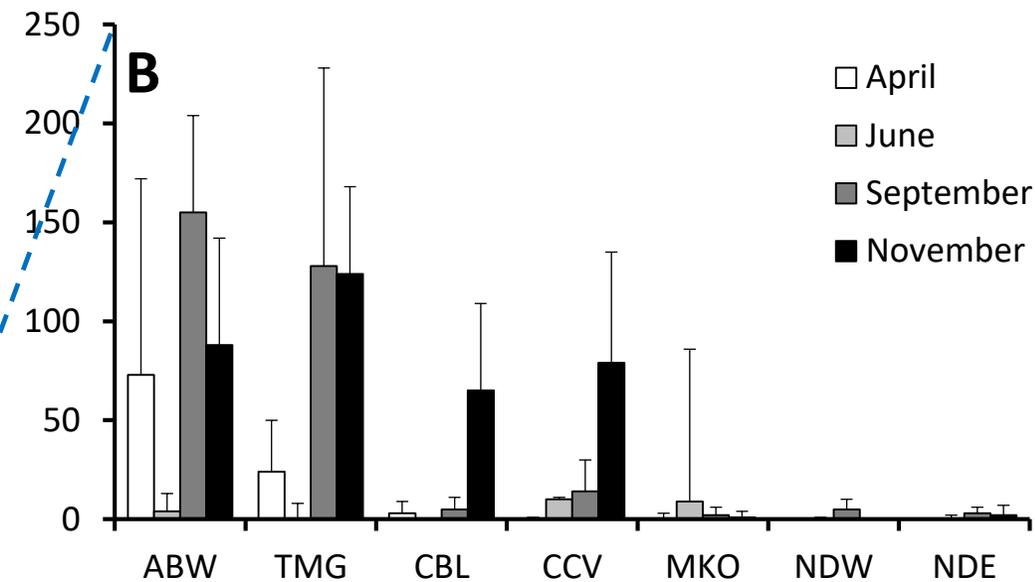
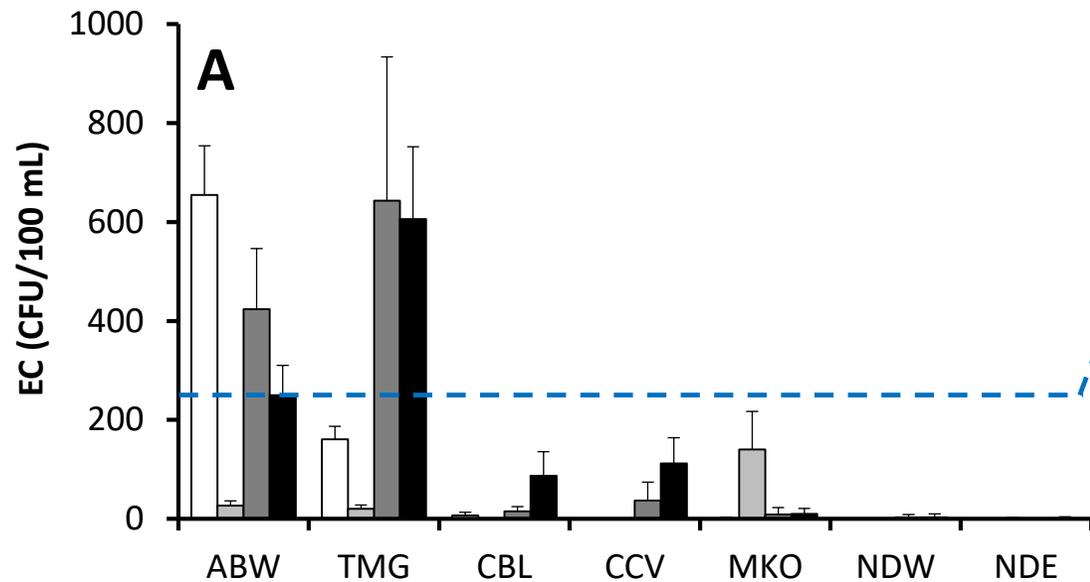


Fig. 5 MPB-D-21-00288\_R1

**Table 1.** FIB contamination (CFU / 100 mL) in water samples from forest rivers and coastal zone of Cap Estérias (*Escherichia coli* EC and fecal streptococci FS). nd: not determined.

Station	April 2018		April 2019	
	EC	FS	EC	FS
ABM	0	58	0	436
ABE	0	2480	355	3600
ABR	0	4960	nd	nd
ABP	0	1425	1360	1322
AAE	nd	nd	0	2800
AZM	nd	nd	40	1160
AAO	27	1030	0	1380
OBM	0	0	nd	nd
OBE	1940	1086	nd	nd
OBG	0	1500	nd	nd
OBA	0	620	0	1250
OVN	120	210	nd	nd
EVO	nd	nd	0	4260
CML	nd	nd	0	1400
IDK	600	11000	0	2050
EGE	nd	nd	0	3160
CMM	nd	nd	0	180
PBA	nd	nd	64	375
ENO	nd	nd	0	0
ECN	nd	nd	0	68
ECS	nd	nd	0	405
ESU	nd	nd	44	412
IDO	nd	nd	0	500

