



HAL
open science

Urban inputs of fecal bacteria to the coastal zone of Libreville, Gabon, Central Western Africa

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

► **To cite this version:**

Christophe Leboulanger, L. Kolanou Biluka, A.R. Nzigou, V. Djuidje Kenmogne, J.L.M. Happi, et al.. Urban inputs of fecal bacteria to the coastal zone of Libreville, Gabon, Central Western Africa. Marine Pollution Bulletin, 2021, 168, 112478 [7 p.]. 10.1016/j.marpolbul.2021.112478 . hal-03276615

HAL Id: hal-03276615

<https://hal.science/hal-03276615>

Submitted on 24 May 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.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

3 Christophe LEBOULANGER ^{1*}, Lévie KOLANOU BILUKA ^{2,3}, Aimé-Roger NZIGOU², Véronique DJUIDJE
4 KENMOGNE^{2,3}, Johann Ludovic Martial HAPPI², Franck Estimé NGOHANG³, Aminata Spanian ELENG⁴,
5 Norbert ONDO ZUE ABAGA², Marc BOUVY¹

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

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 km² (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 μm porosity
132 polycarbonate syringe filters to remove particulate matter. Dissolved ammonium (NH_4^+) and soluble
133 reactive phosphorus (SRP, equivalent to bioavailable orthophosphate 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 (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 (NH_4^+ and 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 ± 99 and 6666 ± 1100 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.

299 **Acknowledgements**

300 This work is a contribution by the WP.2 “Characterization of pollution and analysis of their impacts
301 and risks for biodiversity and public health in the *Arc d’Émeraude*” funded by AFD (French Agency for
302 Development) under the authority of the Gabonese National Agency for National Parks (ANPN).
303 Authors wish to thank the ENEF (Gabonese National School for Water and Forestry), ANPN staff and
304 Dr. Magloir Désiré Mounganga (ANPN / CENAREST) for field sampling support. Research was
305 approved by the CENAREST, Republic of Gabon (agreement AR0010/17). Funding agencies and local
306 authorities played no role in the design, results, analysis and publication of this study. Dr. Marc
307 Pagano (IRD, MIO Marseilles) is warmly acknowledged for help in data analysis. Pierre Lopez (IRD,
308 MARBEC Sète) designed and produced the graphical abstract, and English text was edited by Dr.
309 Dominique Bairstow.

310 **References**

- 311 Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and
312 coastal ecosystem services. *Ecol. Monogr.* 81: 169-193. <https://doi.org/10.1890/10-1510.1>
- 313 Bastaraud A, Cecchi P, Handschumacher P, Altmann M, Jambou R (2020) Urbanization and
314 waterborne pathogen emergence in low-income countries: where and how to conduct surveys?
315 *Int J Environ Res Public Health* 17: 480. doi:10.3390/ijerph17020480
- 316 Bouvy M, Arfi R, Bernard C, Carré C, Got P, Pagano M, Troussellier M (2010) Estuarine microbial
317 community characteristics as indicators of human-induced changes (Senegal River, West Africa).
318 *Estuar Coastal Shelf Sci* 87: 573-582. <https://doi.org/10.1016/j.ecss.2010.02.015>
- 319 Bouvy M, Briand E, M’Boup M, Got P, Leboulanger C, Bettarel Y, Arfi R (2008) Effects of sewage
320 discharges on microbial components in tropical coastal waters (Senegal, West Africa). *Mar*
321 *Freshwater Res* 7: 614-626. <https://doi.org/10.1071/MF07244>
- 322 Camberlin P, Barraud G, Bigot S, Dewitte O, Imwangana FM, Maki Mateso JC, Martiny N, Monsieurs
323 E, Moron V, Pellarin T, Philippon N, Sahani M, Samba G (2019) Evaluation of remotely sensed
324 rainfall products over Central Africa. *Quarterly J Royal Meteorol Soc.* 145: 2115-2138.
325 <https://doi.org/10.1002/qj.3547>
- 326 Cardiec F, Bertrand S, Witt MJ, Metcalfe K, Godley BJ, McClellan C, Vilela R, Parnell RJ, Le Loc’h F
327 (2020) “Too big to ignore”: a feasibility analysis of detecting fishing events in Gabonese small-
328 scale fisheries. *PLoS One* 15(6): e0234091 <https://doi.org/10.1371/journal.pone.0234091>
- 329 Condé-Lamparelli C, Pogreba-Brown K, Verhougstraete M, Zanolli Sato MI, de Castro Bruni A, Wade
330 TJ, Eisenberg JNS (2015) Are fecal indicator bacteria appropriate measures of recreational water
331 risks in the tropics: a cohort study of beach goers in Brazil? *Water Res* 87: 59-68
332 <https://doi.org/10.1016/j.watres.2015.09.001>

333 Djuikom E, Njine T, Nola M, Sikati V, Jugnia LB (2006) Microbiological water quality of the Mfoundi
334 River watershed at Yaoundé, Cameroon, as inferred from indicator bacteria of fecal
335 contamination. *Environ Monit Assess* 122: 171-183 <https://doi.org/10.1007/s10661-005-9172-7>

336 Duarte CM, Agusti S, Barbier E, Britten GL, Castilla JC, Gattuso JP, Fulweiler RW, Hughes TP, Knowlton
337 N, Lovelock CE, Lotze HK, Predragovic M, Poloczanska E, Roberts C, Worm B (2020) Rebuilding
338 marine life. *Nature* 580: 39-51 <https://doi.org/10.1038/s41586-020-2146-7>

339 European Parliament (2006) Directive 2006/7/EC of the European Parliament and of the Council of
340 15 February 2006 concerning the management of bathing water quality and repealing. Directive
341 76/160/EEC. *Official Journal of the European Union* L 64/37

342 Frena M, Stein Santos AP, Souza MRR, Santos Carvalho SS, Madureira LAS, Alexandre MR (2019)
343 Sterol biomarkers and fecal coliforms in a tropical estuary: seasonal distribution and sources. *Mar*
344 *Poll Bull* 139: 111-116 <https://doi.org/10.1016/j.marpolbul.2018.12.007>

345 Garcia Rodrigues J, Conides A, Rivero Rodriguez S, Raicevich S, Pita P, Kleisner K, Pita C, Lopes P,
346 Alonso Roldán V, Ramos S, Klaoudatos D, Outeiro L, Armstrong C, Teneva L, Stefanski S, Böhnke-
347 Henrichs A, Kruse M, Lillebø A, Bennett E, Belgrano A, Murillas A, Sousa Pinto I, Burkhard B,
348 Villasante S (2017) Marine and coastal cultural ecosystem services: knowledge gaps and research
349 priorities. *One Ecosystem* 2: e12290 <https://doi.org/10.3897/oneeco.2.e12290>

350 Goussard JJ, Ducrocq M (2017) Facing the future: conservation as a precursor for building coastal
351 territorial cohesion and resilience. *Aquatic Conserv: Mar Freshw Ecosyst* 27(S1): 151-161
352 <https://doi.org/10.1002/aqc.2823>

353 Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C,
354 Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck
355 R, Watson P (2008) A global map of human impact on marine ecosystems. *Science* 319: 948-952
356 <https://doi.org/10.1126/science.1149345>

357 Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for
358 education and data analysis. *Paleontologica Electronica* 4: 9 pp.

359 Isobe KO, Tarao M, Zakaria MP, Chiem NH, Minh LY, Takada H (2002) Quantitative application of fecal
360 sterols using gas chromatography-mass spectrometry to investigate fecal pollution in tropical
361 waters: Western Malaysia and Mekong Delta, Vietnam. *Environ Sci Technol* 36, 4497-4507
362 <https://doi.org/10.1021/es020556h>

363 Khandeparker L, Chandrasekar Anil A, Naik SD, Gaonkar CC (2015) Daily variations in pathogenic
364 bacterial populations in a monsoon influenced tropical environment. *Mar Poll Bull* 96: 337-343
365 <https://doi.org/10.1016/j.marpolbul.2015.04.051>

366 Koko J, Ambara JP, Ategbo S, Gahouma D (2013) Epidemiology of acute bacterial diarrhea in children
367 in Libreville, Gabon. *Arch Pediatr* 20: 432-433 <https://doi.org/10.1016/j.arcped.2013.01.017>

368 Korajkic A, Wanjugi P, Brooks L, Cao Y, Harwood VJ (2019) Persistence and decay of fecal microbiota
369 in aquatic habitats. *Microbiol Mol Biol Rev* 83: e00005-19 [https://doi.org/10.1128/MMBR.00005-](https://doi.org/10.1128/MMBR.00005-19)
370 19

371 Krepsky N, de Andrade Lino VA, Silva dos Santos F, Naveira CAC (2021) Faecal bacteria density in
372 tropical seawater: the Itanemas' cove case study, Angra dos Reis, Brazil. *Mar Poll Bull* 164: 112027
373 <https://doi.org/10.1016/j.marpolbul.2021.112027>

374 Lewis J (2016) Cleaning up muddy waters – The fight to revive Senegal's Hann Bay. *Environ Health*
375 *Perspect* 124: A92-A97 <https://doi.org/10.1289/ehp.124-A92>

376 Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson
377 CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal
378 seas. *Science* 312: 1806-1810. doi:10.1126/science.1128035

379 Lüthi C, Willetts J, Hoffmann S (2020) Editorial: City-wide sanitation: the urban sustainability
380 challenge. *Front Environ Sci* 8: <https://doi.org/10.3389/fenvs.2020.585418>

381 Lyimo TJ (2008) Microbial and nutrient pollution in the coastal bathing waters of Dar es Salaam.
382 *Aquatic Conserv: Mar Freshwater Ecosyst* 19: S27-S37. <https://doi.org/10.1002/aqc.1037>

383 Moubélé A, Mbonda AP (2017) Les impacts environnementaux du développement urbain de
384 Libreville. In: *Les régions littorales du Gabon*, Pottier P, Menie Ovono Z, Faure FE, Bignoumba GS
385 eds. LETG Nantes Geolittoral & Raponda Walker Gabon, pp. 257-288 (in French)

386 Moynihan MA, Baker DM, Mmochi AJ (2012) Isotopic and microbial indicators of sewage pollution
387 from Stone Town, Zanzibar, Tanzania. *Mar Poll Bull* 64: 1348-1355.
388 <https://doi.org/10.1016/j.marpolbul.2012.05.001>

389 Muniz P, da Silva DAM, Bicego MC, Bromberg S, Pires-Vanin AMS (2015) Sewage contamination in a
390 tropical coastal area (São Sebastião Channel, SP, Brazil). *Mar Poll Bull* 99: 292-300
391 <https://doi.org/10.1016/j.marpolbul.2015.07.046>

392 Padovan A, Kennedy K, Rose D, Gibb K (2020) Microbial quality of wild shellfish in a tropical estuary
393 subject to treated effluent discharge. *Environ Res* 181:108921
394 <https://doi.org/10.1016/j.envres.2019.108921>

395 Rochelle-Newall E, Nguyen TMH, Le TPQ, Sengtaheuanghoung O, Ribolzi O (2016) A short review of
396 fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions.
397 *Frontiers Microbiol* 6: 308. <https://doi.org/10.3389/fmicb.2015.00308>

398 Simard M, Fatoyinbo L, Smetanka C, Rivera-Monroy VH, Castañeda-Moya E, Thomas N, Van der
399 Stocken T (2019) Mangrove canopy height globally related to precipitation, temperature and
400 cyclone frequency. *Nature Geoscience* 12: 40-45 <https://doi.org/10.1038/s41561-018-0279-1>

401 Slanetz LW, Bartley CH (1957) Numbers of enterococci in water, sewage and feces determined by the
402 membrane filter technique with an improved medium. *J Bacteriol* 74: 591-595
403 <https://doi.org/10.1128/JB.74.5.591-595.1957>

404 Snoussi M, Noumi E, Lajnef R, Belila A, Yazidi N, Bakhrouf A (2011) Phenotypic characterization and
405 enterobacteria repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) of
406 *Aeromonas* spp. and *Vibrio* spp. strains isolated from *Sparus aurata* fish farm (Khenis, Tunisia)
407 *African J Microbiol Res* 5: 2920-2928 <https://doi.org/10.5897/AJMR11.321>

408 Troeger C, GBD Diarrhoeal Diseases Collaborators (2017) Estimates of global, regional, and national
409 morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global
410 Burden of Diseases Study 2015. *Lancet Infect Dis* 17: 909-948 [https://doi.org/10.1016/S1473-3099\(17\)30276-1](https://doi.org/10.1016/S1473-3099(17)30276-1)

412 Troussellier M, Got P, Bouvy M, M'Boup M, Arfi R, Lebihan F, Monfort P, Corbin D, Bernard C (2004)
413 Water quality and health status of the Senegal River estuary. *Mar Poll Bull* 48: 852-862
414 <https://doi.org/10.1016/j.marpolbul.2003.10.028>

415 United Nations (2019) *World Urbanization Prospects: The 2018 Revision (ST/ESA/SER.A/420)*. UN
416 Department of Economic and Social Affairs, Population Division. New York: United Nations.

417 United Nations (2020) *Overall total population – World Population Prospects: The 2019 Revision*.
418 United Nations Department of Economic and Social Affairs, Population Division. New York: United
419 Nations.

420 Vukić Lušić D, Lušić D, Pešut D, Mićović V, Glad M, Bilajac L, Peršić V (2013) Evaluation of equivalence
421 between different methods for enumeration of fecal indicator bacteria before and after adoption
422 of the new Bathing Water Directive and risk assessment of pollution. *Mar Poll Bull* 73: 252-257
423 <https://doi.org/10.1016/j.marpolbul.2013.05.012>

424 Walker TJ, Bachoon DS, Otero E, Ramsubhag A (2013) Detection of verotoxin producing *Escherichia*
425 *coli* in marine environments of the Carribean. *Mar Poll Bull* 76: 406-410
426 <https://doi.org/10.1016/j.marpolbul.2013.08.019>

427 Walters G, Ngagnia Ndjabounda E, Ikabanga D, Biteau JP, Hymas O, White LJ, Ndong Obiang AM,
428 Ndong Ondo P, Jeffery KJ, Lachenaud O, Stévant T (2016) Peri-urban conservation in the Mondah
429 forest of Libreville, Gabon: red list assessment of endemic plant species, and avoiding protected
430 area downsizing. *Oryx* 50: 419-430 <https://doi.org/10.1017/S0030605315000204>

431 Wiegner TN, Edens CJ, Abaya LM, Carlson KM, Lyon-Colbert A., Molloy SL (2017) Spatial and temporal
432 pollution patterns in a tropical estuary during high and low river flow conditions. *Mar Poll Bull*
433 114: 952-961. <https://doi.org/10.1016/j.marpolbul.2016.11.015>

- 434 World Health Organization (2018) *WHO recommendations on scientific, analytical and*
435 *epidemiological developments relevant to the parameters for bathing water quality in the Bathing*
436 *Water Directive (2006/7/EC)*. 96 p. WHO, Geneva.
- 437 Wu J, Long SC, Das D, Dorner SM (2011) Are microbial indicators and pathogens correlated? A
438 statistical analysis of 40 years of research. *J Water Health* 9: 265-278
439 <https://doi.org/10.2166/wh.2011.117>

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.

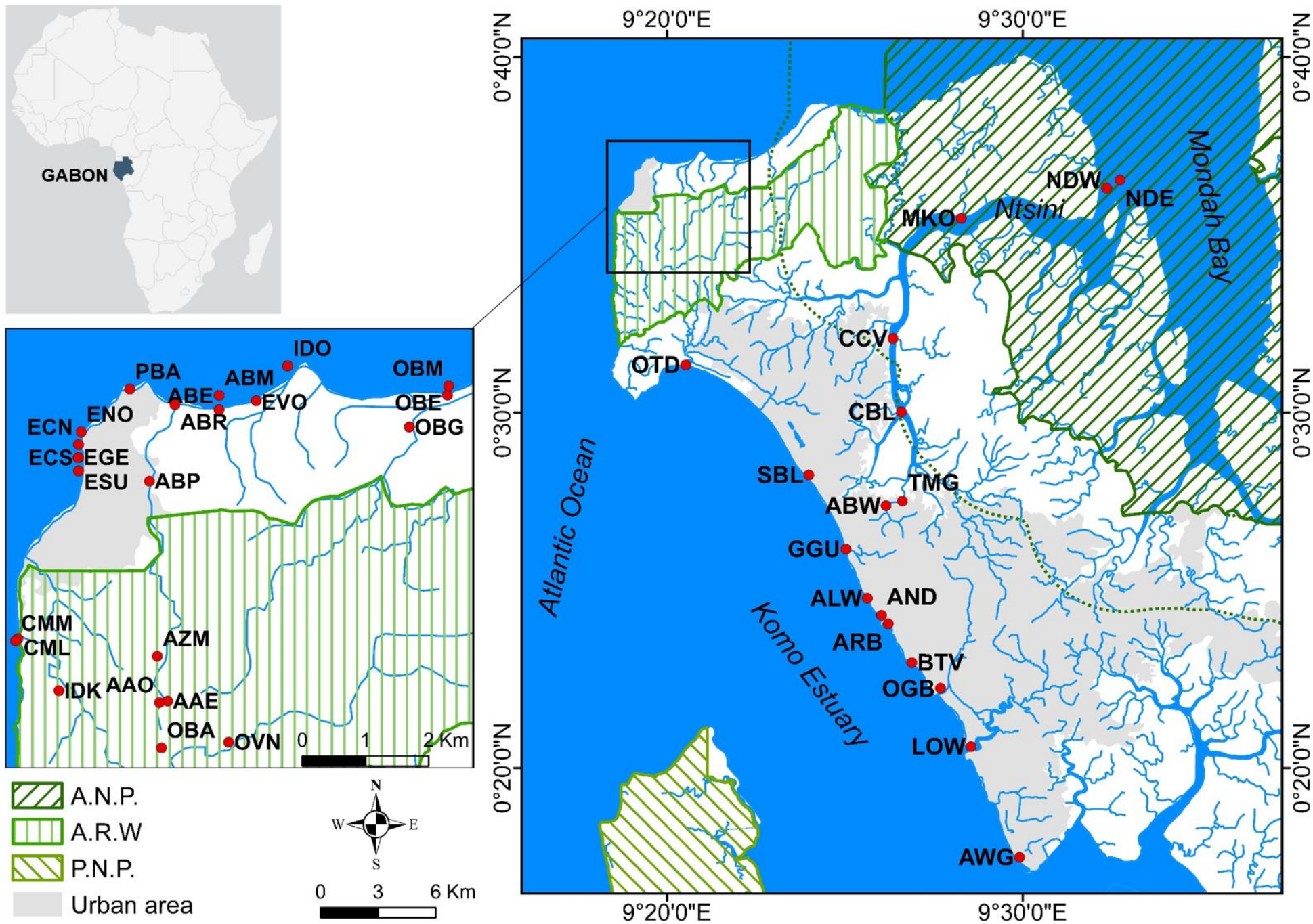
444 **Figure 2.** Concentrations of FIB *Escherichia coli* (A) and fecal streptococci (B) expressed as CFU/100
445 mL in the ten littoral outlets of the Komo estuary sampled within the Libreville area, depending on
446 the sampling month (March-June, 2018). Error bars are given except for samples exceeding 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*; NH_4^+ : ammonium
459 concentration; 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



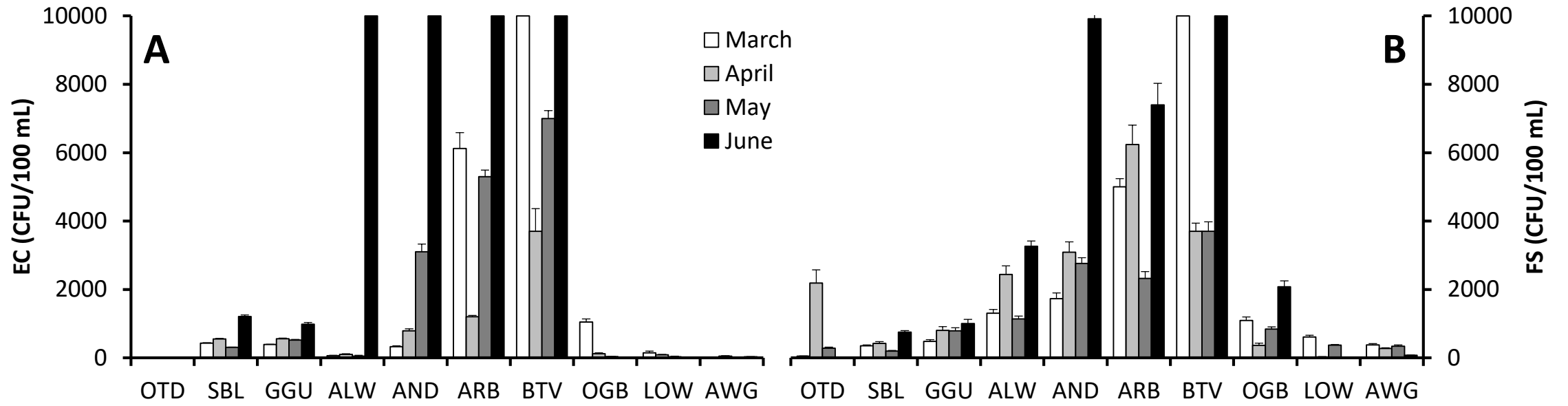


Fig. 2 MPB-D-21-00288_R1

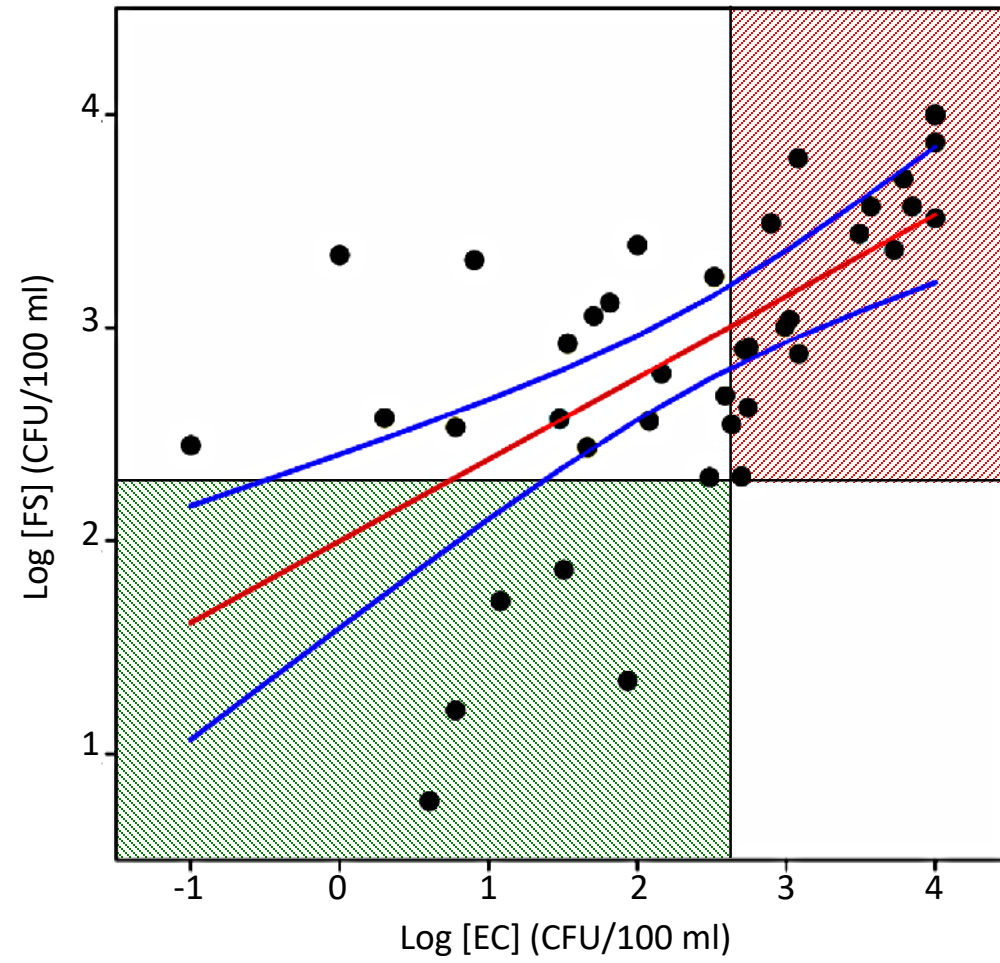


Fig. 3 MPB-D-21-00288_R1

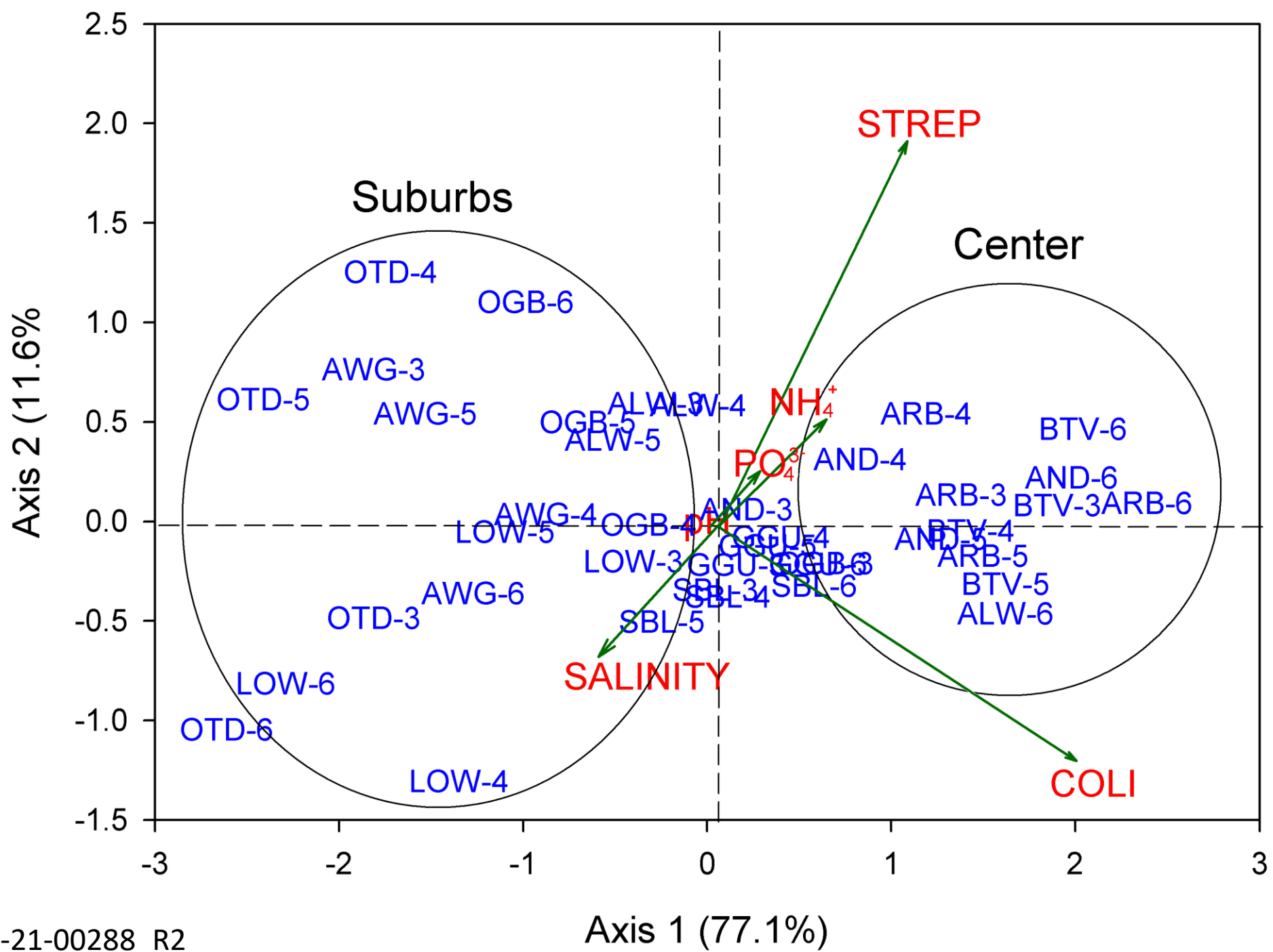


Fig. 4 MPB-D-21-00288_R2

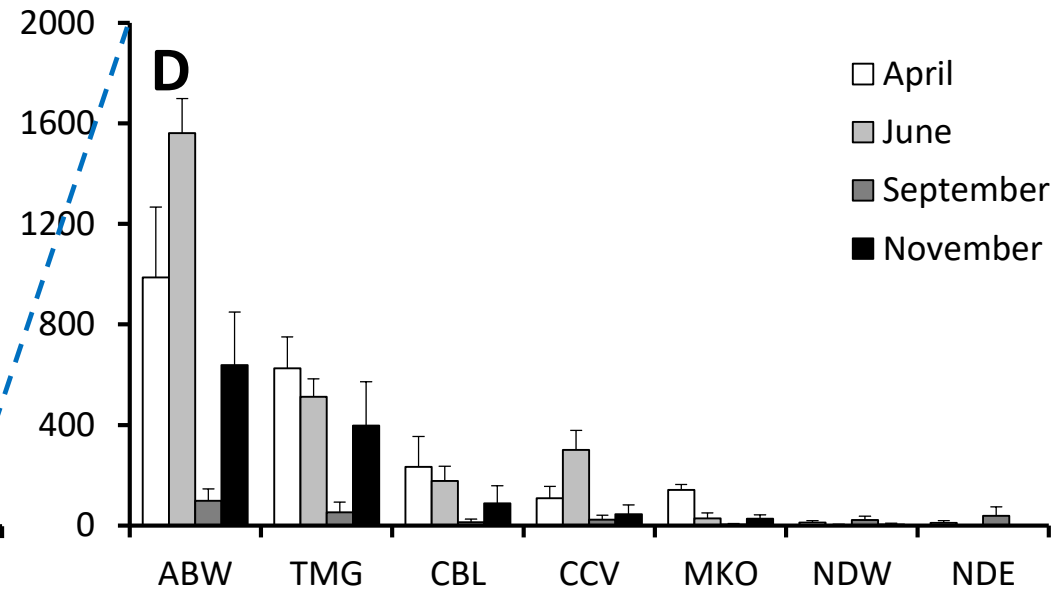
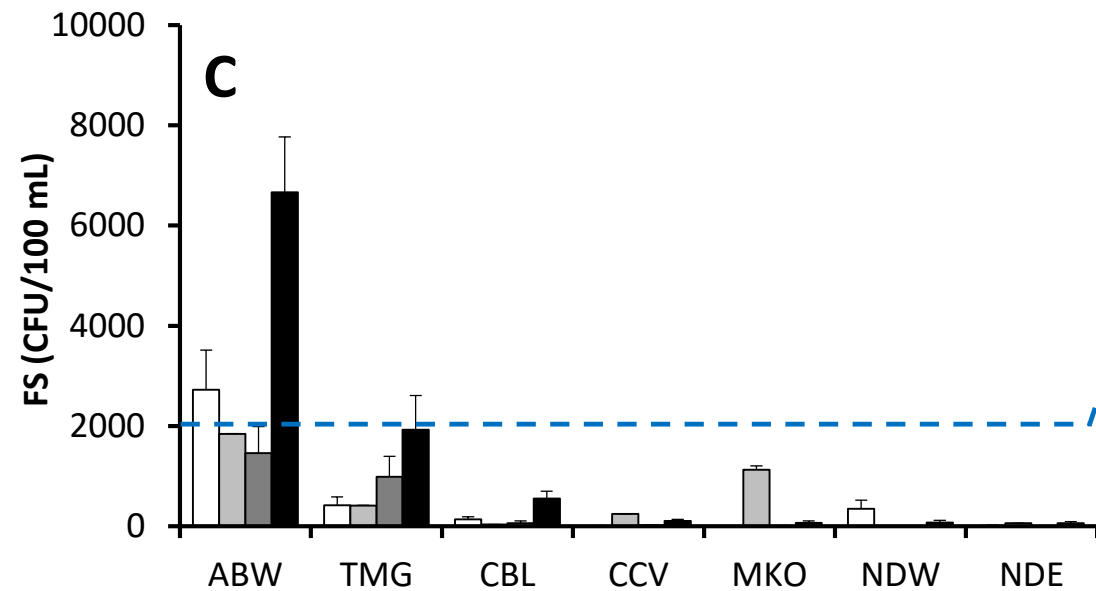
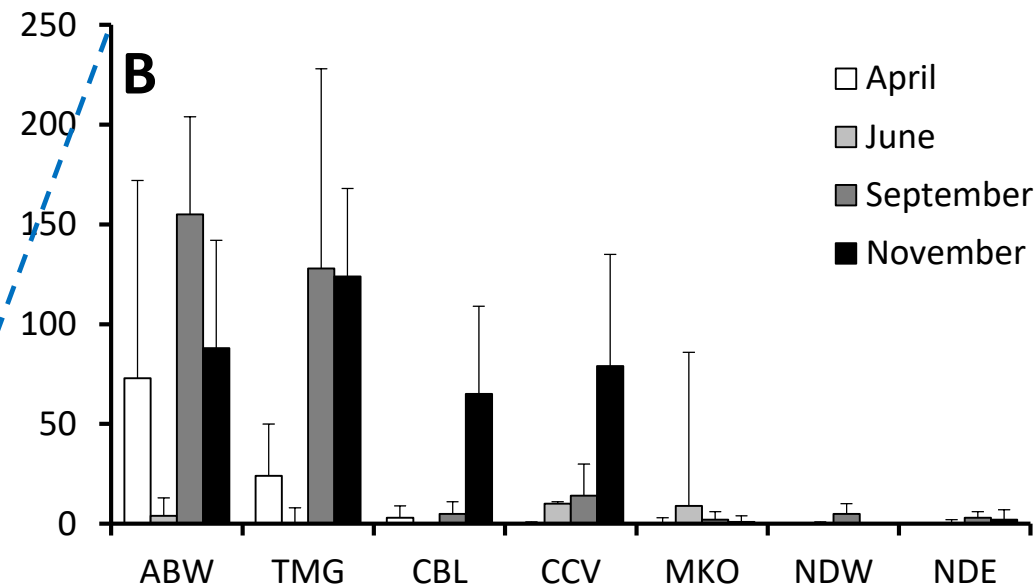
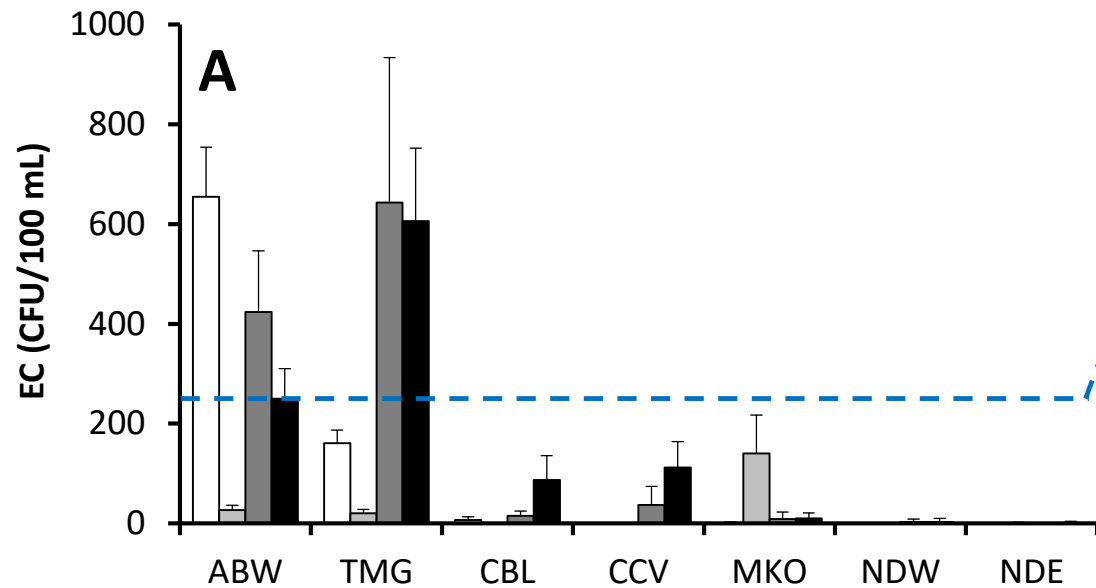


Fig. 5 MPB-D-21-00288_R1

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

