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

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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 NP to the northeast (Walters et al., 2016). Both NPs are characterized by an extended mangrove ecosystem, highlighted for its outstanding biomass and diversity in Gabon (Simard et al., 2019), which spreads from the Mondah Estuary up to the city suburbs in Akanda. The Akanda National Park lies east from Libreville around the Mondah bay, mostly consisting of mangrove forests and channels, 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 to130 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 PO₄³⁻) 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 (NH₄⁺ 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.

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

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

A gradient in waste pressure was clear from the visible accumulation of macro-waste in the urban outlets, increasing in sites near the city center, whereas mangrove roots acted as traps for floating debris even in the most remote protected areas of Akanda National Park. Upper forest rivers were obviously under low human pressure, but solid and liquid waste was collected in their lower part before reaching the seashore. Water contamination by FIB varied accordingly, depending on the 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 densities depending on the sampling month. Maximal EC densities were above the performance range of the method in four of the sampling sites located in the center of Libreville city in June 2018, and one in March 2018 (Fig. 2A). A similar pattern was observed for FS numbers, with two samples out of the measuring range at one site and on two dates (Batavea outlet, Fig. 2B). Lower FIB densities, mostly represented by FS, characterized the locations distant from the city center.

A weak but significant relation ($r^2 = 0.40789$; p = 0.0002) was established between logarithmic 169 170 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.

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

Regarding the FIB contamination in the mangrove protected area in Akanda National Park, seven sites were sampled on the Ntsini channel, representing ca. 27 km of watercourse from Ambowé, close to an urban fishing pier, to Nendé East, at the mouth of the Mondah Bay (Fig. 1). Samples were taken at four dates between April and November 2017, during both rising and ebb tides of the same day. A clear decreasing pattern from up- to downstream was observed for both categories of FIB (Fig. 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, T₉₀ 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.

Beaches and upstream rivers were sampled in April 2018 and April 2019 in water bodies surrounding the settlement of Cap Estérias, including forest streams of the Arboretum Raponda Walker, a protected littoral forest. Out of 29 samples, only one was devoid of measurable FIB contamination (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 dwellers and for the existence of an emblematic fishery for the razor clam, *Solen guinensis*, highly
valued locally as seafood (Cardiec et al., 2020). As noticed for the urban zone of Libreville, sewage
management is absent and will need to be considered in a near future to reduce pollution and safety
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).

Bacterial enumeration by culture-based methods does not consist in a direct assessment of health risk. For example, only a few strains of *E. coli* are known to be toxic to humans, and their detection relies on specific methods such as gene targeting (Snoussi et al., 2011; Walker et al., 2013). Detection 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 waterbone 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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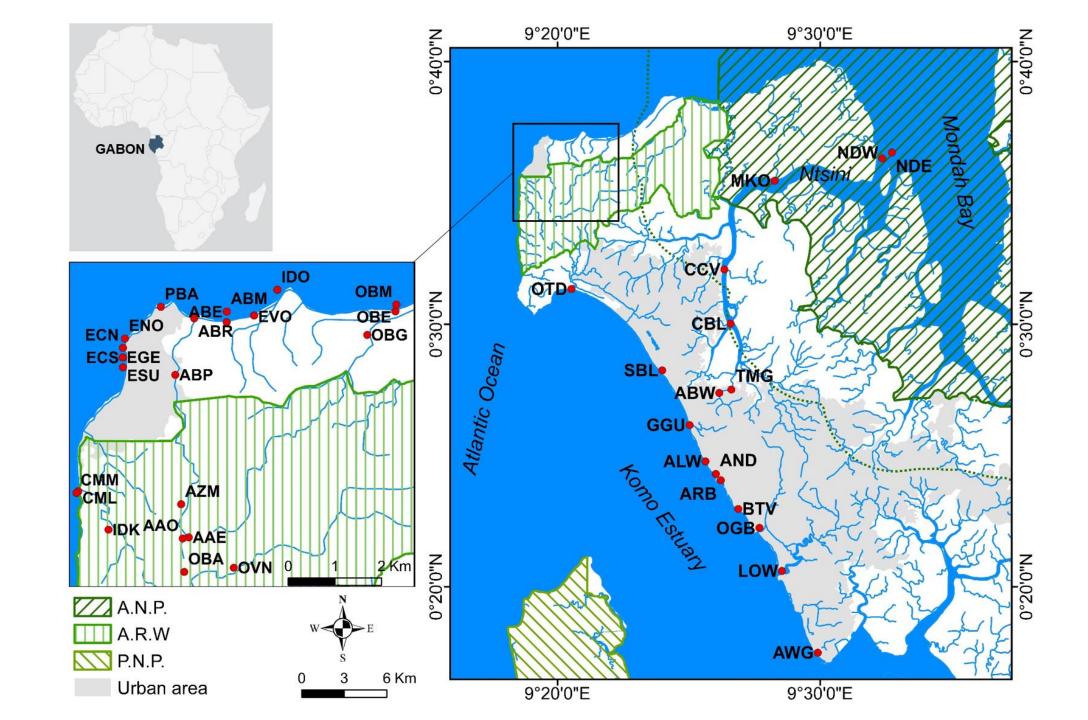
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440 Figures captions

- 441 **Figure 1.** Location of the sampling sites (red circles) in the Libreville area (Estuary province, Gabon).
- A.R.W.: Arboretum Raponda Walker (protected littoral forest); A.N.P.: Akanda National Park; P.N.P.:
 Pongara National Park. Sampling locations are indicated by red circles.
- Figure 2. Concentrations of FIB *Escherichia coli* (A) and fecal streptococci (B) expressed as CFU/100 mL in the ten littoral outlets of the Komo estuary sampled within the Libreville area, depending on the sampling month (March-June, 2018). Error bars are given except for samples exceeding the counting capacity of the method, set at 10,000 CFU / 100 mL.
- Figure 3. Relation between *Escherichia coli* (EC) and fecal streptococci (FS) densities (expressed as log[CFU/100 mL]) in the littoral outlets of the Komo estuary within the Libreville area during the present survey (March-June, 2018). Regression (y = 0.38737 x + 2.0063) is marked by the red line, 95% confidence intervals (bootstrapped, N = 1999) by blue curves. Dashed line indicates 1:1 relation, vertical and horizontal solid lines indicate European Union bathing water guidelines for information, green and orange area being below and above quality criteria for both FIB types respectively.
- Figure 4. Principal component analysis (PCA) on the two first axes, based on environmental
 parameters and FIB concentrations at the outlet of Libreville coastal rivers in the Komo Estuary.
 Eigenvalues for each axis of the PCA are reported. Abbreviations for sampled locations are listed in
 Table 1, sampling dates in 2018 are identified by month numbers (3: March; 4: April; 5: May; 6: June).
 STREP: abundance of fecal streptococci; COLI: abundance of *Escherichia coli*; NH₄⁺: ammonium
 concentration; PO₄³⁻: orthophosphate concentration. Two groups of locations can be defined
 (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.



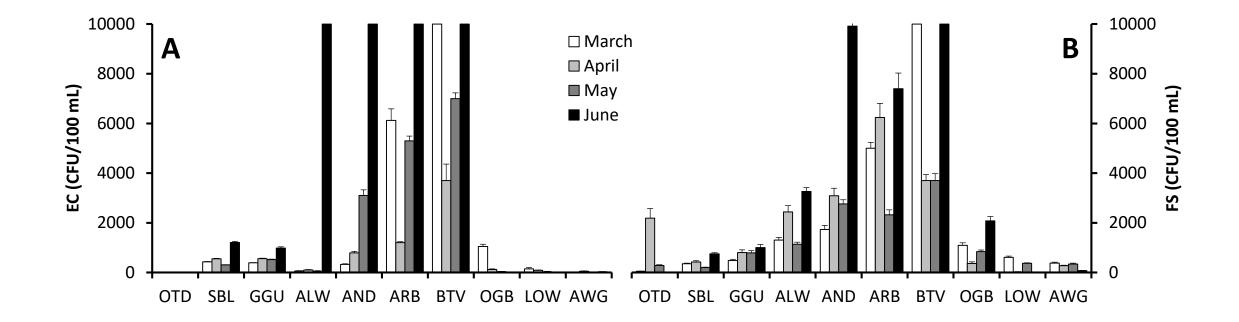
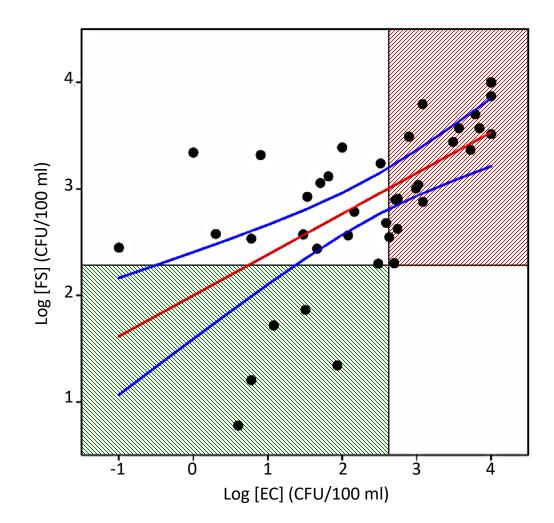


Fig. 2 MPB-D-21-00288_R1



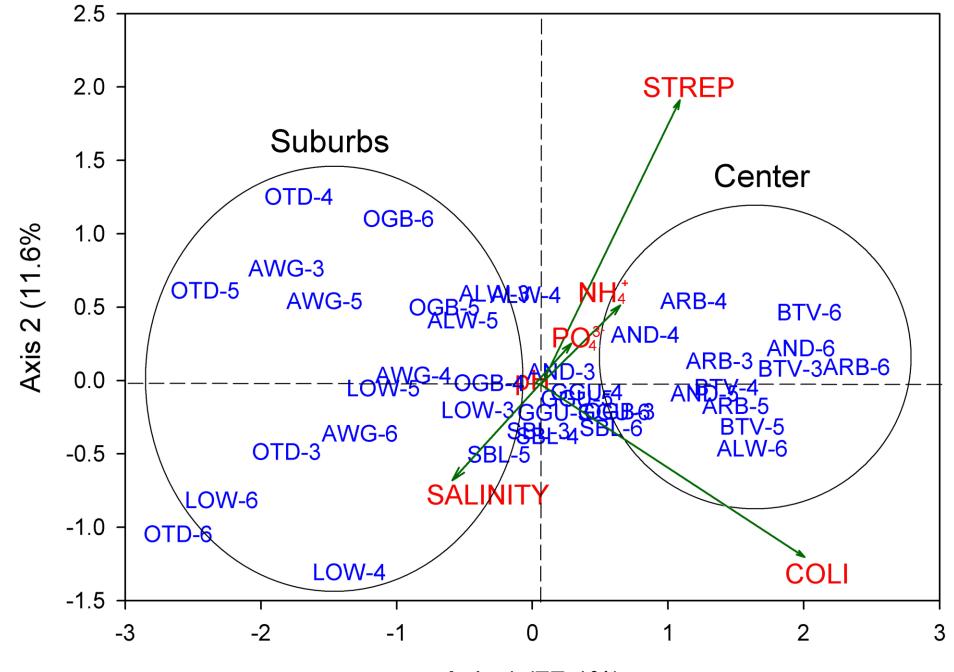


Fig. 4 MPB-D-21-00288_R2

Axis 1 (77.1%)

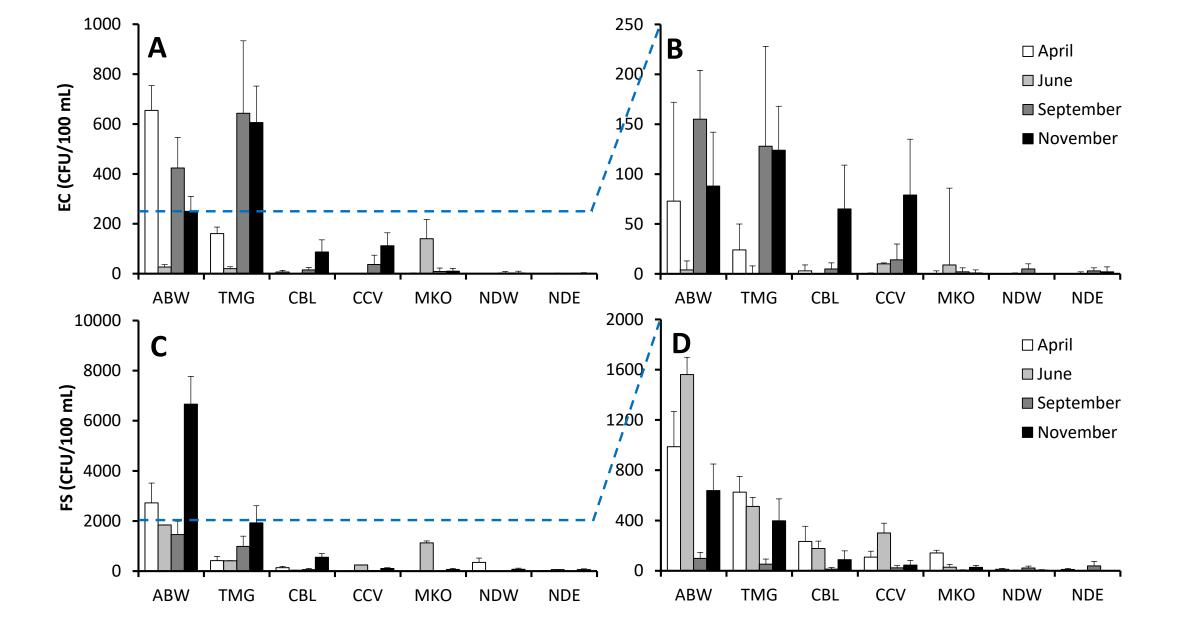


Fig. 5 MPB-D-21-00288_R1

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

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.

