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1 **Assessment of cyanotoxins in water and fish in an African freshwater lagoon**
2 **(Lagoon Aghien, Ivory Coast) and the application of WHO guidelines**

3

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23

24 **Abstract**

25 In comparison with northern countries, limited data are available on the occurrence
26 and potential toxicity of cyanobacterial blooms in lakes and ponds in Sub-Saharan
27 countries. With the aim of enhancing our knowledge on cyanobacteria and their toxins
28 in Africa, we performed a 17-month monitoring of a freshwater ecosystem, Lagoon
29 Aghien (Ivory Coast), which is used for multiple practices by riverine populations and
30 for drinking water production in Abidjan city. The richness and diversity of the
31 cyanobacterial community were high and displayed few variations during the entire
32 survey. The monthly average abundances ranged from 4.1×10^4 cell mL⁻¹ to 1.8×10^5
33 cell mL⁻¹, with higher abundances recorded during the dry seasons. Among the five
34 cyanotoxin families analyzed (anatoxin-a, cylindrospermopsin, homoanatoxin,
35 microcystins, saxitoxin), only microcystins (MC) were detected with concentrations
36 ranging from 0 to 0.364 µg L⁻¹ in phytoplankton cells, from 32 to 1,092 µg fresh weight
37 (FW) kg⁻¹ in fish intestines and from 33 to 383 µg FW kg⁻¹ in fish livers. Even if the MC
38 concentrations in water and fish are low, usually below the thresholds defined in WHO
39 guidelines, these data raise the issue of the relevance of these WHO guidelines for
40 Sub-Saharan Africa, where local populations are exposed throughout the year to these
41 toxins in multiple ways.

42

43 **Key words**

44 Africa, Ivory Coast, cyanobacteria, cyanotoxins, freshwater ecosystem, risk
45 management

46 **Introduction**

47 Eutrophication of freshwater ecosystems due to point and diffuse nutrient pollution is
48 ongoing on the African continent (Jenny et al. 2020). As shown for Lake Victoria (East
49 Africa), this process is associated with the rapid demographic changes taking place on
50 this continent (United Nations 2017) and leads to significant land use changes and
51 increasing pollution pressures on freshwater ecosystems (e.g., Olokotum et al. 2020).
52 Like anywhere else in the world, one would expect that the ongoing eutrophication of
53 African lakes would lead to the development of cyanobacterial blooms in these
54 ecosystems (Nyenje et al. 2010). This has already been well documented in a few
55 large lakes, such as Lake Victoria (e.g., Krienitz et al. 2002; Olokotum et al. 2021), and
56 in lakes and reservoirs located in northern and southern Africa (e.g., Oberholster et al.
57 2010; Matthews and Bernard 2015; Guellati et al. 2017; Hammou et al. 2018).
58 However, in comparison with northern countries, limited data are available on the
59 occurrence of cyanobacterial blooms in lakes and ponds located in intertropical areas
60 (e.g., Merel et al. 2013). In addition, there is also a lack of data on the potential toxicity
61 of these cyanobacterial blooms, with data available from less than half of African
62 countries (e.g., Ndlela et al. 2016; Svircev et al. 2019). In contrast, several reports of
63 freshwater cyanobacterial poisoning in terrestrial wildlife (flamingos and
64 megaherbivores) have been made, mainly in eastern and southern Africa (Ash and
65 Patterson 2022).

66 This lack of knowledge on the occurrence and potential toxicity of cyanobacterial
67 blooms in freshwater ecosystems of numerous sub-Saharan countries is partly
68 explained by the rarity of institutional monitoring of cyanobacteria in freshwater
69 ecosystems, and *vice versa* (Codd et al. 2005). In addition, there are also no
70 management plans, including policy tools, legislation and public communication,

71 dedicated to preventing human exposure to cyanotoxins, despite the potentially high
72 exposure of human populations to these toxins in developing countries. Indeed, the
73 majority of populations living around lakes use raw (untreated) water for multiple
74 purposes, including washing dishes and clothes, bathing, cooking and direct
75 consumption during fishing expeditions (e.g., Effebi et al. 2017; Roegner et al. 2020
76 and 2023; Olokotum et al. 2022).

77 In an attempt to improve our knowledge on threat assessment attributed to
78 cyanobacterial blooms in Sub-Saharan Africa, we performed a 17-month monitoring of
79 a freshwater lagoon, Lagoon Aghien, located in northern Abidjan city (Ivory Coast).
80 This lagoon supplies treated water for the Abidjan district and is used for recreational
81 and multiple domestic activities and for fishing by local populations living in the villages
82 located along its shores. Finally, this assessment led us to discuss the considerations
83 for applying the microcystin guideline values provided by the WHO in African countries
84 and the limits for their use in these countries.

85

86 **Material and Methods**

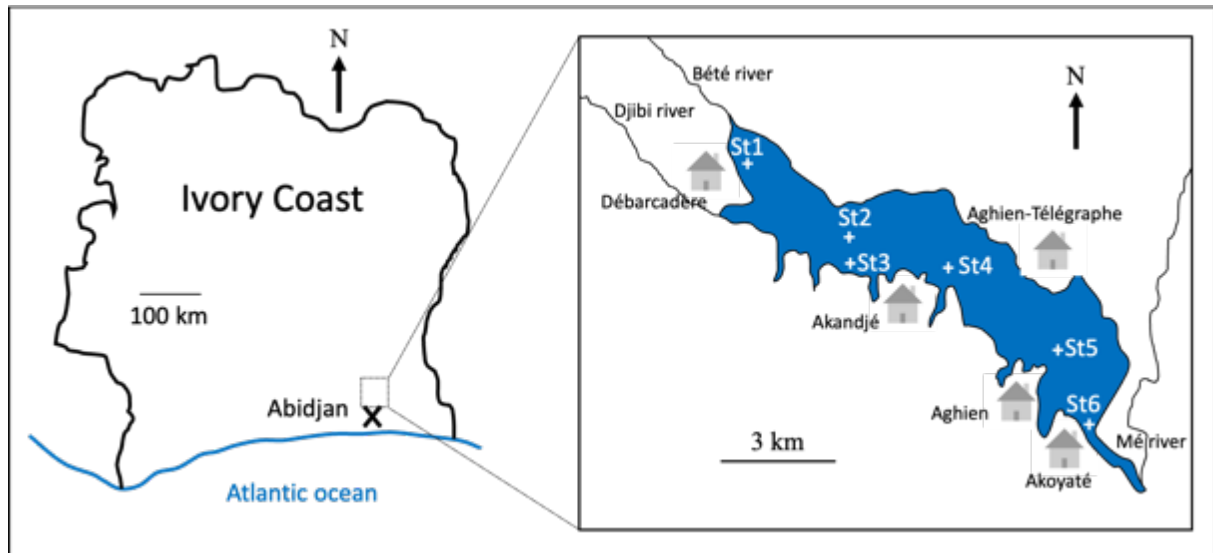
87 **Study site**

88 Lagoon Aghien (5°22'N to 5°26'N and 3°49'W and 3°55'W) is a freshwater ecosystem
89 located in the northern-western part of the Abidjan District (Ivory Coast) (Fig. 1). This
90 lagoon covers a surface area of 19.5 km² with a perimeter of 40.72 km, a volume of 75
91 km³ and a maximum depth of 10 m. More than 12,000 inhabitants live on its shores.

92 The lagoon is drained by three rivers (i.e., rivers Bété, Djibi and Mé) (Fig. 1).
93 The Mé and Bété catchments mainly include natural and agricultural lands, whereas
94 the Djibi catchment contains mostly urbanized areas. The Djibi and Bété Rivers flow
95 directly in the western part of the lagoon, while the Mé River flows in the channel

96 between Lagoon Aghien and Lagoon Potou. These three rivers carry many nutrients
97 (phosphorus and nitrogen), explaining the hypereutrophication of the lagoon (Koffi et
98 al. 2019; Ahoutou et al. 2021).

99



100

101 **Fig. 1** Locations of the six sampling stations in Lagoon Aghien

102

103 Monthly rainfall values were obtained from the averages of the data collected at
104 two weather stations (Anyama and Atchokoi).

105

106 **Sampling strategy of water**

107 As described in Ahoutou et al. (2021), a monthly monitoring survey was performed
108 from December 2016 to April 2018 at five sampling stations (St1, St2, St4, St5 and
109 St6) located on a NW–SE transect and at one additional sampling station (St3) located
110 close to the shoreline area of Akandje village (Fig. 1). Briefly, water samples were
111 collected in the first meter of the water column by using an integrated sampler as
112 described in Laplace-Treyture et al. (2016). The collected water samples were

113 transferred into two clean bottles that were stored in a cool box until they were returned
114 to the laboratory.

115 For cyanobacterial cell counting and identification, 200 mL of raw water was
116 fixed in a formaldehyde solution (5% in final concentration). For the extraction of toxins
117 from cyanobacterial cells, 200 mL of water was filtered through polycarbonate filters
118 (Whatman® Nuclepore), which were stored at $-80\text{ }^{\circ}\text{C}$.

119

120 **Dissolved nutrient analyses**

121 All the nutrient analyses were performed following the instructions of the LCK cuvette test
122 systems provided by of the manufacturer (Hach® Company). The preparation of samples was
123 performed using LCK cuvette test systems LCK 304, 341, 339 and 349 for ammonium (NH_4),
124 nitrites (NO_2), nitrates (NO_3) and soluble reactive phosphorus (SRP) respectively, after the
125 water samples were filtered through nylon membranes (Whatman™, porosity $0.45\text{ }\mu\text{m}$,
126 diameter 47 mm). Colorimetric determinations of the concentrations were made using a HACH
127 DR6000 UV VIS spectrophotometer.

128

129 **Cyanobacteria identification and counting**

130 The identification and counting of cyanobacteria genera were performed with an
131 inverted microscope (Nikon Eclipse TS100) according to the Utermöhl method
132 (Utermöhl 1958) and following the AFNOR 15204 standard. For each sample, a
133 minimum of 30 fields of view, selected at random and distributed over the entire surface
134 of the counting chamber, were observed under the microscope such that at least 500
135 counting units (cells, colonies or filaments/trichomes) were counted. Except for some
136 cyanobacteria, the cells were directly counted. For *Microcystis*, two categories of
137 colonies were considered corresponding to their diameters ($<$ or $>$ $200\text{ }\mu\text{m}$). The mean

138 number of cells per category was estimated under an upright microscope by counting
139 the number of cells in 30 colonies per category after the colonies were gently spread
140 between slides and coverslips. For filamentous cyanobacteria (*Aphanizomenon*,
141 *Cylindrospermopsis/Raphidiopsis*, *Limnothrix*, *Lyngbya*, *Oscillatoria*, *Planktothrix* and
142 *Pseudanabaena*), filament lengths of 100 µm were considered, and the mean cell
143 number per 100 µm of filament was estimated on 30 filaments per genus according to
144 the method described by Catherine et al. (2017). Finally, for all genera, the results of
145 the cell counts are expressed as numbers of cells per mL.

146 The potential ability of the various genera to produce cyanotoxins was identified
147 on the basis of a report published by ANSES (2020).

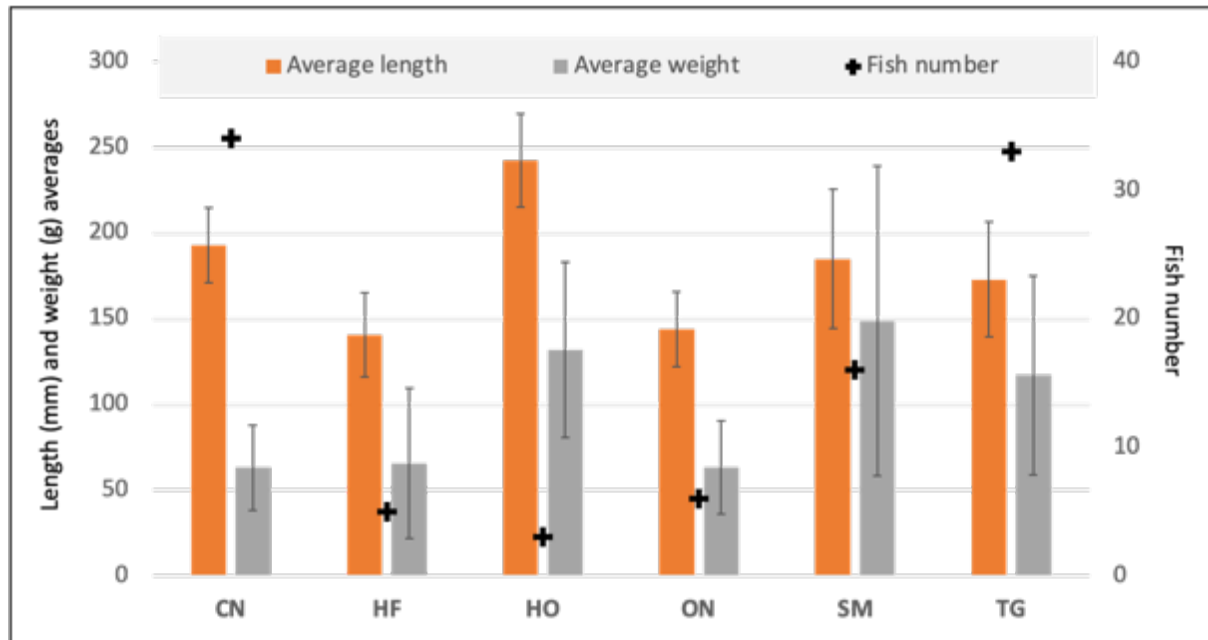
148

149 **Fish**

150 The fish were purchased monthly between May 2017 and April 2018 from fishermen
151 in three villages located around the lagoon (Anyama-Débracadère, Akandjé and
152 Aghien-Télégraphe). They were transported in a cooler to the laboratory, and then (i)
153 the length and weight of each individual were measured (Supplementary Fig. S1; Table
154 1) and (ii) liver, intestinal and muscle tissues were taken from each fish and stored at
155 -80 °C until analysis.

156 A total of 97 fish belonging to six species were collected: 34 *Chrysichthys*
157 *nigrodigitatus* (Claroteidae), 33 *Tilapia guineensis* (Cichlidae), 16 *Sarotherodon*
158 *melanotheon* (Cichlidae), six *Oreochromis niloticus* (Cichlidae), five *Hemichromis*
159 *fasciatus* (Cichlidae) and three *Hepsetus odoe* (Hepsetidae). These fishes belong to
160 four trophic groups (i.e., omnivorous for *T. guineensis*, phytoplanktivorous for *S.*
161 *melanotheon* and *O. niloticus*, zooplanktivorous for *C. nigrodigitatus* and carnivorous

162 for *H. fasciatus* and *H. odoe*) (Blé et al. 2008; Decru et al. 2012; Kidé et al. 2015; Jawad
 163 et al. 2017)
 164



165
 166 **Supplementary Fig. S1** Average length and weight (\pm standard deviation) and fish
 167 number in the six fish species collected in Lagoon Aghien
 168 CN = *Chrysichthys nigrodigitatus*; HF = *Hemichromis fasciatus*; HO = *Hepsetus odoe*;
 169 ON = *Oreochromis niloticus*; SM = *Sarotherodon melanotheron*; TG = *Tilapia*
 170 *guineensis*
 171

172 **Table 1** Total lengths (TL), weights and microcystin (MC) -LR and -RR concentrations
 173 in intestine, liver and muscle of all the fishes collected in Lagoon Aghien
 174 Cn = *Chrysichthys nigrodigitatus*; Hf = *Hemichromis fasciatus*; Ho = *Hepsetus odoe*;
 175 On = *Oreochromis niloticus*; Sm = *Sarotherodon melanotheron*; Tg = *Tilapia*
 176 *guineensis*
 177
 178

Date	Species	TL (mm)	Weight (g)	MC in intestine			MC in liver			MC in muscle
				RR	LR	Total	RR	LR	Total	($\mu\text{g kg}^{-1}$ FW)
May-17	<i>Tg</i>	187	120	0	0	0	0	0	0	
	<i>Tg</i>	193	125	0	0	0	0	0	0	
	<i>Tg</i>	173	101	0	0	0	74	0	74	
	<i>Sm</i>	125	40	0	0	0	77	0	77	
	<i>Sm</i>	130	46	0	0	0	0	0	0	
	<i>Sm</i>	134	43	180	231	411	68	315	383	
	<i>Cn</i>	188	36	0	0	0	0	0	0	
	<i>Cn</i>	179	26	0	0	0	0	0	0	
	<i>Cn</i>	173	45	0	0	0	69	0	69	
Jun-17	<i>Tg</i>	221	198	0	0	0	0	0	0	
	<i>Tg</i>	202	158	0	0	0	298	0	298	
	<i>Tg</i>	193	151	241	276	517	0	0	0	
	<i>Sm</i>	210	205	46	225	271	189	0	189	
	<i>Sm</i>	208	154	165	0	165	0	0	0	
	<i>Sm</i>	191	143	0	0	0	104	0	104	
	<i>Cn</i>	192	49	0	0	0	0	0	0	
	<i>Cn</i>	184	40	0	0	0	0	0	0	
	<i>Cn</i>	203	82	0	0	0	35	0	35	
Jul-17	<i>Sm</i>	226	235	0	0	0	nd	nd	nd	
	<i>Sm</i>	220	240	0	0	0	nd	nd	nd	
	<i>Sm</i>	200	170	113	0	113	nd	nd	nd	
	<i>Tg</i>	250	210	0	0	0	nd	nd	nd	
	<i>Tg</i>	225	250	0	0	0	nd	nd	nd	
	<i>Cn</i>	170	50	0	0	0	nd	nd	nd	
	<i>Cn</i>	170	50	0	0	0	nd	nd	nd	
Aug-17	<i>Cn</i>	210	80	0	0	0	nd	nd	nd	
	<i>Cn</i>	195	60	0	0	0	nd	nd	nd	
	<i>Cn</i>	220	100	0	0	0	nd	nd	nd	
	<i>Tg</i>	148	76	0	0	0	nd	nd	nd	
	<i>Tg</i>	169	112	368	422	790	nd	nd	nd	
	<i>Tg</i>	180	125	396	696	1092	nd	nd	nd	
	<i>Sm</i>	242	290	0	0	0	nd	nd	nd	
	<i>Sm</i>	243	295	34	0	34	nd	nd	nd	
Sep-17	<i>Cn</i>	160	40	158	422	581	nd	nd	nd	
	<i>Tg</i>	125	32	0	0	0	nd	nd	nd	
	<i>Tg</i>	116	24	0	0	0	nd	nd	nd	
	<i>Cn</i>	165	45	0	0	0	nd	nd	nd	
	<i>Cn</i>	162	36	0	0	0	nd	nd	nd	
Oct-17	<i>Cn</i>	190	47	0	0	0	77	0	77	
	<i>Cn</i>	193	73	0	0	0	0	0	0	
	<i>Cn</i>	237	104	0	0	0	0	0	0	
	<i>Tg</i>	200	166	32	0	32	0	0	0	
	<i>Tg</i>	240	250	0	0	0	0	0	0	

	<i>Tg</i>	170	103	0	0	0	0	0	0	0
Nov-17	<i>Cn</i>	190	34	0	0	0	0	0	0	0
	<i>Cn</i>	210	49	0	0	0	0	0	0	0
	<i>Cn</i>	230	89	0	0	0	0	0	0	0
	<i>Tg</i>	197	151	67	0	67	0	0	0	0
	<i>Tg</i>	194	131	81	0	81	0	0	0	0
	<i>Tg</i>	190	145	0	0	0	41	0	41	0
	<i>Sm</i>	185	136	118	0	118	0	0	0	0
	<i>Sm</i>	195	162	0	0	0	84	0	84	0
	<i>Sm</i>	167	92	76	0	76	39	0	39	0
	<i>Hf</i>	150	81	0	0	0	0	0	0	0
	<i>Hf</i>	180	136	0	0	0	0	0	0	0
	<i>On</i>	167	98	0	0	0	0	0	0	0
	<i>On</i>	175	99	0	0	0	0	0	0	0
	Dec-17	<i>Cn</i>	195	39	0	0	0	nd	nd	nd
<i>Cn</i>		210	66	113	0	113	nd	nd	nd	0
<i>Tg</i>		150	77	0	0	0	nd	nd	nd	0
<i>Tg</i>		140	61	0	0	0	nd	nd	nd	0
Jan-18	<i>Cn</i>	212	117	0	0	0	nd	nd	nd	0
	<i>Cn</i>	160	46	0	0	0	nd	nd	nd	0
	<i>Cn</i>	210	69	0	0	0	nd	nd	nd	0
	<i>Tg</i>	135	49	249	269	518	nd	nd	nd	0
	<i>Tg</i>	165	86	0	0	0	nd	nd	nd	0
	<i>Tg</i>	152	77	0	0	0	nd	nd	nd	0
Feb-18	<i>Ho</i>	250	152	0	0	0	0	0	0	0
	<i>Ho</i>	265	170	227	0	227	0	0	0	0
	<i>Ho</i>	212	74	238	3	241	0	0	0	0
	<i>Cn</i>	190	81	0	0	0	0	0	0	0
	<i>Cn</i>	210	84	0	0	0	33	0	33	0
	<i>Cn</i>	179	54	0	0	0	0	0	0	0
	<i>Tg</i>	146	67	139	0	139	0	0	0	0
	<i>Tg</i>	153	79	166	303	469	0	0	0	0
	<i>Tg</i>	131	56	194	161	355	0	0	0	0
	<i>Hf</i>	126	40	0	0	0	0	0	0	0
	<i>Hf</i>	123	36	0	0	0	0	0	0	0
	<i>Hf</i>	124	35	80	114	194	0	0	0	0
	<i>Sm</i>	153	77	221	112	333	0	0	0	0
	<i>Sm</i>	128	43	78	133	211	50	0	50	0
Mar-18	<i>Cn</i>	230	110	0	0	0	0	0	0	0
	<i>Cn</i>	220	82	155	116	272	0	0	0	0
	<i>Cn</i>	163	46	0	0	0	108	0	108	0
	<i>Tg</i>	150	67	0	0	0	132	0	132	0
	<i>Tg</i>	134	49	188	0	188	0	0	0	0
	<i>Tg</i>	135	53	0	0	0	50	158	209	0
	<i>On</i>	125	43	241	130	371	0	0	0	0
	<i>On</i>	137	46	56	0	56	42	0	42	0

	<i>On</i>	133	50	71	0	71	0	0	0	0
	<i>On</i>	125	46	75	0	75	0	0	0	0
Apr-18	<i>Tg</i>	179	112	0	0	0	nd	nd	nd	0
	<i>Tg</i>	166	87	61	0	61	nd	nd	nd	0
	<i>Tg</i>	193	137	154	160	314	nd	nd	nd	0
	<i>Cn</i>	200	95	0	0	0	nd	nd	nd	0
	<i>Cn</i>	195	100	0	0	0	nd	nd	nd	0
	<i>Cn</i>	165	46	0	0	0	nd	nd	nd	0

181
182

183 **Cyanotoxin identification and quantification from phytoplankton cells and fish**

184 The same protocols as those described in Ahoutou et al. (2022) were used for all toxin
185 analyses. Briefly, for intracellular cyanotoxins (in the phytoplanktonic biomass), the
186 extraction was performed from biomass on polycarbonate filters (Whatman®
187 Nuclepore) stored at -80 °C. After thawing, the filters were placed in sterile glass
188 containing 4 mL of 75% methanol and then sonicated on ice with an ultrasound probe
189 (Sonics Vibra Cell). The extracts were centrifuged, and the supernatants were
190 collected and stored at 4 °C. The resulting pellets underwent a second extraction using
191 the same protocol and then were centrifuged again to ensure that all toxins were
192 extracted. These second supernatants were added to the first and centrifuged again.
193 They were further frozen at -80 °C until cyanotoxin analysis.

194 For cyanotoxin extractions from the fish, the method of Manubolu et al. (2018)
195 was used with minor adjustments. For each fish, 100 mg of fresh liver, muscle, and
196 intestinal tissues were used for cyanotoxin extraction. The 100 mg muscle extracts
197 were further previously freeze-dried and underwent ball milling. All samples were
198 extracted twice with BuOH:MeOH:H₂O (1:4:15), submitted to an ultrasonic probe
199 (Sonics Vibra Cell, 130 Watt, 20 kHz, 60%, 30 sec, three cycles) and centrifuged
200 (15 300 G, 10 min, 4 °C). The collected supernatants underwent final centrifugation
201 (15 300 G, 10 min, 4 °C) and were stored at -80 °C until analysis.

202 The identification and quantification of the cyanotoxins in the water and fish
203 samples were performed by ultrahigh-performance liquid chromatography in tandem
204 (Elute UHPLC—Bruker) coupled with high-resolution mass spectrometry (Compact
205 QTOF—Bruker) (UHPLC–MS/MS). All samples were separated on a C18 stationary
206 phase column (Acclaim RSLC Polar Advance 2, 2.2 μm , Thermo Fisher® 2.1 Å~ 100
207 mm) along a linear gradient of acidified acetonitrile and acidified ultrapure water and
208 then analyzed between 50 and 1500 m/z in positive BbCID (MS-MS/MS) mode,
209 alternating at 2 Hz between low and high-energy MS and MS2 modes, respectively,
210 with a mass accuracy of less than 0.5 ppm. The injection volume and data acquisition
211 time were 4 μL and 21 min, respectively. The elution rate was 0.5 $\mu\text{L min}^{-1}$. The
212 analytical standards of the cyanotoxins investigated were saxitoxin (STX) (CAS 35554-
213 086), anatoxin-a (ATX) (CAS 64285-06-9), homoanatoxin (HTX) (CAS 142926-86-1),
214 nodularin (NOD) (CAS 118399-22-7), cylindrospermopsin (CYL) (CAS 143545-90-8),
215 and seven variants of microcystins (MC) (variant MC-LR, -LA, -LF, -LW, -LY, -RR and
216 -YR corresponding to CAS 101043-37-2; CAS 96180-79-9; CAS 154037-70-4; CAS
217 157622-02-1; CAS 123304-10-9; CAS 11755-37-4; and CAS 10164-48-6,
218 respectively), all certified one year, purity >99% (Novakit®). Data were integrated using
219 TASQ 1.1 software (Bruker®). Cyanotoxins were identified according to (i) retention
220 time, (ii) molecular mass, (iii) isotopic pattern, and (iv) diagnostic ions. They were then
221 quantified according to the area under the peak signal determined for analytical
222 standards. *Phormidium favosum* (PMC 240.05), *Raphidiopsis raciborskii* (PMC 99.03),
223 *Aphanizomenon gracile* (PMC 638.10), and *Microcystis aeruginosa* (PMC 728.11)
224 strains were used as positive controls for ATX, CYL, STX and MC, respectively. Except
225 for MC-RR, MC-LR, and MC-YR, other MC structural variants were quantified

226 according to MC-LR equivalence, calculated from the regression curves of the MC-LR
227 analytical standard.

228 For phytoplankton, the LOD and LOQ were equal to 0.0003 and 0.001 $\mu\text{g L}^{-1}$ of
229 raw water for MC-RR and 0.001 and 0.004 $\mu\text{g L}^{-1}$ for MC-LR, respectively. For fish
230 tissues, the LOD and LOQ were equal to 1.3 and 12.8 $\mu\text{g kg}^{-1}$ fresh weight (FW) for
231 MC-RR and 0.5 and 4.8 $\mu\text{g kg}^{-1}$ for MC-LR, respectively.

232

233 **Statistical analyses**

234 Wilcoxon pairwise tests, sign tests, ordinary least squares regression and multiple
235 linear regression (MLR) were performed by using PAST software v4.11.

236

237 **Results**

238 **Spatial and temporal variations in cyanobacteria abundances and biovolumes**

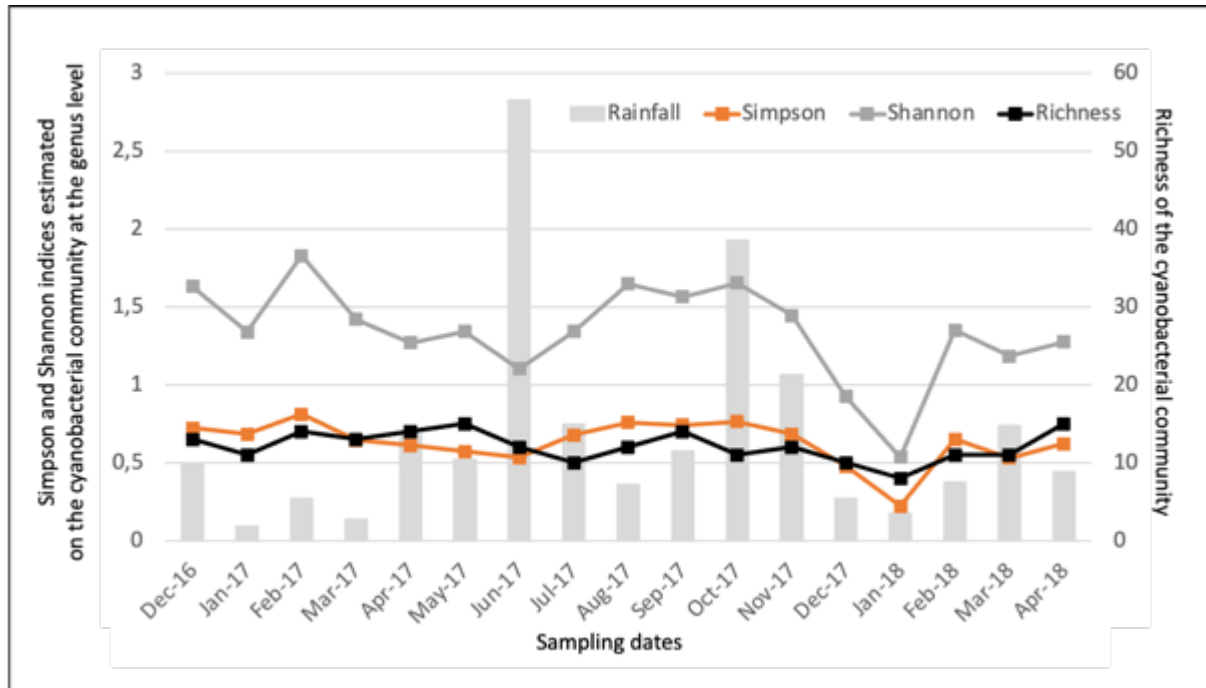
239 A total of 15 cyanobacterial genera were found during the monitoring of the lagoon:
240 *Aphanizomenon*, *Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Dolichospermum*,
241 *Gomphosphaeria*, *Leptolyngbya*, *Limnothrix*, *Lyngbya*, *Merismopedia*, *Microcystis*,
242 *Oscillatoria*, *Planktothrix*, *Pseudanabaena* and *Raphidiopsis* (previously
243 *Cylindrospermopsis*) (Supplementary Table S1).

244

245 **Supplementary Table S1** Cyanobacterial abundances (cell mL^{-1}) and potential toxicity
246 of the 15 cyanobacterial genera identified during the 17-month monitoring performed
247 at six sampling stations in Lagoon Aghien
248

254 indices in January 2018. These low values in richness and diversity were associated
255 with a high dominance of the genus *Limnothrix* in the cyanobacterial community (Fig.
256 2 and 3).

257



258

259 **Fig. 2** Monthly variations in the richness and diversity (evenness, Shannon and
260 Simpson indices calculated on the average cyanobacterial abundances at the six
261 sampling stations) of the cyanobacterial community estimated at the genus taxonomic
262 level in Lagoon Aghien. Monthly cumulative rainfall values (cm) have been added to
263 the figure.

264

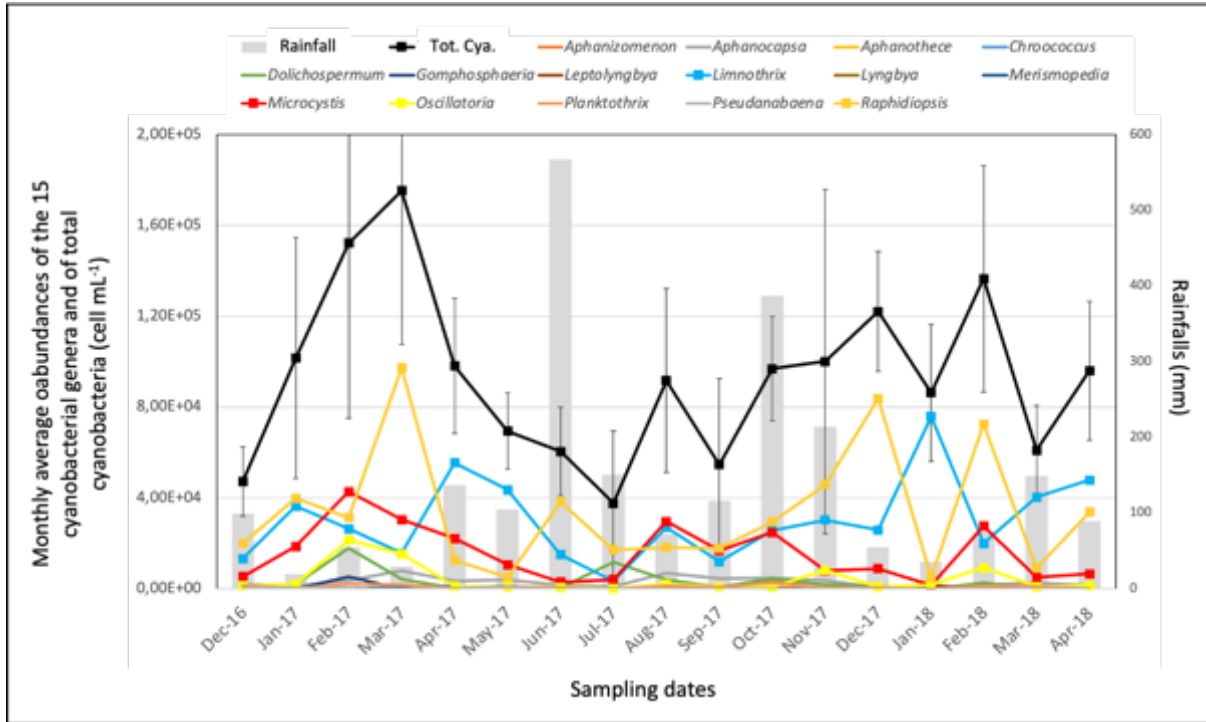
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266

267 The total cyanobacterial abundances (sum of all genera) estimated during the
268 monitoring survey ranged from 1.6×10^4 to 3.1×10^5 cell mL⁻¹ (Supplementary Table S1),
269 and monthly average abundances ranged from 4.1×10^4 cell mL⁻¹ in July 2017 to
270 1.8×10^5 cell mL⁻¹ in March 2017. As shown in Figure 3, two main peaks of total
271 cyanobacteria abundances were observed in February-March 2017 and February
272 2018. The four dominant cyanobacterial genera in terms of cellular abundance were
Raphidiopsis, *Limnothrix*, *Microcystis* and *Oscillatoria*. The main peak abundances of

273 these four genera were found from January to April 2017 and from December 2017 to
 274 February 2018 when rainfall was low (Fig. 3).

275

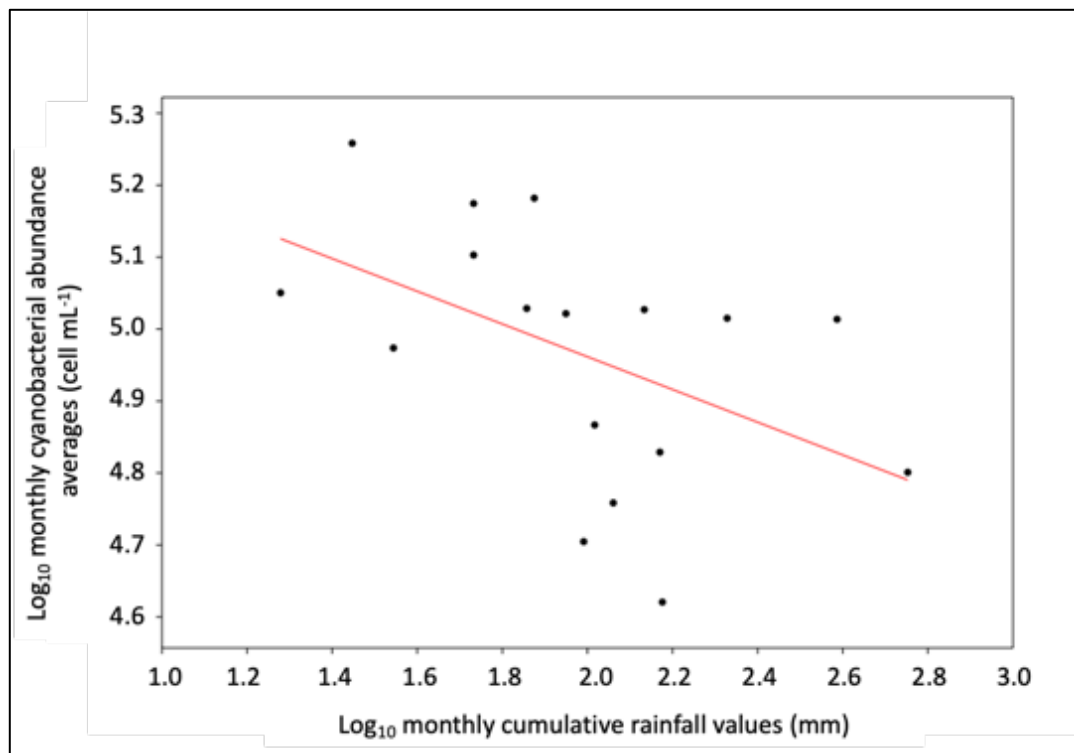


276

277 **Fig. 3** Variations in the monthly average abundances of the total cyanobacteria
 278 abundances (black curve) and of each cyanobacterial genus abundance (colored
 279 curves) (mean values from the six sampling stations) and variations in the cumulative
 280 monthly rainfall (gray bar chart). Standard deviations have only been added for the
 281 monthly averages of total cyanobacteria abundances. Total cya = Total cyanobacteria
 282 abundance.

283

284 A negative correlation was found between monthly variations occurring in the
 285 monthly cumulative rainfall and those occurring in the total cyanobacterial abundance
 286 averages ($r^2=0.24$; ordinary least squares regression, $p<0.05$) (Supplementary Fig.
 287 S2).



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289 **Supplementary Fig. S2** Correlation between Log₁₀ monthly cyanobacterial
 290 abundance averages and Log₁₀ monthly cumulative rainfall values

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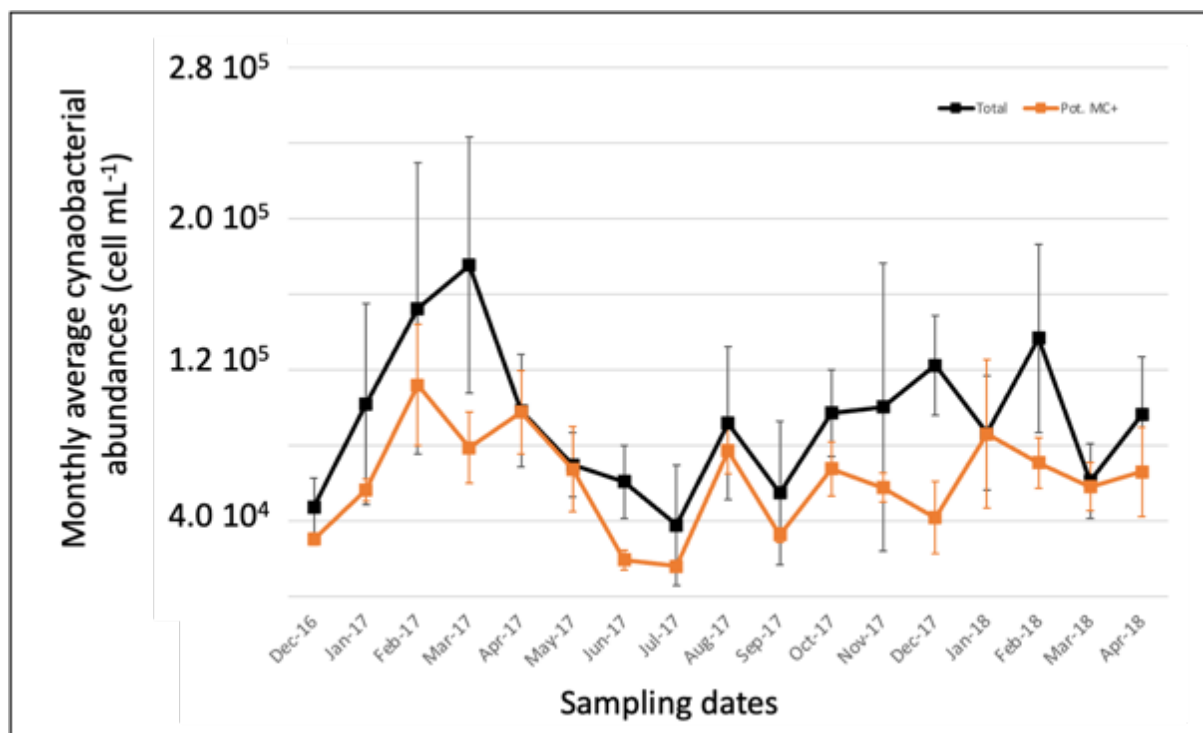
292

293 Finally, among the 15 cyanobacteria genera observed in the lagoon, twelve are
 294 known to produce cyanotoxins. More specifically, four of them are known to produce
 295 ATX or HTX, five are known to produce CYL, nine are known to produce MC and four
 296 are known to produce STX (Supplementary Table S1).

297 As observed for the variations in the total cyanobacterial abundances, the
 298 variations in the monthly averages of the potentially MC-producing cyanobacteria
 299 abundances (*Aphanocapsa*, *Dolichospermum*, *Leptolyngbya*, *Limnothrix*,
 300 *Merismopedia*, *Microcystis*, *Oscillatoria*, *Planktothrix* and *Pseudanabaena*) were
 301 characterized by a first peak of abundance at the beginning of 2017 followed by a
 302 strong decrease in June-July 2017 and a progressive increase in average abundance
 303 values until the end of the monitoring (Supplementary Fig. S3).

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Supplementary Fig. S3 Variations in the monthly average abundances of all cyanobacteria and of cyanobacteria potentially able to produce microcystins (mean values from the six sampling stations) during the monitoring of Lagoon Aghien. Total = monthly average abundances of all cyanobacteria; Pot. MC+ = monthly average abundances of cyanobacteria potentially able to produce microcystins

314 Finally, as shown in Fig. 4A and by a Wilcoxon pairwise test (Supplementary
315 Table S2), Station 6 and, to a lesser extent, Station 3 were characterized by lower
316 monthly average abundances of cyanobacteria potentially able to produce MC.

317

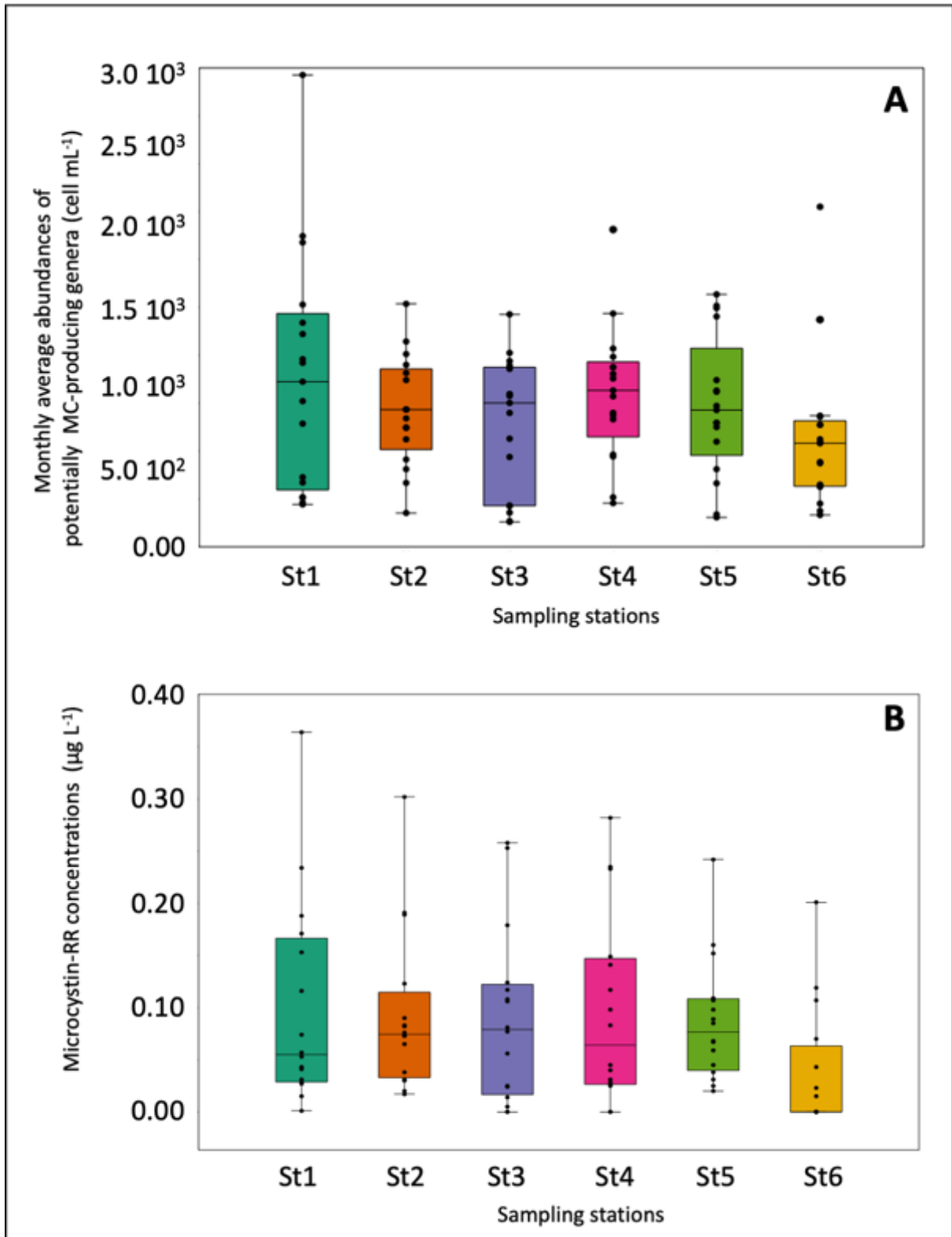
318 **Supplementary Table S2** p values of the Wilcoxon paired test performed on the
319 monthly average abundances of cyanobacteria potentially able to produce
320 microcystins at the six sampling stations during the monitoring of Lagoon Aghien (bold
321 characters = significant p values)

322

Stations	St1	St2	St3	St4	St5	St6
St1	-	0,43	0,05	0,61	0,21	0,01
St2		-	0,26	0,46	0,96	0,04
St3			-	0,02	0,21	0,52
St4				-	0,40	0,01
St5					-	0,16
St6						-

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Fig. 4A & B Box plot of the variations in the monthly average abundances of potentially MC-producing genera (A) and in MC-RR concentrations (B) recorded at the six sampling stations in Lagoon Aghien

333 **Spatial and temporal variations in intracellular cyanotoxin concentrations**

334 During the survey, intracellular STX, ATX, HTX, NOD and CYL were not detected. Two
335 MC congeners, MC-RR and MC-LR, were detected throughout the year, but MC-LR
336 concentrations were always under the limits of quantification.

337 MC-RR was detected in cyanobacterial cells during all monitoring (Figure 5).
338 The MC-RR concentrations ranged from 0 to 0.364 $\mu\text{g L}^{-1}$ (mean value during the
339 monitoring = 0.08 $\mu\text{g L}^{-1}$) and were not correlated with the total abundances of
340 potentially MC toxigenic cyanobacteria ($R^2=0.01$). There was no correlation between
341 MC-RR concentrations and the cell abundances of each of the nine genera potentially
342 able to produce MC. Finally, the MLR analysis performed by using MC concentration
343 as the dependent variable and cell abundance of each potentially MC-toxigenic
344 cyanobacteria as the independent variables, showed that MC-RR concentrations were
345 positively affected by *Microcystis* cell abundance (cell mL^{-1}) and negatively affected by
346 *Oscillatoria* cell abundance (cell mL^{-1}) ($p<0.05$). The regression model obtained by
347 MLR was: $\text{MC-RR} = 0.072 + 1.22 \cdot 10^{-6} (\text{Microcystis}) - 2.99 \cdot 10^{-6} (\text{Oscillatoria})$.

348 The comparison of these MC-RR concentrations at the six sampling sites shows
349 that these concentrations were significantly lower at station 6 (median concentration =
350 0.000 $\mu\text{g L}^{-1}$, respectively) than at the other stations (median concentrations between
351 0.055 and 0.079 $\mu\text{g L}^{-1}$) (Fig. 4B and Supplementary Table S3).

352
353 **Supplementary Table S3** p values of the paired sign tests performed on the
354 intracellular MC-RR concentrations recorded at the six sampling stations (S1 to S6)
355 during the monitoring of Lagoon Aghien (bold characters = significant p values)
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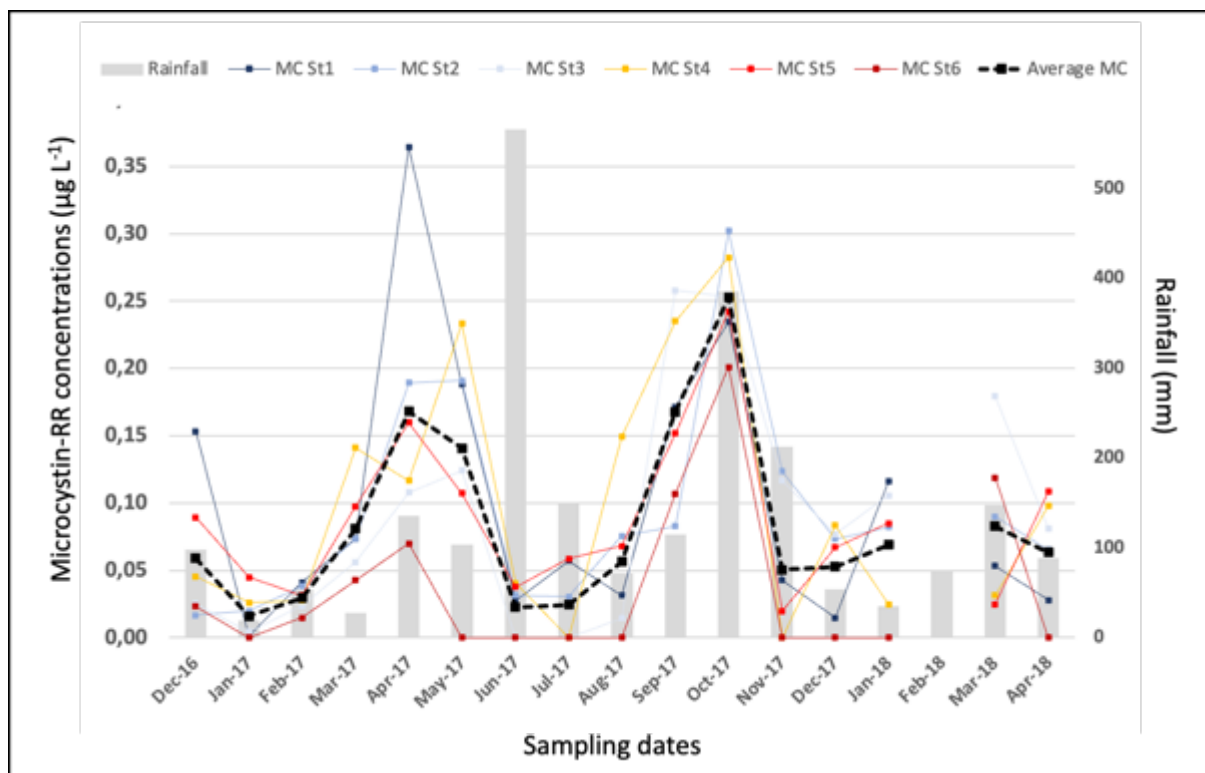
Stations	St1	St2	St3	St4	St5	St6
St1	-	0.80	0.80	0.80	0.80	0.00
St2		-	0.46	0.80	0.80	0.02
St3			-	0.12	0.80	0.00
St4				-	0.80	0.00
St5					-	0.00
St6						-

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359 The monthly variations in MC-RR concentrations were broadly similar at all
 360 sampling stations, with a first peak in April-May 2017, followed by a sharp decrease in
 361 June, and then a second peak in September-October 2017, followed by a sharp
 362 decrease in November 2017 (Fig. 5). These two peaks in MC-RR concentration
 363 followed an increase in nitrate concentrations in the lagoon (Supplementary Fig. S4).

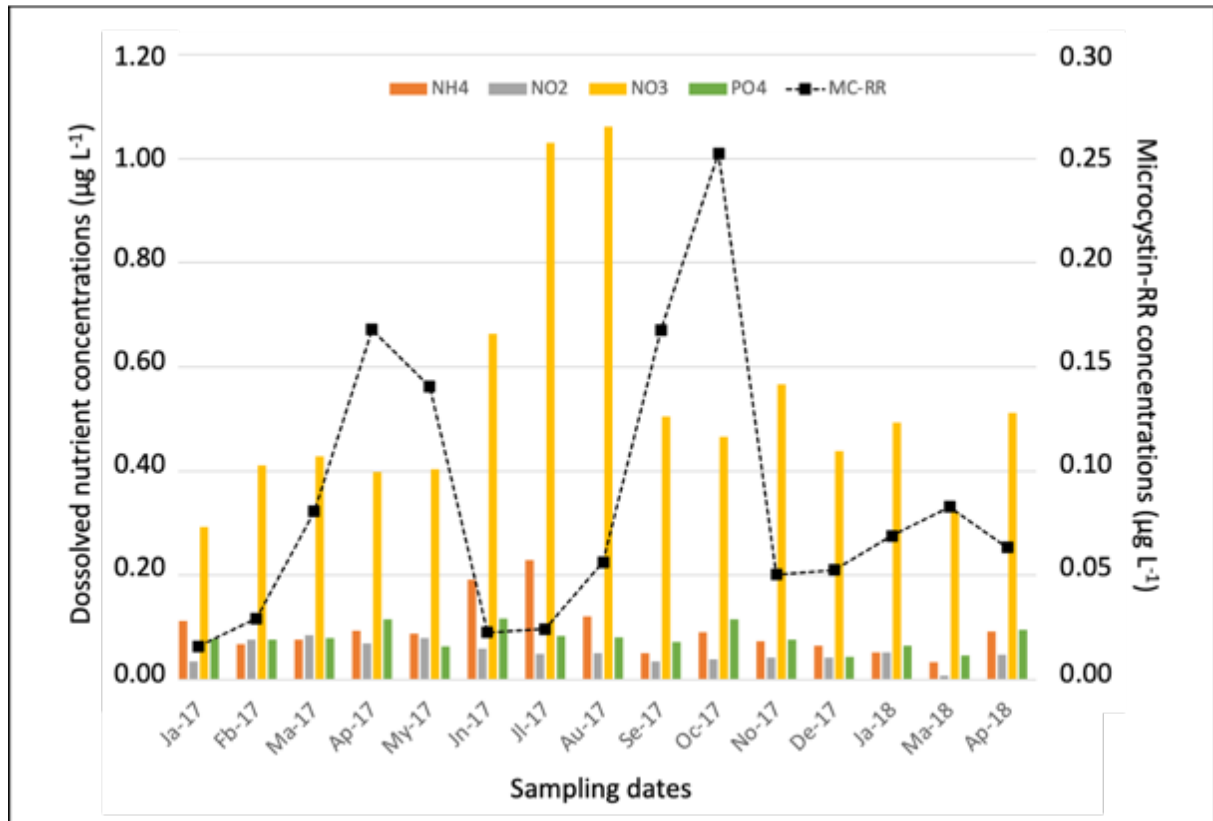
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366 **Fig. 5.** Variations in the intracellular microcystin-RR concentrations at the six sampling
 367 stations and of rainfall values during the monitoring of Lagoon Aghien (no value in
 368 February 2018). MC St1-6= Microcystin concentrations at sampling stations 1 to 6.
 369 Average MC = Average MC concentrations at the six sampling stations.

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Supplementary Figure S4. Variations in the intracellular microcystin-RR and dissolved nutrient average concentrations during the monitoring of Lagoon Aghien (no value in February 2018)

378 Temporal variations in microcystin concentrations in fish tissues

379 No STX, ATX, HTX, NOD or CYL were detected in any of the fish tissues analyzed,
380 while MC-RR and MC-LR were frequently detected in the intestines and livers of all
381 fish species but never in muscle tissues (Table 1). MC-RR and/or MC-LR were
382 quantified in 32 of the 97 intestines analyzed (33%) and in 18 of the 61 livers analyzed
383 (30%) (Table 1).

384 Among the 61 fish for which MC concentrations were quantified in liver and/or
385 in intestine, MC-RR/LR were quantifiable (i) both in intestine and liver in 8% of the fish
386 for which intestines and liver were analyzed, (ii) only in liver for 28% and (iii) only in
387 intestine for 23% of them (Table 1). Total MC concentrations (MC-RR + MC-LR)

388 ranged between 32 and 1,092 $\mu\text{g kg}^{-1}$ FW in the intestines and between 33 and 383
389 $\mu\text{g kg}^{-1}$ FW in the livers (Table 1).

390 As shown in Table 2, MC was found in a higher proportion of the intestines in
391 phytoplanktivorous fish (56 and 67% for *O. niloticus* and *S. melanotheron*, respectively)
392 than in other dietary regimes (from 9 to 39% for all the other fish species). Moreover,
393 there was a significant difference (Sign test, $p < 0.05$) in the monthly averages of
394 intestine MC concentrations of phytoplanktivorous-omnivorous fish and
395 zooplanktivorous-carnivorous fish, with these concentrations being higher in the
396 intestines of phytoplanktivorous-omnivorous fish. There were no significant differences
397 (Kruskal–Wallis test) in the MC concentrations estimated in the livers of *T. guineensis*
398 (omnivorous), *S. melanotheron* (phytoplanktivorous) and *C. nigrodigitatus*
399 (zooplanktivorous).

400

401 **Table 2** The proportions of the intestines and livers containing microcystins in the six
402 fish species sampled in Lagoon Aghien

403 C = carnivorous fish; Z = zooplanktivorous fish; P = phytoplanktivorous fish; O = omnivorous
404 fish

405

	Intestine	Liver
<i>Chrysichthys nigrodigitatus</i> (Z)	3/34 (9%)	5/18 (28%)
<i>Hemichromis fasciatus</i> (C)	1/5 (20%)	0/5 (0%)
<i>Hepsetus odoe</i> (C)	1/3 (33%)	0/3 (0%)
<i>Tilapia guineensis</i> (O)	13/33 (39%)	5/18 (28%)
<i>Sarotherodon melanotheron</i> (P)	9/16 (56%)	7/11 (64%)
<i>Oreochromis niloticus</i> (P)	4/6 (67%)	1/6 (17%)

406

407

408 **Discussion**

409 *Cyanobacterial community composition and dynamics*

410 Fifteen cyanobacterial genera were observed during the survey of Lagoon Aghien,
411 which is within the same range as data obtained in other tropical freshwater
412 ecosystems, such as those reported by Olokotum et al. (2022), who identified 16
413 cyanobacterial genera in two embayments of Lake Victoria (Uganda), and by Dalu and
414 Wasserman (2018), who identified 13 cyanobacterial genera in a reservoir in
415 Zimbabwe. In contrast with temperate areas, few variations in the richness of the
416 cyanobacterial community were observed during the Lagoon Aghien survey, probably
417 because of the temporal stability of the water temperature of the lagoon (between 26.3
418 °C in July 2017 and 31.6 °C in March 2017; Ahoutou et al. 2021). Studies performed
419 in temperate areas have shown that the decrease in water temperature is one of the
420 main factors associated with a decrease in richness in phytoplankton communities
421 (e.g., Catherine et al. 2016; Maberly et al. 2022).

422 Among these 15 genera, four were dominant: *Raphidiopsis* (formerly
423 *Cylindrospermopsis*), *Microcystis*, *Oscillatoria* and *Limnothrix*. The first three genera
424 are the most frequently reported in studies performed in Sub-Saharan Africa (Svircev
425 et al.2019), while *Limnothrix* is mainly found in shallow and eutrophic lakes of
426 temperate areas (e.g., Rucker et al. 1997; Wiedner et al. 2002), even if it is also able
427 to proliferate in tropical ecosystems, as shown in Brazil by Soares et al. (2009). The
428 highest cyanobacterial abundances were generally associated with the genus
429 *Raphidiopsis*, which is known to have high fitness at tropical latitudes and to be able
430 to outcompete other cyanobacterial genera under these environmental conditions
431 (e.g., Burford et al. 2016; Jia et al. 2020). Finally, we found that the variations in total
432 cyanobacterial abundances were negatively correlated with rainfall, as previously

433 observed in other tropical freshwater ecosystems, this negative relationship being
434 probably explained by the water dilution of cyanobacteria combined with high flushing
435 rates (e.g., Barros et al. 2017; Giani et al. 2020). As shown by a canonical correspondence
436 analysis performed by Ahoutou et al. (2021), only 22% of the variation in biovolume values of
437 the dominant phytoplankton genera (including *Raphidiopsis* and *Limnothrix*) in the Lagoon
438 Aghien phytoplankton community could be explained by variations in environmental variables.

439

440 *Toxin production*

441 In Lagoon Aghien, 12 of the 15 cyanobacteria genera observed can potentially
442 produce ATX, HTX, CYL, MC and/or STX (ANSES 2020), but only MC was found in
443 the phytoplankton biomass. These data are in agreement with those reported in the
444 reviews of Mowe et al. (2015) and Svircev et al. (2019), showing that MC are the most
445 frequent cyanotoxins on the African continent during cyanobacterial blooms. Despite
446 the high abundances of the genus *Raphidiopsis*, known to be a potential producer of
447 CYL and/or STX (Vico et al. 2020), these two cyanotoxins were not found in Lagoon
448 Aghien water. In two papers dealing with the production of CYL in Sub-Saharan Africa,
449 all the *Raphidiopsis* strains isolated in Senegal and Uganda were shown to lack the
450 genes involved in the biosynthesis of this toxin (Berger et al. 2006; Haande et al. 2008).
451 These data should also be considered in light of the recent study of Vico et al. (2020),
452 which suggested by genomic and phylogenetic analyses that central Africa was the
453 original dispersion center of *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* and that
454 these African populations were not able to produce CYL.

455 The intracellular MC concentrations in the water were quite low, always $<0.4 \mu\text{g}$
456 $\text{equi. MC-LR eq L}^{-1}$ and were not correlated with the total abundances of
457 cyanobacterial genera known to be able to produce MC or with the abundance of each
458 of these genera. On the other hand, our MLR analysis showed that MC-RR

459 concentrations were negatively affected by *Oscillatoria* cell abundances, but positively
460 affected by *Microcystis* cell abundances. This finding is consistent with the fact that
461 this genus is considered to be the major MC-producing genus in Africa (see reviews of
462 Mowe et al. 2015; Chia et al. 2022). It is known that the variations in MC concentration
463 are partly explained by the variations in the proportions of MC-producing (MC+) and
464 non-MC-producing (MC-) strains in *Microcystis* populations (e.g., Briand et al. 2009;
465 Suominen et al. 2017). Their respective proportions were not estimated in our study,
466 and few data are available in Africa on this topic. Haande et al. (2007) found that only
467 four of 24 *M. aeruginosa* isolates were able to produce MC, and Ballot et al. (2014)
468 found that only one of 16 *M. aeruginosa* isolates was able to produce MC. These first
469 data are interesting to consider, but knowing that there is very high variability in
470 Europe/North America in the variations of MC+/MC- proportions during a bloom event
471 or from one bloom to another (e.g., Briand et al. 2009; Sabart et al. 2009; Bozarth et
472 al. 2010), much more data are necessary to get a real idea of the variability of these
473 proportions in cyanobacterial populations in Sub-Saharan Africa.

474 The average abundances of potentially MC-producing cyanobacteria and of MC
475 concentrations in water were lower at sampling Station 6. As shown by Ahoutou et al.
476 (2022), the values of some physico-chemical parameters, such as turbidity and
477 phytoplankton community composition and biomass, were significantly different at
478 Station 6, probably because this station is placed under the direct influence of the Mé
479 river and Potou channel (Koffi et al. 2019).

480 Another point to consider for explaining these low MC concentrations in Lagoon
481 Aghien concerns nitrogen availability. In their review, Mowe et al. (2015) reported a
482 positive correlation between N:P ratio values and MC concentrations from data
483 collected in several tropical lakes. It is also well known that an increase in N supply

484 leads to an increase in MC cell quotas and concentrations (e.g., Chaffin et al. 2018;
485 Horst et al. 2014; Wagner et al. 2021). During this monitoring of Lagoon Aghien, the
486 N:P ratio values ranged between 7 and 14 (Ahoutou et al. 2020), which corresponds
487 to the low N:P ratio and low microcystin concentrations reported by Mowe et al. (2015).
488 It is interesting to note that in the present study the two MC peaks followed an increase
489 in nitrate concentration, suggesting a possible link between these events. Moreover,
490 during mesocosm experiments performed in Lagoon Aghien (Ahoutou et al. 2022), we
491 found that the addition of nitrogen, alone or combined with phosphorus in the
492 mesocosms filled with the water of the lagoon, stimulated MC production by
493 cyanobacteria. Taken together, these results are interesting to consider in light of the
494 data provided by Chianu et al. (2012) showing that nitrogen fertilizer consumption is
495 low in African farming systems compared to northern countries. This suggests that an
496 increase in the use of nitrogen fertilizers on this continent might lead to increasing MC
497 concentrations in African freshwater ecosystems in the future.

498 Few data are available on fish contamination by MC in Africa (Abdallah et al.
499 2021, Roegner et al. 2023), but our data are interesting to compare with those of
500 Semyalo et al. (2010), who dealt with the MC contamination of Nile tilapia (*O. niloticus*)
501 in two Ugandan lakes (Lake Mburo and Lake Victoria). The MC concentrations in the
502 cyanobacterial biomass in Lake Mburo ranged between 0.006 and 0.26 $\mu\text{g eq. MC-LR}$
503 L^{-1} and were close to those recorded in Lagoon Aghien (between 0.02 and 0.25 $\mu\text{g MC}$
504 L^{-1}). These MC concentrations were higher in Murchison Bay (Lake Victoria), with
505 values ranging between 0.2 and 0.7 $\mu\text{g eq. MC-LR L}^{-1}$, depending on the season. In
506 the fish intestines, the highest MC concentrations recorded in the *O. niloticus* sampled
507 in the two Ugandan lakes were >300 and $>390 \mu\text{g.kg}^{-1}$ FW in Lake Mburo and
508 Murchison Bay, respectively, whereas the highest MC concentration recorded in

509 Lagoon Aghien was 1,092 $\mu\text{g.kg}^{-1}$ FW when considering all the fish and 371 $\mu\text{g.kg}^{-1}$
510 FW when considering only *O. niloticus*. MC was most frequently found in the intestine
511 and liver of phytoplanktivorous fish, consistent with the consumption and presence of
512 cyanobacterial cells in the fish intestine and subsequent accumulation in the liver, the
513 target organ of MCs. An interesting point was that in carnivorous fish (*H. fasciatus*, *H.*
514 *odoe*), MC was only quantified in the liver and not in the intestine. This is probably
515 related to their mode of intoxication via contaminated prey (no direct ingestion of
516 cyanobacterial cells), the short length of their intestines and their rapid digestion
517 time, allowing MC to be rapidly assimilated after prey digestion and directed to the liver
518 for accumulation and metabolism. Such differences in MC accumulation in the gut and
519 liver of phytoplanktivorous versus carnivorous fish have been previously reported by
520 Ibeling et al. (2005), Zhang et al. (2009) and Nyakairu et al. (2010).

521 Although similar MC concentrations were found in the fish intestines in Uganda
522 and Ivory Coast, no MC was found in the fish muscle tissues in Lagoon Aghien, while
523 low concentrations ($<6 \mu\text{g.kg}^{-1}$ FW) were found in these tissues in fish from Mburo and
524 Victoria Lakes. In the same way, MC concentrations ranging between 0.5 and 1,917
525 $\mu\text{g.kg}^{-1}$ FW were found in the muscle tissue of various fish species (including *O.*
526 *niloticus*) collected in several Ugandan lakes by Poste et al. (2011), and some of these
527 MC concentrations exceeded the tolerable daily intake guideline defined by the WHO
528 for chronic exposure to MC. More recently, Roegner et al. (2023) found in the Winam
529 Gulf (Lake Victoria, Kenya) that the hazard quotients for fish consumed by young
530 children were 5 to 10 times higher than the permissible levels.

531 This absence of MC in muscle tissues of fish collected in Lagoon Aghien is
532 intriguing knowing that similar MC concentrations in water and in fish intestine were
533 found in this lagoon and in two Ugandan lakes (Semyalo et al. 2010), where MC was

534 found in muscle tissues. High MC concentrations in fish muscle have been frequently
535 estimated by ELISA and this method may overestimate these MC concentrations (see
536 Ibelings et al. 2021). This could partly explain the lack of MC detection in the muscle
537 tissue of fish from Lagoon Aghien, but the same analytical approach (LC–MS/MS) as
538 ours was used in the study of Semyalo et al. (2010), where MC was found in fish
539 tissues. However, in our study, our LOQ values were quite high (12.8 $\mu\text{g kg}^{-1}$ FW for
540 MC-RR and 4.8 $\mu\text{g kg}^{-1}$ FW for MC-LR), while the LOQ was not provided by Semyalo
541 et al. (2010). Consequently, the existence of moderate MC contamination in the muscle
542 of fish in Lagoon Aghien cannot be ruled out. Furthermore, only free MC was quantified
543 in our study, whereas much of the accumulated MC may be covalently bound in animal
544 tissues requiring specific extraction protocols (Neffling et al. 2010, Bouteiller et al.
545 2021). Finally, as shown in the review paper of Martins and Vasconcelos (2009), fish
546 are efficient in detoxifying MC, depending on the species and on the environment. In
547 contrast with fish living in freshwater ecosystems from temperate areas, which are
548 exposed to MC for only a few months, we showed that the fish community of Lagoon
549 Aghien is contaminated throughout the year by MC, which could have led this
550 community to develop active processes for the detoxification of MC.

551 All these data on toxin production by cyanobacteria in Lagoon Aghien raise the
552 issue of health hazards for populations living around the lagoon. The WHO has
553 provided guideline values for lifetime and short-term exposure to MC-LR by drinking
554 water and for exposure by recreational activities (1, 12 and 24 $\mu\text{g L}^{-1}$, respectively)
555 (Chorus and Welker 2021). In France, more restrictive guideline values (0.1 $\mu\text{g L}^{-1}$ for
556 drinking water and 0.3 $\mu\text{g L}^{-1}$ for recreational activities) have been adopted because
557 the French Agency for Food, Environmental and Occupational Health and Safety

558 (ANSES) has taken into account the findings provided by Chen et al. (2001) showing
559 reprotoxic effects of MC-LR during chronic low-dose exposure of mice.

560 The WHO TDI for microcystin yields a threshold concentration of microcystin in
561 fish of 24 $\mu\text{g kg}^{-1}$ FW for a consumer weighing 60 kg and consuming 100 g of fish daily
562 (Poste et al. 2011). Knowing that no MC was found in the muscle of the six fish species
563 studied and even if we cannot exclude the presence of a low MC content in fish
564 muscles, it is likely that the exposure risk by fish consumption of human populations
565 living around Lagoon Aghien is probably limited. However, local populations consume
566 some small fish species with viscera (i.e., *Pellonulla leonesis*, which was not sampled
567 in this study), which could potentially contribute to their exposure to MC, since MC
568 were found in the intestines of all the species targeted in this study.

569 Taking into account all these guidelines, human populations living around the
570 lagoon seem to be moderately exposed to adverse health effects of MC because MC
571 concentrations in water and fish are generally below the WHO thresholds. However, in
572 agreement with Roegner et al. (2020), we must question the relevancy of these
573 guideline values, which have been established in developed countries where water
574 uses are very different and have consequences on the cyanotoxin exposure level. For
575 example, around Lagoon Aghien, children play for several hours every day of the first
576 twelve years of their life in water continuously containing approximately 0.2 $\mu\text{g L}^{-1}$ of
577 MC, as we found, for example, between April 2017 and 2018. Nontreated lagoon water
578 is also used for meal preparation, to wash children since the first weeks of their life, to
579 wash dishes and clothes, etc. In addition, these populations eat fish from the lagoon
580 almost every day, with fish being the main source of protein for them (Ahi 2021; Effebi
581 et al. 2017). Daily exposure to low MC concentrations by different exposure routes

582 over more than 10 years is not taken into account in the current guidelines, making
583 children in particular a potentially very vulnerable population.

584 Even if guidelines adapted to developing countries were provided by the WHO,
585 they would be far from being used in Sub-Saharan African countries, where there is
586 usually no regular monitoring of cyanobacteria and their toxins in freshwater
587 ecosystems. As observed for Lagoon Aghien and Lake Guiers (Senegal), which are
588 used for the production of drinking water for the cities of Abidjan and Dakar,
589 respectively, and face problems with cyanobacteria, the two main difficulties for the
590 implementation of long-term monitoring of these resources are (i) the competition
591 between national water institutions for the leadership of such a program and (ii) the
592 sustainable funding of this monitoring, none of the ministries concerned agreeing to
593 ensure it (Mitroi et al. 2022). Beyond the resolution of these governance difficulties,
594 another key point in Africa will also be to inform the local populations and make them
595 aware of the health hazards associated with cyanobacterial blooms. Finally, even if
596 guidelines adapted to the local water practices are provided and monitoring and
597 awareness of local populations are performed, the issue of the provision of alternative
598 resources (in water, proteins, etc.) will be the last key step needed to reduce the
599 exposure of these populations to MC.

600

601

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607

608

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854 **Statements and Declarations**

855 **Ethical Approval:** Not applicable.

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871 **Data availability.** All the data generated during this study are provided in
872 supplementary tables

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