

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

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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

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# **Abstract**

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In comparison with northern countries, limited data are available on the occurrence and potential toxicity of cyanobacterial blooms in lakes and ponds in Sub-Saharan countries. With the aim of enhancing our knowledge on cyanobacteria and their toxins in Africa, we performed a 17-month monitoring of a freshwater ecosystem, Lagoon Aghien (Ivory Coast), which is used for multiple practices by riverine populations and for drinking water production in Abidjan city. The richness and diversity of the cyanobacterial community were high and displayed few variations during the entire survey. The monthly average abundances ranged from 4.1x10<sup>4</sup> cell mL<sup>-1</sup> to 1.8x10<sup>5</sup> cell mL<sup>-1</sup>, with higher abundances recorded during the dry seasons. Among the five cyanotoxin families analyzed (anatoxin-a, cylindrospermopsin, homoanatoxin, microcystins, saxitoxin), only microcystins (MC) were detected with concentrations ranging from 0 to 0.364 µg L<sup>-1</sup> in phytoplankton cells, from 32 to 1,092 µg fresh weight (FW) kg<sup>-1</sup> in fish intestines and from 33 to 383 µg FW kg<sup>-1</sup> in fish livers. Even if the MC concentrations in water and fish are low, usually below the thresholds defined in WHO guidelines, these data raise the issue of the relevance of these WHO guidelines for Sub-Saharan Africa, where local populations are exposed throughout the year to these toxins in multiple ways.

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## Key words

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

## Introduction

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

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

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

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

# **Material and Methods**

## Study site

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

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

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



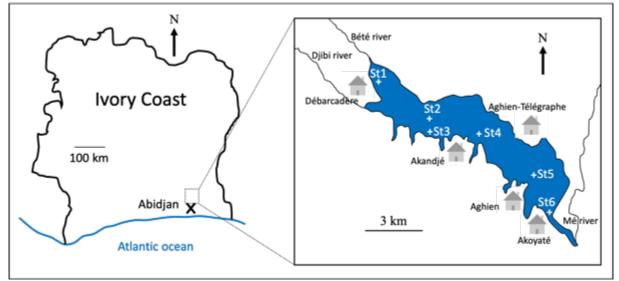


Fig. 1 Locations of the six sampling stations in Lagoon Aghien

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

# Sampling strategy of water

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

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

For cyanobacterial cell counting and identification, 200 mL of raw water was fixed in a formaldehyde solution (5% in final concentration). For the extraction of toxins from cyanobacterial cells, 200 mL of water was filtered through polycarbonate filters (Whatman® Nuclepore), which were stored at -80 °C.

# **Dissolved nutrient analyses**

All the nutrient analyses were performed following the instructions of the LCK cuvette test systems provided by of the manufacturer (Hach® Company). The preparation of samples was performed using LCK cuvette test systems LCK 304, 341, 339 and 349 for ammonium (NH<sub>4</sub>), nitrites (NO<sub>2</sub>), nitrates (NO<sub>3</sub>) and soluble reactive phosphorus (SRP) respectively, after the water samples were filtered through nylon membranes (Whatman<sup>TM</sup>, porosity 0.45 μm, diameter 47 mm). Colorimetric determinations of the concentrations were made using a HACH DR6000 UV VIS spectrophotometer.

# Cyanobacteria identification and counting

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

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

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

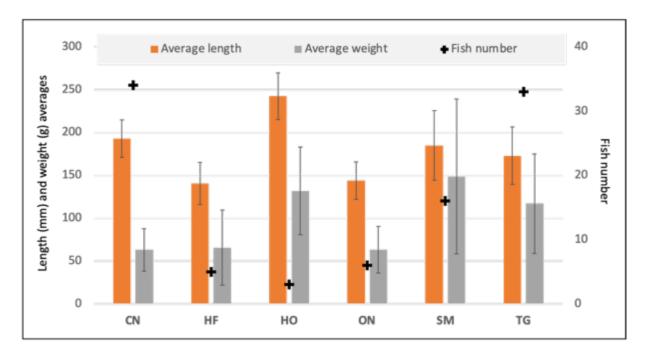
#### Fish

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

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

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





**Supplementary Fig. S1** Average length and weight (± standard deviation) and fish number in the six fish species collected in Lagoon Aghien CN = Chrysichthys nigrodigitatus; HF = Hemichromis fasciatus; HO = Hepsetus odoe; ON = Oreochromis niloticus; SM = Sarotherodon melanotheron; TG = Tilapia quineensis

**Table 1** Total lengths (TL), weights and microcystin (MC) -LR and -RR concentrations in intestine, liver and muscle of all the fishes collected in Lagoon Aghien Cn = Chrysichthys nigrodigitatus; Hf = Hemichromis fasciatus; Ho = Hepsetus odoe; Ho = Chrysichthys nigrodigitatus; Ho = Chrysichthys nigrodigita

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

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

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

# Cyanotoxin identification and quantification from phytoplankton cells and fish

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

For cyanotoxin extractions from the fish, the method of Manubolu et al. (2018) was used with minor adjustments. For each fish, 100 mg of fresh liver, muscle, and intestinal tissues were used for cyanotoxin extraction. The 100 mg muscle extracts were further previously freeze-dried and underwent ball milling. All samples were extracted twice with BuOH:MeOH:H<sub>2</sub>O (1:4:15), submitted to an ultrasonic probe (Sonics Vibra Cell, 130 Watt, 20 kHz, 60%, 30 sec, three cycles) and centrifuged (15 300 G, 10 min, 4 °C). The collected supernatants underwent final centrifugation (15 300 G, 10 min, 4 °C) and were stored at -80 °C until analysis.

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

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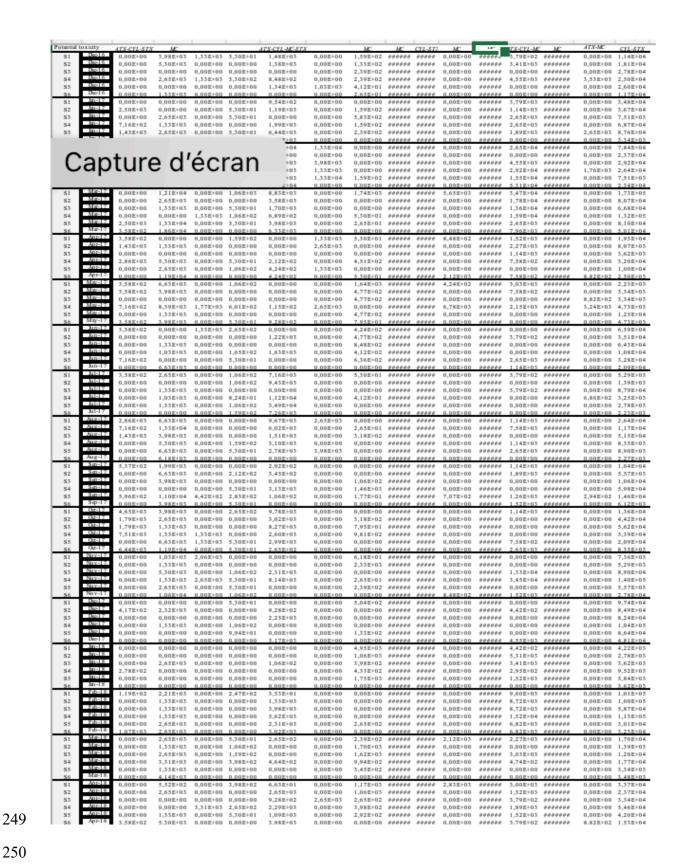
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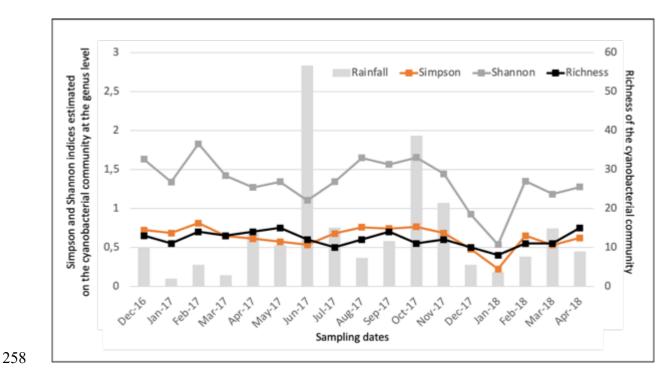
226 according to MC-LR equivalence, calculated from the regression curves of the MC-LR 227 analytical standard. 228 For phytoplankton, the LOD and LOQ were equal to 0.0003 and 0.001 µg L<sup>-1</sup> of raw water for MC-RR and 0.001 and 0.004 µg L<sup>-1</sup> for MC-LR, respectively. For fish 229 230 tissues, the LOD and LOQ were equal to 1.3 and 12.8 µg kg<sup>-1</sup> fresh weight (FW) for MC-RR and 0.5 and 4.8 µg kg<sup>-1</sup> for MC-LR, respectively. 231 232 233 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: Aphanizomenon, Aphanocapsa, Aphanothece, Chroococcus, Dolichospermum, 240 Gomphosphaeria, Leptolyngbya, Limnothrix, Lyngbya, Merismopedia, Microcystis, 241 242 Planktothrix, Pseudanabaena Oscillatoria, and Raphidiopsis (previously 243 Cylindrospermopsis) (Supplementary Table S1). 244 245 Supplementary Table S1 Cyanobacterial abundances (cell mL<sup>-1</sup>) and potential toxicity of the 15 cyanobacterial genera identified during the 17-month monitoring performed 246 247 at six sampling stations in Lagoon Aghien 248



Few variations were found in the richness and diversity (Shannon and Simpson indices) of the cyanobacterial community at the genus level during the 17-month monitoring of Lagoon Aghien (Fig. 2), except for the decrease in the two diversity

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





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

The total cyanobacterial abundances (sum of all genera) estimated during the monitoring survey ranged from 1.6x10<sup>4</sup> to 3.1x10<sup>5</sup> cell mL<sup>-1</sup> (Supplementary Table S1), and monthly average abundances ranged from 4.1x10<sup>4</sup> cell mL<sup>-1</sup> in July 2017 to 1.8x10<sup>5</sup> cell mL<sup>-1</sup> in March 2017. As shown in Figure 3, two main peaks of total cyanobacteria abundances were observed in February-March 2017 and February 2018. The four dominant cyanobacterial genera in terms of cellular abundance were *Raphidiopsis*, *Limnothrix*, *Microcystis* and *Oscillatoria*. The main peak abundances of

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

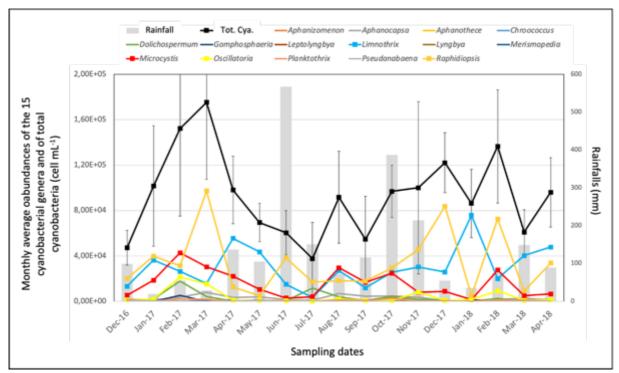
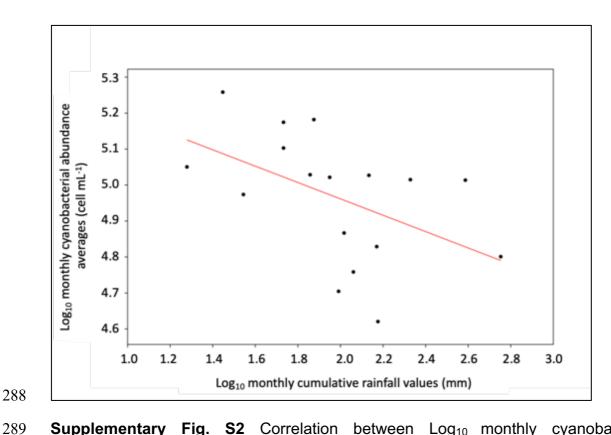


Fig. 3 Variations in the monthly average abundances of the total cyanobacteria abundances (black curve) and of each cyanobacterial genus abundance (colored curves) (mean values from the six sampling stations) and variations in the cumulative

monthly rainfall (gray bar chart). Standard deviations have only been added for the monthly averages of total cyanobacteria abundances. Total cya = Total cyanobacteria

abundance.

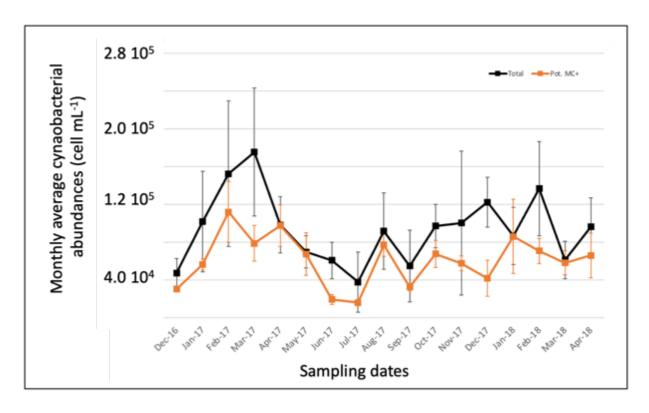
A negative correlation was found between monthly variations occurring in the monthly cumulative rainfall and those occurring in the total cyanobacterial abundance averages ( $r^2$ =0.24; ordinary least squares regression, p<0.05) (Supplementary Fig. S2).



**Supplementary Fig. S2** Correlation between Log<sub>10</sub> monthly cyanobacterial abundance averages and Log<sub>10</sub> monthly cumulative rainfall values

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

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

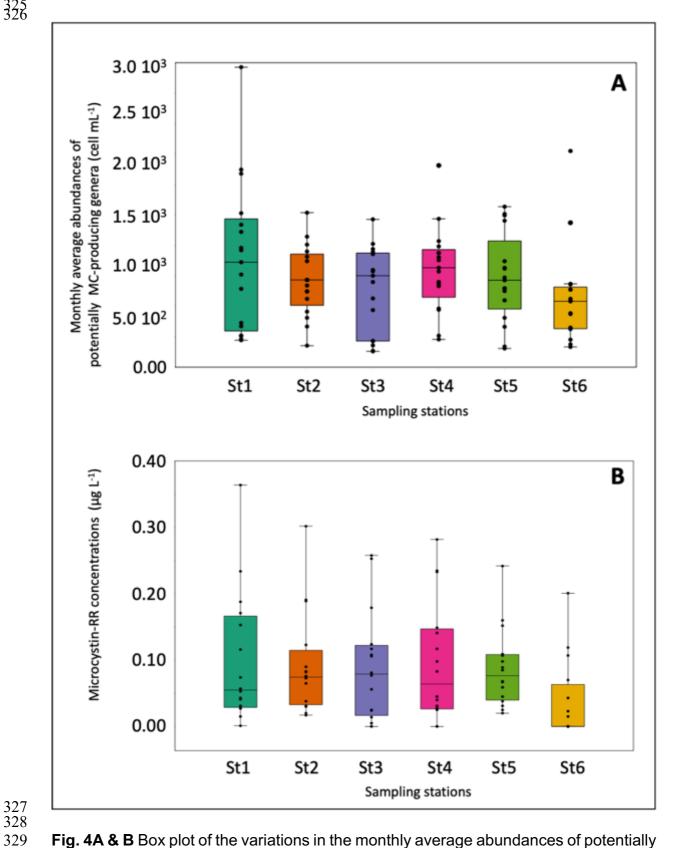


**Supplementary Fig. S3** Variations in the monthly average abundances of all cyanobacteria and of cyanobacteria potentially able to produce microcystins (mean values from the six sampling stations) during the monitoring of Lagoon Aghien Total = monthly average abundances of all cyanobacteria; Pot. MC+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

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

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

<b>Stations</b>	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						-



**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

Spatial and tempo	oral variations	in intracellular d	cvanotoxin	concentrations
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During the survey, intracellular STX, ATX, HTX, NOD and CYL were not detected. Two MC congeners, MC-RR and MC-LR, were detected throughout the year, but MC-LR concentrations were always under the limits of quantification.

MC-RR was detected in cyanobacterial cells during all monitoring (Figure 5). The MC-RR concentrations ranged from 0 to 0.364 μg L<sup>-1</sup> (mean value during the monitoring = 0.08 μg L<sup>-1</sup>) and were not correlated with the total abundances of potentially MC toxigenic cyanobacteria (R<sup>2</sup>=0.01). There was no correlation between MC-RR concentrations and the cell abundances of each of the nine genera potentially able to produce MC. Finally, the MLR analysis performed by using MC concentration as the dependent variable and cell abundance of each potentially MC-toxigenic cyanobacteria as the independent variables, showed that MC-RR concentrations were positively affected by *Microcystis* cell abundance (cell mL<sup>-1</sup>) and negatively affected by *Oscillatoria* cell abundance (cell mL<sup>-1</sup>) (p<0.05). The regression model obtained by MLR was: MC-RR= 0.072 +1.22 10<sup>-6</sup> (*Microcystis*) - 2.99 10<sup>-6</sup> (*Oscillatoria*).

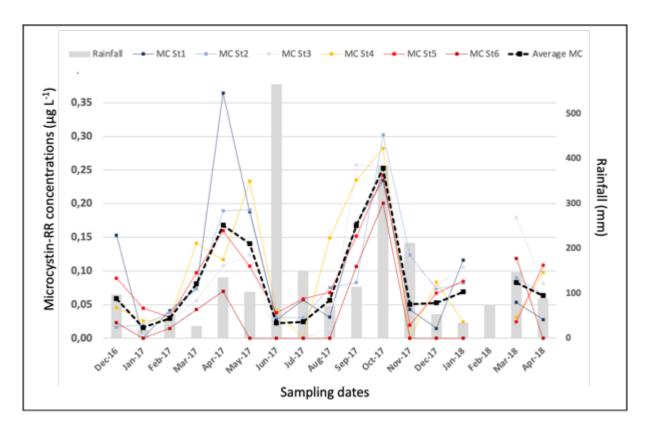
The comparison of these MC-RR concentrations at the six sampling sites shows that these concentrations were significantly lower at station 6 (median concentration =  $0.000 \mu g L-1$ , respectively) than at the other stations (median concentrations between 0.055 and  $0.079 \mu g L^{-1}$ ) (Fig. 4B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

**Supplementary Table S3** p values of the paired sign tests performed on the intracellular MC-RR concentrations recorded at the six sampling stations (S1 to S6) during the monitoring of Lagoon Aghien (bold characters = significant p values)

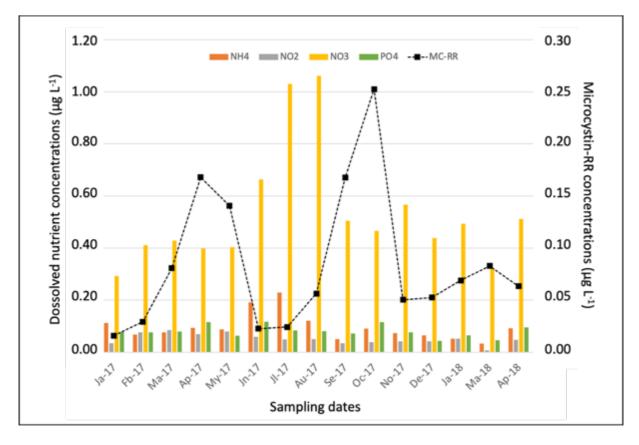
<b>Stations</b>	St1	St2	St3	St4	St5	St6
St1	-	0.80	0.80	0.80	0.80	0.00
St2		_	0.46	0.80	0.80	0.02
St3			-	0.12	0.80	0.00
St4				-	0.80	0.00
St5					-	0.00
St6						-

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





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



**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)

## Temporal variations in microcystin concentrations in fish tissues

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

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

ranged between 32 and 1,092  $\mu$ g kg<sup>-1</sup> FW in the intestines and between 33 and 383  $\mu$ g kg<sup>-1</sup> FW in the livers (Table 1).

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

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

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

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fish

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

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# **Discussion**

Cyanobacterial community composition and dynamics

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

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

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

# Toxin production

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

The intracellular MC concentrations in the water were quite low, always <0.4 µg equi. MC-LR eq L<sup>-1</sup> and were not correlated with the total abundances of cyanobacterial genera known to be able to produce MC or with the abundance of each of these genera. On the other hand, our MLR analysis showed that MC-RR

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

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

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

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

Few data are available on fish contamination by MC in Africa (Abdallah et al. 2021, Roegner et al. 2023), but our data are interesting to compare with those of Semyalo et al. (2010), who dealt with the MC contamination of Nile tilapia (*O. niloticus*) in two Ugandan lakes (Lake Mburo and Lake Victoria). The MC concentrations in the cyanobacterial biomass in Lake Mburo ranged between 0.006 and 0.26 μg eq. MC-LR L<sup>-1</sup> and were close to those recorded in Lagoon Aghien (between 0.02 and 0.25 μg MC L<sup>-1</sup>). These MC concentrations were higher in Murchison Bay (Lake Victoria), with values ranging between 0.2 and 0.7 μg equi. MC-LR L<sup>-1</sup>, depending on the season. In the fish intestines, the highest MC concentrations recorded in the *O. niloticus* sampled in the two Ugandan lakes were >300 and >390 μg.kg<sup>-1</sup> FW in Lake Mburo and Murchison Bay, respectively, whereas the highest MC concentration recorded in

Lagoon Aghien was 1,092 μg.kg<sup>-1</sup> FW when considering all the fish and 371 μg.kg<sup>-1</sup> FW when considering only *O. niloticus*. MC was most frequently found in the intestine and liver of phytoplanktivorous fish, consistent with the consumption and presence of cyanobacterial cells in the fish intestine and subsequent accumulation in the liver, the target organ of MCs. An interesting point was that in carnivorous fish (*H. fasciatus*, *H. odoe*), MC was only quantified in the liver and not in the intestine. This is probably related to their mode of intoxication via contaminated prey (no direct ingestion of cyanobacterial cells), the short length of their intestines and their rapid digestion time,allowing MC to be rapidly assimilated after prey digestion and directed to the liver for accumulation and metabolism. Such differences in MC accumulation in the gut and liver of phytoplanktivorous versus carnivorous fish have been previously reported by Ibeling et al. (2005), Zhang et al. (2009) and Nyakairu et al. (2010).

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

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

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

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All these data on toxin production by cyanobacteria in Lagoon Aghien raise the issue of health hazards for populations living around the lagoon. The WHO has provided guideline values for lifetime and short-term exposure to MC-LR by drinking water and for exposure by recreational activities (1, 12 and 24 µg L<sup>-1</sup>, respectively) (Chorus and Welker 2021). In France, more restrictive guideline values (0.1 µg L<sup>-1</sup> for drinking water and 0.3 µg L<sup>-1</sup> for recreational activities) have been adopted because the French Agency for Food, Environmental and Occupational Health and Safety

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

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

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

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

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

# Acknowledgments

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on previous versions of the manuscript. All authors read and approved the final manuscript.

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