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Article

Characterization of potential threats from cyanobacterial toxins in Lake Victoria embayments and during water treatment

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Abstract: Africa's water needs are often supported by eutrophic waterbodies dominated by cyanobacteria posing health threats to riparian populations from cyanotoxins, and Lake Victoria is no exception. In two embayments of the lake (Murchison Bay and Napoleon Gulf), cyanobacterial surveys were conducted to characterize the dynamics of cyanotoxins in lake water and water treatment plants. Forty-six cyanobacterial taxa were recorded and out of these 14 were considered potentially toxigenic (i.e., from the genera *Dolichospermum, Microcystis, Oscillatoria, Pseudanabaena* and *Raphidiopsis*). A higher concentration (ranging from 5-10 µg MC-LR equiv. L^{-1}) of microcystins (MC) was detected in Murchison Bay compared to Napoleon Gulf, with a declining gradient from the inshore (max. 15 µg MC-LR equiv. L^{-1}) to the open lake. In Murchison Bay, an increase either in *Microcystis* sp. biovolume and MC was observed over the last two decades. Despite high cell densities of toxigenic *Microcystis* and high MC concentrations, the water treatment plant in Murchison Bay efficiently removed the cyanobacterial biomass, intracellular and dissolved MC to below the lifetime guideline value for exposure via drinking water (< 1.0 µg MC-LR equiv. L^{-1}). Thus, the potential health threats stem from the consumption of untreated water and recreational activities, along the shores of the lake embayments. MC concentrations were predicted from *Microcystis* cell numbers regulated by environmental factors such as solar radiation, wind speed in the N-S direction and turbidity. Thus, an early warning through microscopical counting of *Microcystis* cell numbers is proposed to better manage health risks from toxigenic cyanobacteria in Lake Victoria.

Keywords: Drinking water; rapid sand filtration; Recreational areas; Exposure; *Microcystis* and *Dolichospermum*; Microcystins.

Key Contribution: This study reports microcystins (MC) concentrations in lake water and during water treatment for two contrasting embayments of Lake Victoria in Uganda. Despite higher MC concentrations in raw water, MC was effectively removed by the water treatment plant.

1. Introduction

For decades, the increase in cyanobacterial dominance and regime shifts in freshwater ecosystems has been linked to a multitude of increasing human activities in the catchment resulting in greater nutrient pollution pressures [1]. Moreover, this dominance has been predicted to increase globally because of climate change [2]. The changes will be system-specific, depending on the mean depth of the mixed water layer, the light penetration and light absorption characteristics specific to the cyanobacterial taxa [3]. The cyanobacterial blooms have attracted attention due to their impact on the aquatic ecosystem services including water supply, fish food, recreational activities [4] and the health threats associated with cyanotoxins [5].

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Lake Victoria, the second-largest freshwater lake in the world, is a major source of water for the riparian populations and supports one of the largest freshwater fisheries in the world (1 million tons of fish annually) but increasing pollution has threatened it through widespread eutrophication [6]. After the occurrence of fish kills in Nyanza Gulf that were attributed to suspended algae and detritus coupled with low dissolved oxygen [7], several studies have focused on phytoplankton dynamics, cyanobacteria and cyanotoxins in the open lake and the embayments (for review, see [8]). Most of the studies on cyanotoxins in Lake Victoria have detected microcystins (MC), produced by dominant cyanobacteria of this lake, most importantly *Microcystis* spp. and, possibly *Dolichospermum* spp. An earlier study by Kotut et al. [9] conducted during a *Dolichospermum* bloom, examined water samples for potential anatoxins (ATX), but these toxins were undetectable in both environmental samples and isolated strains. More recently, very low concentrations of cylindrospermopsins (CYN) and nodularins (NOD) have been reported in the southern part of Lake Victoria [10].

Since the first report of MC occurrence in Nyanza Gulf (Kenya) at a concentration < 1 µg MC-LR equiv. L^{-1} [11], several studies have reported higher MC concentrations across the embayments of Lake Victoria. For instance, from the inner part of Nyanza Gulf a maximum of 274 (133) µg MC-LR equiv. L^{-1} has been reported from surface samples dominated by *Microcystis* spp. In patches formed in the open water even up to 2 mg MC-LR equiv. L^{-1} has been reported, probably through concentrating buoyant *Microcystis* colonies along Langmuir spirals [12]. In general, high spatial variability has been observed and MC concentration did not exceed 5 µg MC-LR equiv. L^{-1} in most of the embayments, i.e., < 2 µg MC-LR equiv. L^{-1} in the Napoleon Gulf and Murchison Bay [8,13] and < 4 µg MC-LR equiv. L^{-1} in closed bays in Tanzania [8,14]. Therefore, these studies have shown that the Lake Victoria riparian populations are exposed to variable levels of toxigenic cyanobacteria and MCs.

The embayments of Lake Victoria serve as the source of water and sources of abstraction for most water treatment facilities [8]. Therefore, the location and technology of the water intake, as well as the efficiency of the flocculation-clarification, rapid sand filtration, and chlorination treatment, are important factors in the water treatment process [4]. The major exposure routes for cyanotoxins to lakeside populations include drinking and other domestic use (both treated and untreated water), eating contaminated fish and food supplements, and recreational activities [4]. The dangers of cyanotoxins originate from both cell-bound and free-dissolved toxins [4,15]. Cyanotoxins such as MC, NOD, CYN and ATX which are cell-bound can be released into the water upon cyanobacterial cell lysis [16]. For example, up to 90% of the CYNs were found dissolved in water in temperate lakes dominated by *Aphanizomenon* sp. [17]. Thus, it is generally accepted that dissolved cyanotoxins are the main threat to humans through the consumption of untreated water for domestic use or the accidental consumption of water during recreation [5]. These exposure routes have been largely studied in the Northern hemisphere of the globe where national regulations to prevent health risks from cyanotoxins have been established, which contrasts with the south [18]. Currently, populations in the East African countries with access to Lake Victoria lack community awareness of the dangers of exposure to cyanotoxins, and, have no regular monitoring or regulations mitigating the effects of cyanotoxins from treated or untreated water.

In the present work, we characterized the conditions and relative risks associated with toxigenic cyanobacteria and their toxins for human populations for a one-year period (Nov 2017 – Oct 2018) from lake water (raw water) at two embayments of Lake Victoria in Uganda, Murchison Bay (MB) and Napoleon Gulf (NG), and during water treatment (Nov 2016 – Jan 2017). The two embayments differ in eutrophication status, with higher nutrient concentrations in MB than in NG, and show contrasting abundances of the dominant toxigenic cyanobacteria: *Microcystis aeruginosa* and *M. flos-aquae* in MB and *Dolichospermum circinale* and *Planktolyngbya circumcreta* in NG [19]. From the point of view of sanitation, no studies have been done on both the source of cyanotoxin exposure (dynamics in lake water and removal during water treatment) and the associated health hazards. We monitored the dynamics and the spatial variability of toxigenic cyanobacteria and cyanotoxins in lake water from the inshore to the abstraction point of the water treatment plants (WTPs) as well as in the open lake. We also determined the removal of MC after water treatment in two WTPs, one in MB and one in NG, supplying treated water for the cities Kampala and Jinja, respectively. In considering extracellular MC, we also measured the intracellular MC level and the potential cyanobacterial cell lysis during the water treatment process. Afterwards, the potential risks associated with the toxigenic cyanobacteria and their toxins for human populations using raw and treated water from these two embayments have been discussed.

2. Results

2.1. Toxigenic vs *non-toxigenic cyanobacteria diversity and biovolume*

From the data of both surveys, up to 46 cyanobacteria taxa were recorded with 14 potentially toxigenic taxa belonging to five genera: *Dolichospermum, Microcystis, Oscillatoria, Pseudanabaena*, and *Raphidiopsis* (previously *Cylindrospermopsis*). Most of the cyanobacterial species identified so far were considered non-toxigenic (27 taxa from MB and 26 from NG: **Figure 1**). Comparing the two embayments, a higher

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cyanobacterial biovolume was observed in MB (0.9 × 10^{-4} to 45.0 mm^3 L^{-1}; mean ± SD = 2 ± 4.2 mm^3 L^{-1}) than NG (0.4 × 10^{-4} to 22 mm^3 L^{-1}; mean ± SD = 0.5 ± 1.4 mm^3 L^{-1}; Mann-Whitney test, p<0.0001). In MB, higher cyanobacterial biovolumes were recorded from inshore samplings than in the water treatment abstraction point and open lake stations (Friedman X^2 = 4987.8, p< 0.00001 with the Nemenyi post hoc test). However, in NG no significant differences in the cyanobacteria biovolume were observed from the inshore to the open lake stations (repeated ANOVA, F = 0.618, p = 0.603; **Figure 1**).

In MB, the most dominant toxigenic cyanobacteria belonged to the genus *Microcystis* with *M. aeruginosa* (0.5 – 31.4 mm^3 L^{-1}, mean ± SD = 7.3 ± 6.7 mm^3 L^{-1}; 18.7% of the total cyanobacteria biovolume) and *M. flos-aquae* (0.4 – 30 mm^3 L^{-1}, mean ± SD = 6.6 ± 6.7 mm^3 L^{-1}; 17.9% of the total cyanobacteria biovolume). In MB, the highest biovolume of toxigenic cyanobacteria was recorded from the inshore and recreational stations (0.6 - 28.2 and 1.2 - 29.8 mm^3 L^{-1}, respectively). The non-toxigenic cyanobacteria made the most significant contribution to the cyanobacterial biovolume (56.4%) ranging from 8.9 ×10^{-5}-45 mm^3 L^{-1}, mean ± SD = 1 ± 3 mm^3 L^{-1}, dominated by *Chroococcus turgidus* in the recreational area (**Figure 1**). Temporal dynamics of the most abundant toxigenic cyanobacteria in MB were dominated by *Microcystis* spp. at the inshore and recreational sites (with the highest biovolume observed in September (inshore station) and November (recreational station) (**Figure S1**).

In NG, the dominant potentially toxigenic cyanobacterium was *D. circinale* at 0.1 – 8.6 mm^3 L^{-1}, mean ± SD = 2.3± 1.9 mm^3 L^{-1} (36% of the total cyanobacteria biovolume) followed by *M. flos-aquae* 0.3 – 2.9 mm^3 L^{-1}, 0.7 ± 0.6 mm^3 L^{-1} (6.1 % of the total cyanobacteria biovolume). The toxigenic cyanobacterium *Oscillatoria tenuis* (22 mm^3 L^{-1}) was detected once with high frequency in November 2017 (62.3% of the total cyanobacteria biovolume) at the inshore station. The non-toxigenic cyanobacterial biovolume ranged from 0.4 × 10^{-4} to 5.5; 0.3 ± 0.7 mm^3 L^{-1} (47% of the total cyanobacteria biovolume) dominated by *Planktolyngbya circumcreta* (0.5 to 5.5; 2 ± 1.3 mm^3 L^{-1}; 72% of the non-toxigenic cyanobacteria biovolume) in the recreational station (**Figure 1 and Figure S1**).

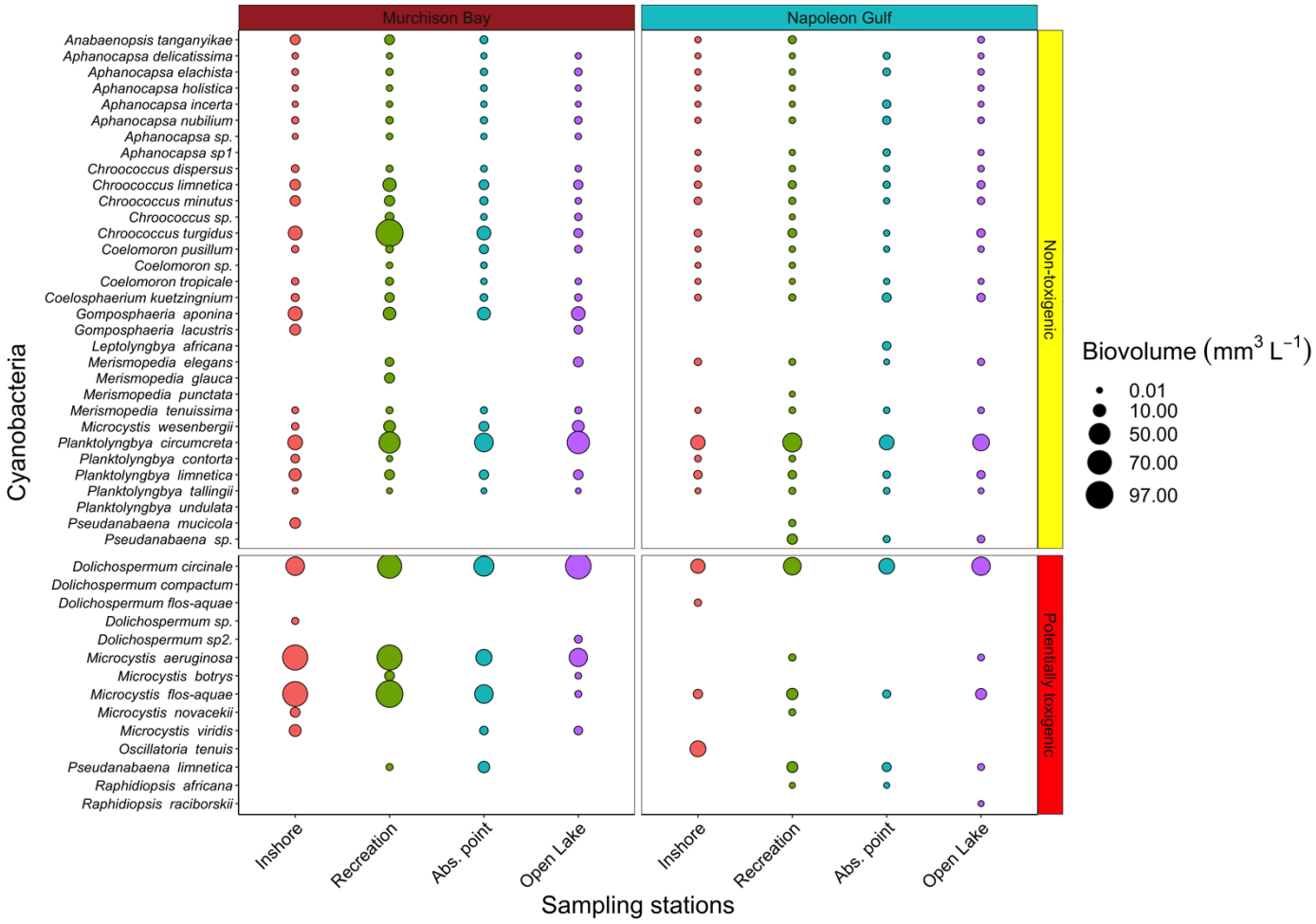

Figure 1. Potentially toxigenic and non-toxigenic cyanobacteria recovered from the lake survey in the sampling stations: i) inshore, ii) recreational area, iii) water treatment abstraction point (Abs. point), iv) open lake, in Murchison Bay and Napoleon Gulf. Among the 46 cyanobacteria species, 14 were considered potentially toxigenic. Circle sizes are proportional to the total biovolume (mm^3 L^{-1}) of each taxon. (Data collected between Nov 2017 - Oct 2018, n=120).

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Since our survey on MC concentrations during water treatment (see below) was performed for three months (Nov 2016-Jan 2017) only, seasonal variation in cyanobacteria biovolume in raw water could not be considered. Thus, it became important to investigate seasonality of cyanobacteria community composition for a longer period. Comparing the water treatment abstraction points and samples from the WTPs for both study periods, no significant differences were observed between the two surveys for toxigenic cyanobacteria biovolumes (t-test, $p = 0.04$; **Figure S2**). Fifteen cyanobacterial taxa were found including three potentially toxigenic taxa (*M. aeruginosa, M. flos-aquae* and *Dolichospermum* sp.) and 12 non-toxigenic cyanobacteria (**Figure 2**). The taxonomic composition recorded during the two study periods was similar (see **Figures 1 and 2**). The water treatment abstraction point of MB-Gaba was dominated by cyanobacteria (15.9 ± 3.5 mm^3 L^{-1}; >90% of total phytoplankton biovolume) with *Microcystis* spp. (12.3 ± 2.6 mm^3 L^{-1}) representing >70% of the total cyanobacterial biovolume in the raw water (**Figure 2A**). At the NG-Walukuba abstraction point, cyanobacteria were dominated by the non-toxigenic *Planktolyngbya* (4.5 ± 1.3 mm^3 L^{-1}; 46% of the total cyanobacterial biovolume) and the toxigenic *Microcystis* spp. (4.4 ± 1.7 mm^3 L^{-1}; 44% of the total cyanobacterial biovolume) followed by the non-toxigenic *Aphanocapsa* (0.8 ± 0.2 mm^3 L^{-1}; 8% of the total cyanobacterial biovolume). Other genera such as *Dolichospermum* sp., *Chroococcus* sp. and *Merismopedia* sp. contributed <1% of the cyanobacterial biovolume (**Figure 2**).

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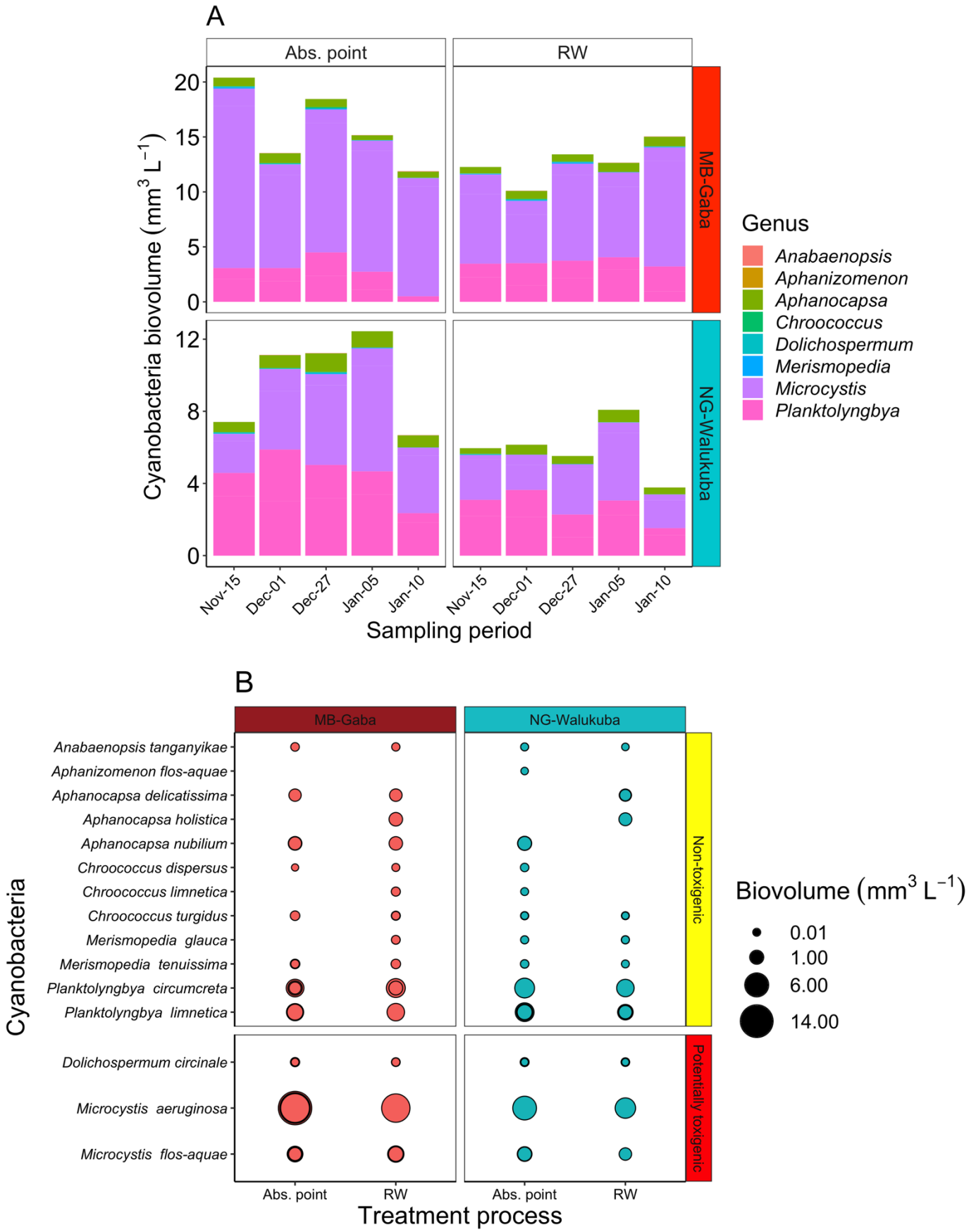

Figure 2. Cyanobacterial biovolume and composition in the Abs. point and raw water (RW) abstracted water (right panel) in MB-Gaba and NG-Walukuba water treatment plants. (A) Temporal dynamics of cyanobacteria genera; (B) Proportion of potentially toxigenic and non-toxigenic cyanobacteria. (Data collected between Nov 2016 - Jan 2017, n=90).

2.2. Cyanotoxins occurrence in the Lake Victoria embayments and during water treatment

Among all the samples analysed (n = 210) including the lake (n=120) and WTPs (n=90) all were negative for ATX, CYN, and STX, but 120 samples were found to contain MC (see below), and two were found positive

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for homoanatoxin-a (HTX) at the inshore station in MB (at low HTX concentrations, <0.04 HTX equiv. L^{-1}). During the field survey, 99 samples, (82.5%, n=120) were found to contain MC. The main MC congeners in MB and NG included MC-RR and MC-LR (**Figure 3**). Total concentrations of MC were significantly higher among samples obtained from MB than those from NG (Mann-Whitney test, $p<0.001$; **Figure 3A**). In MB, the MC concentrations showed a decreasing gradient from the inshore (0.15 - 11.7; mean ± SD = 2.01 ± 2.37 µg MC-LR equiv. L^{-1}) and recreational stations (0.18 - 14.8; 2.34 ± 4.03 µg MC-LR equiv. L^{-1}) to the WTP abstraction point (0.04 - 1.41; 0.62 ± 0.47 MC-LR equiv. L^{-1}; ANOVA, $p = 0.0128$) and open lake stations (0.02 - 0.91; 0.22 ± 0.25 MC-LR equiv. L^{-1}, ANOVA, $p<0.05$). In NG, the mean MC concentrations were rather low (<LOQ – 0.2 µg MC-LR equiv. L^{-1}) with no significant difference across the sampling stations (ANOVA, $p = 0.904$; **Figure 3A**). In MB MCs were detected throughout the year but in NG MCs mostly occurred during the first half-of the study period, i.e. Nov 2017 – April 2018 (**Figure S3**).

From the water treatment abstraction point and during treatment, 21 samples (23.3%, n= 90) were found positive for MC. For MB-Gaba abstraction point and raw water, six MC congeners including MC-RR, MC-YR, MC-LR, [Asp^3]-MC-RY, [$MeAsp^3$]-MC-RY, and [$NMeSer^7$]-MC-YR were regularly detected. In NG-Walukuba, four congeners were detected at the abstraction point including MC-LR, MC-RR, MC-YR and [$NMeSer^7$]-MC-YR (**Figure 3D**). For the WTP survey both intracellular and dissolved MC were differentiated. In general, intracellular MC concentrations at the two WTP abstraction points were found comparable to MC concentrations observed inshore (**Figure 3B**), i.e., the mean concentrations of intracellular MC were 1.22 ± 0.36 µg MC-LR equiv. L^{-1} at MB-Gaba and 0.36 ± 0.46 µg MC-LR equiv. L^{-1} at NG-Walukuba. For MB-Gaba only, the MC concentration increased to 3.61 ± 0.48 µg MC-LR equiv. L^{-1} in raw water (**Figure 3B**). Accordingly, the concentration of dissolved MC as determined via ELISA were significantly higher in MB-Gaba than in NG-Walukuba (**Figure 4A**). However, for NG-Walukuba dissolved MC were consistently below the LOD for LC-MS (**Figure 4B**). In addition, there was an increase in dissolved MC during certain treatment steps in MB-Gaba, especially coagulation and flocculation, but in general dissolved MC was drastically decreased until the final treatment step. Indeed, MC has been detected in final treated water only once (0.14 µgL^{-1} on 4th January 2017). In the diluted fraction MCs were constituted of MC-LR, MC-YR, [$MeAsp^3$]-MC-RY and [$NMeSer^7$]-MC-YR (**Figure 4C, Table S1 and Table S2**).

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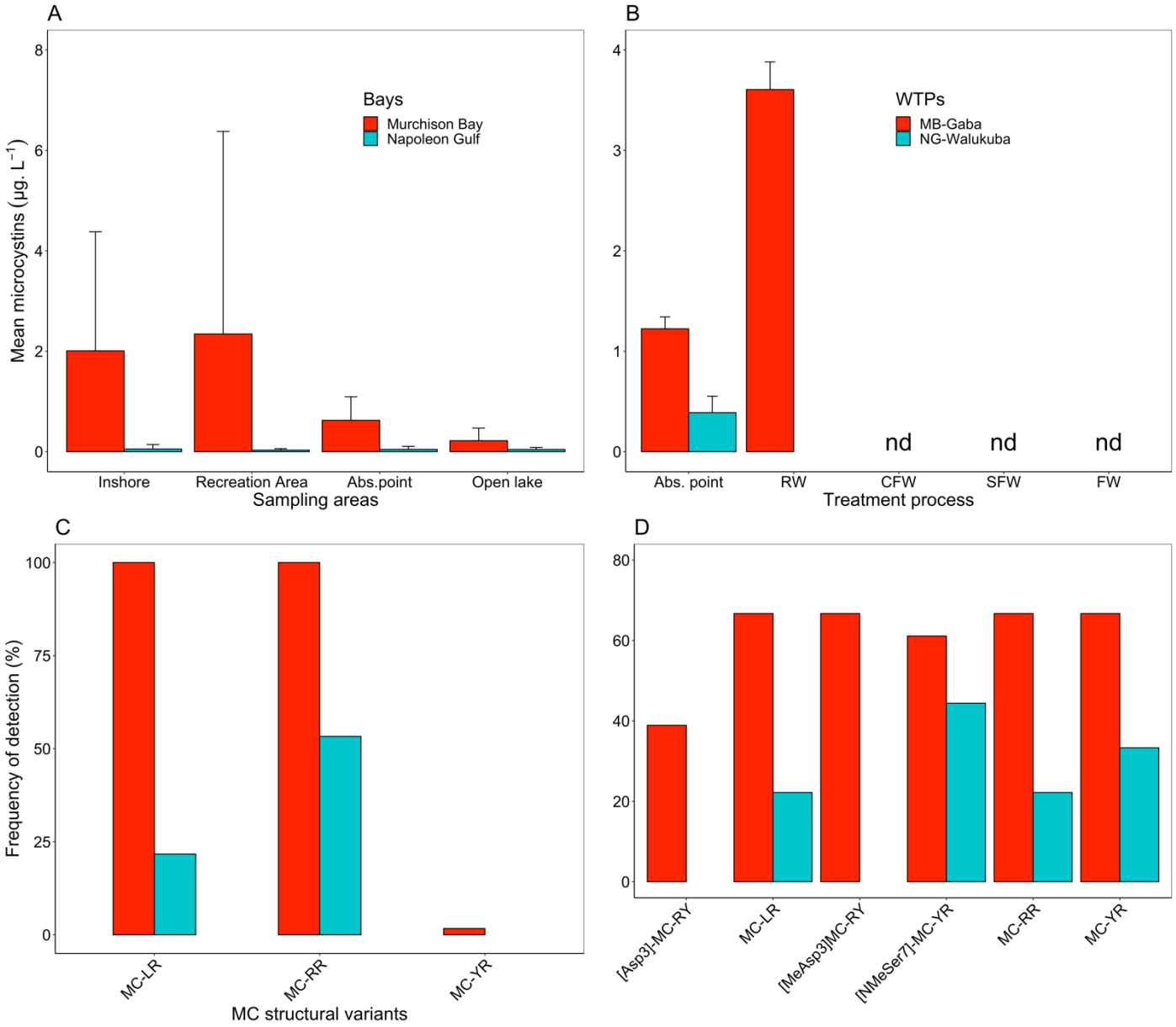

Figure 3. Spatial variation in the intracellular MC concentration (mean ± SD), (A) at i) inshore stations in the bays, ii) recreational areas, iii) water treatment abstraction points (Abs. point), and iv) open lake in Murchison Bay (MB) and Napoleon Gulf (NG), (between Nov 2017 -Oct 2018, n=120), (B) during the water treatment processes from lake water to final water in MB-Gaba and NG-Walukuba (between Nov 2016 – Jan 2017, n=90). Note the different scales in the y-axis. The proportion of the structural variants of MC detected during the lake survey (C) and from the lake water and raw water abstracted into the WTPs. (n=18), from MB-Gaba and NG-Walukuba (B). Abbreviations: LW=Lake water, WTP=Water treatment plant, RW=Raw water, CFW=Coagulated and flocculated water, SFW=Sand filtered water, FW=Final water, nd=not detected.

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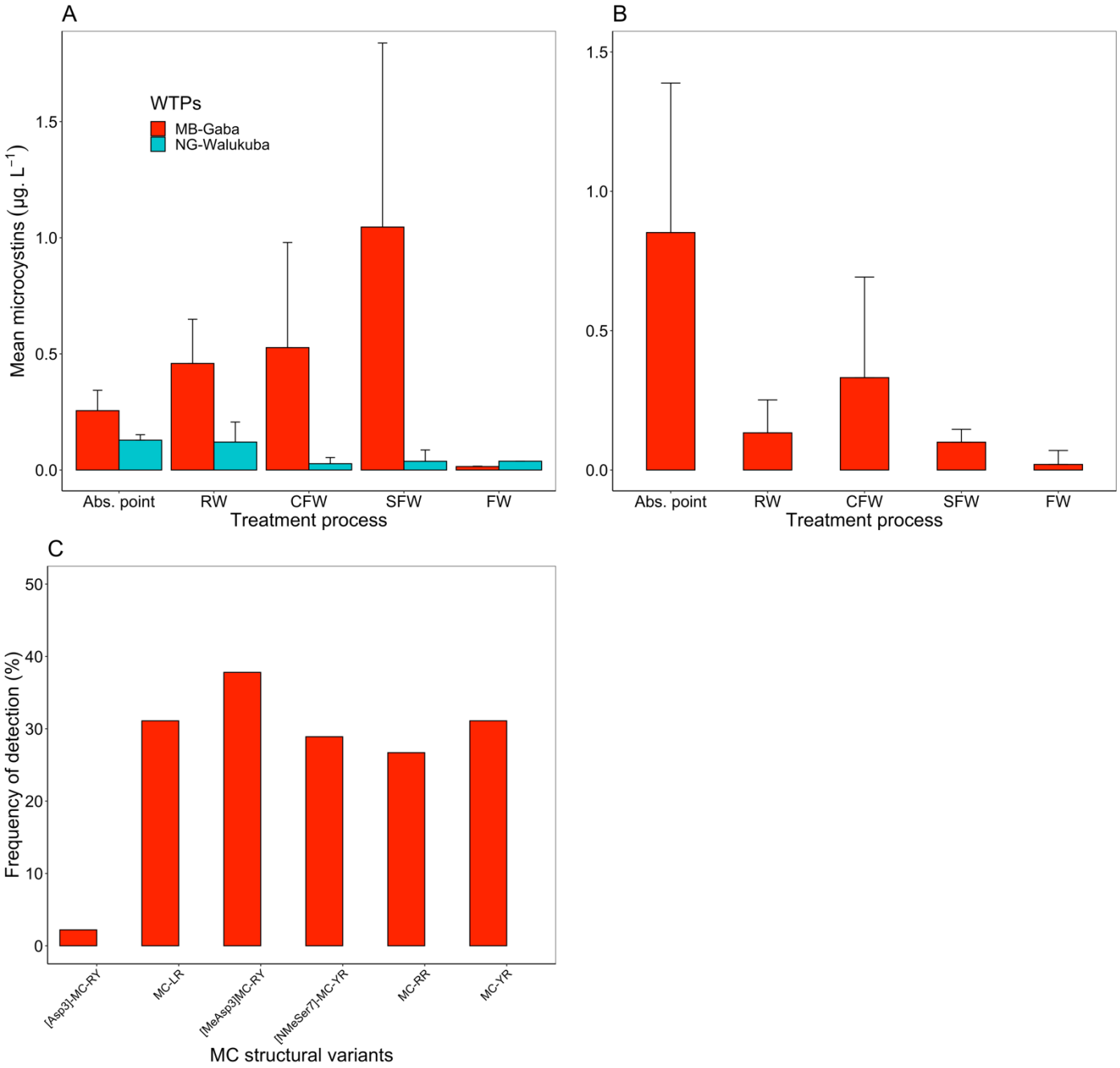

Figure 4. Concentration of dissolved MC (mean ± SD) as detected by ELISA (A) and LC-MS (B) from abstraction points in MB and NG, and during the treatment process in MB-Gaba and NG-Walukuba WTPs. (C) Proportion of the structural variants of MC detected in the dissolved water from MB-Gaba (n=45). Note: there was no detection of dissolved MC in NG-Walukuba. Abbreviations: Abs. point = water treatment abstraction point, WTP=Water treatment plant, RW=Raw water, CFW=Coagulated and flocculated water, SFW=Sand filtered water and FW=Final water.

2.3. Relationships between predictive variables and microcystins

Regarding the limnological variables selected to predict MC concentration (**Table 1**), turbidity ranged from 0.2 -197 NTU, and the dissolved N:P ratio ranged from 0.16 – 149.1 with a mean of (18.2±22.3). Among the biological variables, the average biovolumes and cell numbers of *Microcystis* were higher than *Dolichospermum*. The meteorological variables such as rainfall pattern during the sampling days varied from no rain (dry season sampling) to maximum rainfall of 61.1 mm /day and an average of 11 mm for the sampling period. The primary wind directions were from north to south (N-S), from east to west (E-W), and from northeast to southeast (NE-SE). In particular, during the study period (Nov 2017 - Oct 2018), 30.4% of the sampling days had an average wind speed of 4.3 km s^{-1} in the N-S direction, followed by 26.1% of sampling days with a wind speed of 3.5 km s^{-1} in the E-W direction and 21.7% days with wind of 5.4 km s^{-1} in the S-SE direction. The average wind speed ranged from 3.1 - 8.1 with a mean of 4.5±1.1 km.s^{-1} while the mean solar radiation was 291±81 Wm^2 day^{-1} for the past 10 days before sampling (D10).

Taking all data together (n=120) the parameters wind speed and wind direction, mean intensity of solar radiation, and biovolume of *Microcystis* and *Dolichospermum* explained 55.2% of the variance of the partial least

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squares (PLS) model for MC concentrations (PLS model coefficients not shown). When considering the cell numbers of biological parameters in the PLS model, wind speed, direction, mean solar radiation and *Microcystis* cell numbers explained 49.6% variance in MC concentrations (**Table 1**). Furthermore, there was a significant correlation between *Microcystis* in MB and MC concentration (abundance data: r = 0.57, p<0.0001; biovolume data: r = 0.62, p<0.0001) with no significant correlation between *Dolichospermum* (abundance or biovolume) in NG and MC concentration.

Prediction of MC was primarily related to the abundance of *Microcystis* spp. (Support Vector Machines with Linear Kernel, kappa = 0.7877, p<0.001; **Figure 5**). When *Microcystis* abundance was >200,000 cells mL^{-1} (or equivalent of 13.7 mm^3L^{-1}) there was a 100% probability of observing MC. When *Microcystis* abundance was between 58,000 (the equivalent of 3.9 mm^3L^{-1}) and 200,000 cells mL^{-1}, the MC concentrations were significantly related to mean solar radiation (>248 Wm^2 day^{-1}) and mean wind speed (<4.8 km s^{-1}), in the north to south (N-S) direction. And, when *Microcystis* abundance was <58,000 cells mL^{-1}, mean solar radiation intensity for the past 10 days before sampling (D10), turbidity (>17.4 NTU) and abundance of *Dolichospermum* were correlated with low concentrations of MC (**Figure 5**).

Table 1. Standardized parameter coefficients from the global Partial Least Square (PLS) Regression models using log_{10} transformed variables for MC concentration, and correlation with environmental and biological parameters in NG and MB, Lake Victoria. Data from NG and MB were pooled together, and Pearson product-moment partial correlation coefficient (r) was used. Significant variables are shown in bold, p-values are indicated by asterisks: ***, p < 0.001; *, p < 0.05.

Parameters	Min- Max (Mean±SD)	Regression coefficient (Log_{10})	Correlation Coefficient
Intercept		-2.044	
SRP (µg L^{-1})	0.56 – 380.3 (13.4±38.8)	0.055	0.31
NH_4 (µg L^{-1})	0.01 – 1206.8 (49.3±150.9)	0.041	0.32
NO_3^- (µg L^{-1})#	1.11 – 947.4 (73.5±127)	–	0.02
NO_{2-} (µg L^{-1})#	0.00 –47.2 (4.7±7.1)	–	0.24
N:P ratio	0.16 – 149.1 (18.2±22.3)	-0.093	0.02
Turbidity (NTU)	0.02 – 197.2 (18.2±22.3)	–	0.42
Rainfall (mm)	0 – 61.1 (11.2±13.9)	0.004	0.06
Wind speed (km s^{-1})	3.1 – 8.1 (4.5±1.1)	**-0.703***	0.31
Wind direction	N-S, E-W, S-N, N-NE (S-SE, E-W)	**0.035***	0.18
Solar Radiation (Wm^2 day^{-1})	181.7 – 394.7 (290.9±80.9)	**1.021*****	0.60
Microcystis biovolume (mm^3L^{-1})	0.27 -69 (7.3±9.4)	–	0.62
Microcystis cell number	39,000 – 1,061,206 (106,277±148,826)	**0.031****	0.57
Dolichospermum biovolume (mm^3L^{-1})	0.07 – 20.6 (3.9±4.1)	–	0.22
Dolichospermum cell number	645 – 182,627 (33,822±34,975)	0.008	0.24
R^2		0.49	

#Dissolved fractions of N used for the calculation of the N:P ratio applied in the PLS model.

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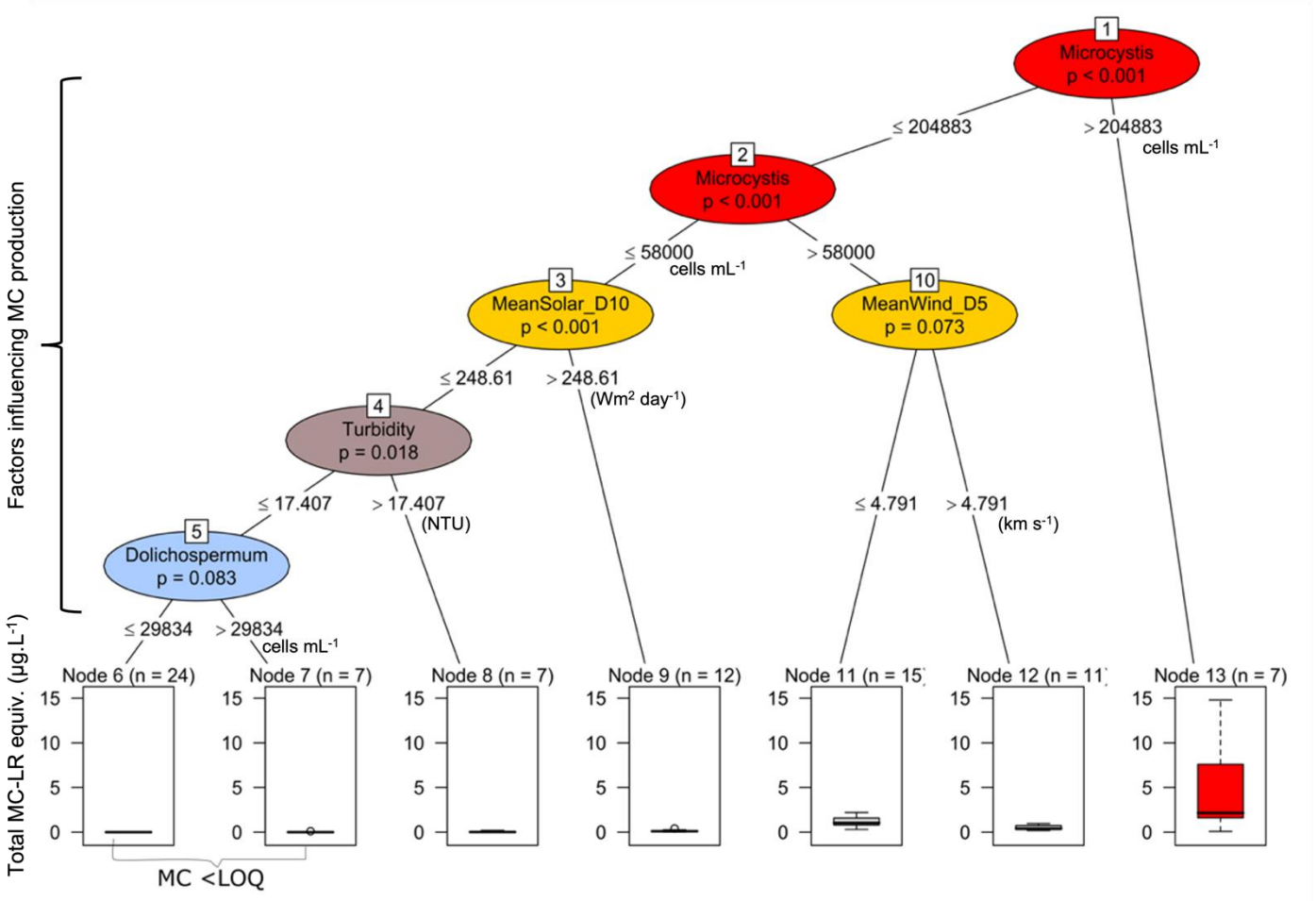

Figure 5. Decision support tree for prediction of MC concentrations in lake water from Murchison Bay and Napoleon Gulf, northern Lake Victoria. Note: LOQ = 0.04 µg L^{-1} ; MeanWind_D5 = the mean wind speed for the past five days (km s^{-1}) (N-S direction) and MeanSolar_10 = mean solar irradiance for the past ten days before sampling (Wm^2 day^{-1}). (Data collected between Nov 2017 - Oct 2018, n=120). Colours: Red = 0.08 – 14.8 µg MC-LR $^{-1}$; Yellow = LOQ – 4.5 µg MC-LR $^{-1}$; Brown and Blue = LOQ – 0.91 µg MC-LR $^{-1}$

3. Discussion

Toxigenic cyanobacteria and cyanotoxins in the Lake Victoria embayments

The predominant toxigenic cyanobacteria in the two embayments of northern Lake Victoria during the studied periods were *Microcystis* in MB and *Dolichospermum* and *Microcystis* in NG. These two genera are among the most common toxigenic genera in aquatic freshwater ecosystems, followed by *Raphidiopsis* (previously *Cylindrospermopsis*) [5]. The *Microcystis* biomass (expressed in biovolume) between April 2003 and March 2004 formed 0.3±0.1 mm^3 L^{-1} making 20.1% of total cyanobacteria biovolume (1.6±0.7 mm^3 L^{-1}) in inner MB [20]. In another study between May and June, 2004 *Microcystis* biovolume ranged from 3.1 to 23.2 mm^3 L^{-1} [21], and in 2017-2018 (this study) it varied between 0.5 – 69 mm^3 L^{-1} (10.5±10.6 mm^3 L^{-1}). Considering these data, it suggests that there is an increase of *Microcystis* sp. biomass (six-fold) in MB, over the last two decades.

Analogously the range of *Dolichospermum* biovolume in the mid-2000s was 8.6 – 15 mm^3 L^{-1} [21] but varied between 0.4 – 20.6 mm^3 L^{-1} (this study). In NG, *Microcystis* biovolume varied between 3.1 – 23.2 mm^3 L^{-1} in the mid-2000s to 0.18 – 31.4 mm^3 L^{-1} (this study), but the *Dolichospermum* biovolume declined from 9.8 – 83 mm^3 L^{-1} to 0.1 – 8.63 mm^3 L^{-1}. This suggests that either during the mid-2000s a *Dolichospermum* bloom event occurred in NG or there was a shift over these two decades of cyanobacterial dominance from *Dolichospermum* to *Planktolyngbya*. This shift between the two genera, heterocystous *Dolichospermum* vs non-heterocystous *Planktolyngbya* could reflect increased inorganic nitrogen inputs *i.e.*, via terrestrial run-off.

Our study also revealed the presence of HTX at the inshore station of MB. This neurotoxic cyanotoxin is produced by cyanobacteria from the genera *Dolichospermum, Aphanizomenon, Oscillatoria, Phormidium* and *Planktothrix* [22]. In this study, among the potential HTX producers, only *Dolichospermum* was observed, thus the detected HTX was possibly associated with this genus. For instance, the *Dolichospermum* biovolumes were 8.6 and 5.1 mm^3 L^{-1} during June and September 2018 when HTX were detected from the inshore areas in MB. In the future, the suggested HTX occurrence in L. Victoria embayment should be evaluated by strain isolation.

The main cyanotoxins detected in lake water and during water treatment included MCs with the congeners, MC-RR, MC-YR, MC-LR, [Asp^3]-MC-RY, [MeAsp^3]-MC-RY, and [NMeSer^7]-MC-YR, which have

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been recorded already during previous studies in these embayments, as well as partly from other parts of the lake (see review [8]). However, while the congeners were similar, in this study for the MB embayment the MC concentrations were higher than those reported previously (0.2 - 15 µg MC-LR equiv. L^{-1} in this study compared to 0.2 – 0.7 µg MC-LR equiv. L^{-1} recorded during the mid-2000s (particularly from 2004 to 2005) [23]; and 0 - 1.6 µg MC-LR equiv. L^{-1} between 2007 and 2008 [13]. This increase in MC concentrations might be associated with the six-fold increase in the mean *Microcystis* biovolume (10.5±10.6 mm^3 L^{-1}) in MB. On the contrary, in NG, the concentrations of MC reported in this study corresponded to those reported in the late 2000s estimated between 0 and 1.5 µg MC-LR equiv. L^{-1} [13,21,24]. Obviously, the change in cyanobacterial community composition from *Dolichospermum* dominance to *Planktolyngbya* dominance did not affect MC concentrations in NG since this genus does not produce MC [25].

Factors explaining MC concentrations

The most important drivers explaining MC concentrations are cyanobacterial species composition, followed by physiological factors related to cellular growth such as light intensity and rising temperature [26], and the concentrations and forms of N and P [27,28]. For various lakes in Uganda, Poste et al. [24] showed that high TP, low TN:TP ratios and high cyanobacteria biomass all positively influenced MC concentrations. Recently, Krausfeldt et al. [29], indicated that although N sustained the biomass of toxigenic cyanobacteria, different forms of N can induce physiological changes consequently causing variations in the MC concentrations. Aside from physiological effects, all these drivers are triggers of growth of the toxigenic cyanobacteria, *Microcystis* and *Dolichospermum*, and thus also influence the production of MC through cell division [30]. Earlier studies by Roegner et al. [31] did not report potential predictors of MC presence or an increased MC concentration in another embayment, Kisumu Bay, Lake Victoria (Kenya). During our study period, the variation in MC concentration was associated with the biovolume of *Microcystis* spp. and mean solar radiation, while changes in mean wind speed and direction (N-S and N-NE) were also related to the incidence of toxigenic *Microcystis* sp. and MC in the two embayments. From the decision tree (**Figure 5**), it can be inferred that MC concentration was increasing at lower wind speed (<4.8 kms^{-1}) blowing in the N-S direction, suggesting that the wind was blowing the scums from the inshore to the open water. In general, wind-driven currents are important in the horizontal and vertical distribution (sinking or floating) of *Microcystis* which depend on the colony size [32] and physical density of the *Microcystis* cells [33]. During the study period, the wind direction most frequently was N-S oriented (30.4%), i.e., moving surface water from the inshore areas to the open lake. In contrast, the open lake had less chance to be blown into the bays (S-N) and to the shorelines (S-NE) since only 4.3% of the sampling days had wind blowing in these directions. Thus, the southward wind might have influenced the movement of *Microcystis* scum from the inshore areas to the open water and thus reduced MC concentrations.

In general, it is known that *Microcystis* growth is favoured by eutrophic and polymictic conditions because *Microcystis* form buoyant colonies that can float up quickly and use the light for photosynthesis more efficiently than other non-buoyant taxa like diatoms [34]. For example, in Nyanza Gulf of Lake Victoria (Kenya), the growth of the chain-forming diatom *Aulacoseira granulata* has been found positively related to transparency (Secchi depth) while the growth of *Microcystis* sp. was found negatively related [35]. Marshall et al. [36] predicted an overall increase in temperature by 2°C in northern Lake Victoria by 2055 which will cause also increased physical stratification of the main basin and possibly favour the growth of buoyant cyanobacteria such as *Microcystis* and support cyanobacteria dominance in general.

In addition, studies by Okello et al. [21] suggested that the concentration of MC per *Microcystis* cell was causally dependent on the quantity of MC-producing genotypes, *i.e.,* those carrying the *mcyB* gene which is indicative of MC synthesis. The same authors also reported a relationship between MC concentrations and total *Microcystis* cell numbers, which was also found in this study. It was reported that for individual strains, high light availability increased MC synthesis in *M. aeruginosa* by increasing gene transcription [37]. Under high light intensity, the higher level of MC was related to the higher transcription levels of both *mcy*B and *mcy*D [38]. In our study neither the genetic basis nor genetic regulation of MC biosynthesis has been tested, but besides *Microcystis* biovolume correspondingly the factor of light availability, i.e., through lower turbidity (or increased transparency) and average solar radiation (a proxy for both light and temperature) were found positively related to MC concentration.

The fate of MC during water treatment

In general WTPs are susceptible to cyanotoxin contamination but data on cyanotoxin occurrence in treated water produced in the tropics is scanty [4,39]. Earlier studies in Lake Victoria focused on intracellular toxins [8,31] but less considered the risks of extracellular toxins. This study monitored both intra- and extracellular MC occurring through a conventional water treatment installed at the two embayments susceptible to cyanobacterial blooms. Chorus and Bartram [39] reported that WTPs in general using

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coagulation, clarification, and rapid sand filtration for surface water treatment are effective at removing cyanobacterial cells but only partially effective at removing cyanotoxins. Indeed, rapid sand filtration can be considered effective in removing intracellular MC through the removal of cyanobacterial cells. For instance, in this study, chlorophyll-a concentration recorded from raw water (47.6±7.7 μg.L^{-1} in Gaba III and 12.3±5.42 μg.L^{-1} in Walukuba) declined by >90% in final water (2.7±1.8 μg.L^{-1} in Gaba and 2.3±0.4 μg.L^{-1} in Walukuba), suggesting that sestonic algae and cyanobacterial cells were effectively removed. In addition, our data showed that intracellular MC were detected in 62.6 % and 33.3% of the raw water samples from MB-Gaba III and NG-Walukuba, respectively, but were not detected anymore in final water.

On the other hand, rapid sand filtration may be more susceptible to cell lysis [4,39]. Therefore, water authorities in charge of conventional WTPs might face a challenge arising from dissolved MC. In this study, when raw water MC concentrations was 0.46±0.19 μgL^{-1}, we observed increase of dissolved MC during rapid sand filtration. This is in agreement with studies in Finland where Tarczynska et al. [40] found that on average 0.8 μg.L^{-1} of MC were detected in treated water, suggesting that pre-chlorination, coagulation, and rapid sand filtration were ineffective in removing dissolved MC from treated water. Similarly, Lahti et al. [41] also reported that rapid sand filtration was less effective in removing dissolved MC due to the occasional occurrence of MC in treated water below the lifetime guideline value for exposure via drinking water (WHO 2021). As a result, the release of MC from cells should receive more attention during water treatment via rapid sand filtration. For effective removal of dissolved MC, an additional process is required, e.g., Ho et al. [42], reported complete degradation of MC on biologically active sand filters within four days, attributed to high bacterial activity. Another effective method to remove dissolved MC has been slow sand filtration or filtration through granular activated carbon [43] however, both methods are less common in water treatment in East Africa and the global south.

For MB-Gaba dissolved MC detected in the sand-filtered final water, was possibly associated with filter clogging and more frequent backwashing per day (min. 2 times per day). Possibly a higher cyanobacteria biomass in the abstracted raw water cause a higher dissolved MC concentration. Nevertheless, the total dissolved MC concentration in treated water was found always below the lifetime guideline value for exposure via drinking water (WHO 2021, i.e. <1.0 μg MC-LR equiv. L^{-1}). However, since an increase in *Microcystis* biovolume in raw water in the future cannot be excluded, regular monitoring of dissolved MC during the water treatment process is recommended to better understand potential health threats over longer periods.

Multiple exposure routes to toxigenic cyanobacteria and cyanotoxins

The primary threat to humans is via direct exposure to toxigenic cyanobacteria and cyanotoxins through drinking water [4], mainly from contaminated untreated or treated water [31]. In our study the two WTPs efficiently purified raw water containing toxic cyanobacteria. However, probably through cell lysis (after pre-chlorination and coagulation) free intracellular MC was released into the water and were composed of five or six structural congeners. Besides the most frequent MC-RR, -YR and -LR variants the [NMeSer^{7}]-MC-YR congener was found frequently. The toxicity of this rare MC congener has not been studied, and thus, it is advisable to use MC-LR equivalents to estimate long-term and short-term exposure. It should be mentioned that chlorination of raw water or treated water has been reported to be associated with the formation of undesirable degradation by-products and isomers such as monochloro-MC, monochloro-dihydroxy-MC, and tri-halo-menthanes [44]. Thus, in the future, if an increased concentration of free MC is recorded in finished water, investigations on the potential chlorination degradation by-products would be required as well.

To guarantee the elimination of MC from treated water, the actual toxin concentrations in the source water should be monitored for use in more efficient water treatment methods and further reduce health risks. Early warning parameters such as cell numbers or pigments could be used to trigger an action plan for safety during water treatment if thresholds of toxigenic cyanobacteria and cyanotoxins in the source water are exceeded.

In addition, for Uganda, the use of untreated water by local populations engaging in recreational activities is considered an important exposure route. For example, in this study the highest concentration of MC in MB (15 μg MC-LR equiv. L^{-1} recorded at the recreational areas coincided with a *Microcystis* cell density of 1.45 × 10^{6} cells mL^{-1}. Thus, the nearshore recreational areas, which are easily accessible by the locals, expose them to toxic cyanobacteria and cyanotoxins. In general, the observed *Microcystis* cell densities (*M. flos-aquae*, 859,690 ± 582,749 cells mL^{-1} and *M. aeruginosa*, 792,609 ± 418,230 cells mL^{-1}) exceeded the frequently cited alert level of 100,000 cells mL^{-1} when assessing health risk and regular monitoring is recommended [39]. A general increase of MC-producing *Microcystis* in MB would suggest that the probability of exceeding the WHO lifetime guideline value of 24 μg MC L^{-1} in recreational water will increase as well. Thus, monitoring of *Microcystis* cell abundance could be used as an early warning tool both for recreational services providers and WTP operators [45]. Consequently, measures to reduce the threats of deliberate and accidental consumption of toxigenic cyanobacteria during recreation, and eventually adjustments in the dosages of the chemicals used in the water

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treatment process are recommended [46]. These measures could involve warning signs and the closure or discontinuation of swimming or bathing. So far these measures have been effective in a few developed countries such as the Netherlands [46] but have not been used in the south including Uganda. For WTP operation and due to seasonal variability in toxigenic cyanobacteria and the high cost of toxin monitoring, it is deemed necessary to adopt simple, rapid methods such as cell counting of the dominant toxigenic taxa of *Microcystis*.

4. Conclusions

This study is the first to examine the seasonal and spatial diversity, and dynamics of MC in lake water used for water treatment in Murchison Bay and Napoleon Gulf embayments in Lake Victoria. Although MC were reduced by both WTPs, the high abundance of toxigenic cyanobacteria within the lake water, and the possible occurrence of the cyanotoxins MC pose a potential health threat to riparian communities. The risk of daily consumption of untreated water by locals and people engaging in recreation requires an early warning especially during cyanobacterial bloom events. The increase of MC concentration has been related to the biovolume of *Microcystis*, influenced through solar radiation, mean wind speed (N-S direction) but also turbidity in the water column. Thus, early warning methods such as regular *Microcystis* biovolume estimation and sensitization of people during bloom events are proposed to prevent health threats from toxigenic cyanobacteria. According to the results of this study, we recommend not to use lake water for domestic purpose (cooking, drinking, washing) but rather use treated water and reduce recreational activities during *Microcystis* bloom events.

5. Materials and Methods

5.1. Study sites and sampling design

The two bays of Lake Victoria, Murchison Bay (MB) and Napoleon Gulf (NG) were selected for this study because of their contrasting ecological conditions and contrasting abundances and diversity in toxigenic cyanobacteria [19] (**Figure 6**). MB is a shallow (maximum sampled depth = 18 m) and closed bay receiving mainly point source pollutants while NG, although similarly shallow (18.1 m), is an open gulf and hosts the major drainage of Lake Victoria (Source of River Nile). These bays are the main water source for local inhabitants who are not connected to piped (treated) water using lake water and raw water processed by the WTPs.

To investigate the dynamics of cyanobacteria and cyanotoxins concentrations within the two embayments of MB and NG, temporal-spatial variability was monitored monthly (n = 12) between November 2017 and October 2018, at four stations, i) the inshore station, ii) the recreational area, iii) in the lake water at the abstraction point of the WTP, and iv) open lake (**Figure 6**). At the various sampling stations, depth-integrated samples were obtained using a horizontal water sampler and transported in cooling boxes to the laboratory for filtration using GF/C filters under low vacuum pressure for cyanotoxin analysis (see below).

In order to estimate cyanobacteria biovolume composition and MC during water treatment, sampling was done weekly (n = 9) between November 2016 and January 2017 at the Gaba III plant (MB-Gaba) located in MB and at the Walukuba plant (NG-Walukuba) in NG (**Figure 6**). Although both plants use the conventional methods of water treatment of coagulation, rapid sand filtration and chlorination, differences exist between the capacity of the WTPs and additional treatment steps applied in the Gaba III plant. The main differences are that MB-Gaba III abstracts raw water about 4,100 $m^3.hr^{-1}$ from 1.5 km offshore at a depth of 8 m while NG-Walukuba abstracts about 880 $m^3.hr^{-1}$ from 600 m offshore at a depth of 4 m within the water column. In addition, MB-Gaba plant undertakes pre-chlorination of the raw water and pH control of the final treated water using calcium carbonate ($CaCO_3$). Sampling was performed to monitor intracellular and dissolved MC concentration during different steps of the water treatment: i) in the lake water at the abstraction point, ii) in the raw water at the entry point of the WTP, iii) after flocculation and sedimentation, iv) after sand filtration, v) in final water after chlorination (**Table 2**).

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Table 2. Main characteristics of the two water treatment plants (Gaba III and Walukuba) and physical-chemical characteristics of water (mean ±SD) collected during the water treatments processes between November 2016 and January 2017. MB: Murchison Bay. NG: Napoleon Gulf (sample size= 90, 45 for each WTP).

Water treatment plant	Capacity ($m^3.day^{-1}$)	Piped water connection	Demand (m^3/day)	Treatment steps		Physico-chemical parameters during the treatment process				
						Lake water (abstraction point)	Raw water (Entry point of the WTP)	Flocculation water	Sand Filter	Final
MB_Gaba	80,000 but supplementing Gaba I & II water treatment plants	315,897 connections supplying 2 millions of people	300,000	Pre-chlorination, coagulation, clarification, rapid sand filtration, pH correction and chlorination	pH	7.95±0.27	7.57±0.10	7.20±0.29	7.17±0.28	7.02±0.19
					Conductivity ($\mu S.cm^{-1}$)	117.96±2.13	118.98±3.72	128.81±5.49	129.21±5.90	130.99±5.27
					Temperature(°C)	26.10±0.28	26.11±0.43	26.03±0.37	25.98±0.33	26.41±0.42
					Dissolved oxygen ($mg.L^{-1}$)	5.00±0.67	5.92±0.39	7.14±0.08	7.36±0.07	7.07±0.12
					Dissolved oxygen (%)	71.04±9.56	85.01±8.31	101.76±1.66	103.88±1.22	101.28±1.21
NG_Walukuba	50,000 but producing only 30,100	28,881 connections supplying over 500,000 people	30,000	Coagulation, clarification, rapid sand filtration and chlorination	pH	8.94±0.32	7.84±0.23	7.49±0.38	7.44±0.18	7.63±0.22
					Conductivity ($\mu S.cm^{-1}$)	101.98±1.20	103.92±6.73	105.18±6.79	102.72±2.78	109.24±4.34
					Temperature(°C)	27.05±0.34	27.28±0.58	27.27±0.37	26.97±0.35	27.16±0.21
					Dissolved oxygen ($mg.L^{-1}$)	6.55±1.02	3.77±0.93	3.90±0.64	3.24±0.80	6.35±0.63
					Dissolved oxygen (%)	94.25±15.09	55.58±12.97	56.69±9.52	46.98±11.82	92.09±8.86

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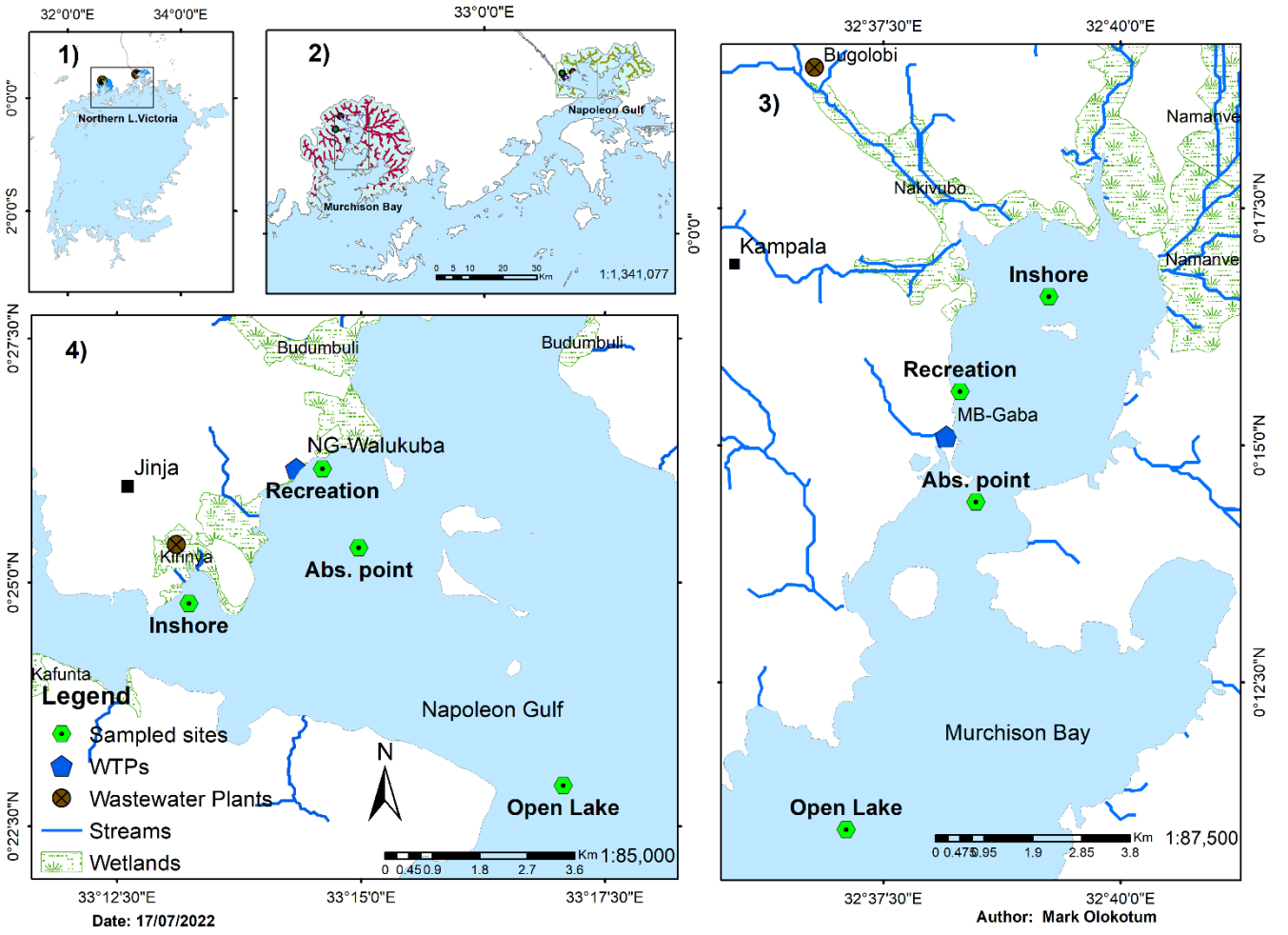

Figure 6. Location of the two study areas in Murchison Bay (MB) and Napoleon Gulf (NG) (1) and close up with tributaries and wetlands (2) and the location of sampling sites (Inshore, Recreation, water treatment abstraction points (Abs. point) and open lake; in MB (3) and NG (4). The maximum sampling depth in MB for sites were as follows: Inshore 1.4 - 2.5 m (1.84±0.34), Recreation =2 - 2.7 m (2.3±0.3), water treatment abstraction points (Abs. point) =9.4-18.4 m (14±2.33) and Open Lake = 12 -14 m (12.7±0.5). In NG the sampling depths were as follows: Inshore =5.4 - 6.5 m (6.1±0.3), Recreation =5.7- 7.2 m (6.5±0.5), water treatment abstraction points (Abs. point) = 11.4- 15.8 m (13.1 ±1.2) and Open Lake =16.7-21.5 m (18.1±1.3). Note: The details on water treatment are described in **Table 2.**

5.1. Meteorological, physical-chemical, and biological variables

Daily meteorological data (rainfall, air temperature, wind speed and solar radiation) for October 2017 to October 2018 were obtained from the Jinja and Kampala weather stations operated by the Uganda National Meteorological Authority (UNMA) and are described in Olokotum et al. [19]. In addition, wind direction data for the same period was acquired from https://www.timeanddate.com.

For each sampling date in the lake, several physical-chemical variables (water depth, Secchi depth, temperature, pH, electrical conductivity, dissolved oxygen, and turbidity measured in-situ) and soluble reactive phosphorus (SRP), nitrate (NO_3-N), nitrite (NO_2-N), ammonium (NH_4-N), soluble reactive silica (SRSi), total phosphorus and total nitrogen) were measured at Analytical laboratory of the National Fisheries Resources Research Institute (NaFIRRI) following standard operating procedures as described in Olokotum et al. [19].

For cyanobacterial communities 20 ml of the water samples from the lake and the subsequent steps in water treatment were fixed using Lugol's solution [47], stored away from light. Later, 2-5 mL of the fixed water samples were sedimented for 6-12 hours [47] before identification and enumeration. Cyanobacteria taxonomic identification, and taxa abundance and biovolume estimation were performed based on geometric shapes described in Sun and Liu [48]. The cyanobacteria species were identified using a taxonomic guide and keys as described in Talling [49], Komárek and Anagnostidis [50], and Cronberg and Annadotter [51], and Chl-a concentration was used as a guide to estimate the volume of sample that should be sedimented. Total phytoplankton biovolume was positively correlated with Chl-a (R^2 = 0.55, p< 0.0001) [19]. When Chl-a concentration was low (≤ 20 µg L^{-1}), 5 mL were sedimented. When it was high (≥ 20 µg L^{-1}), 2-3 mL were sedimented. In addition, *Microcystis* was first counted as colonies from which the number of cells per colony was estimated based on the size of the colony and cell size. Later the samples containing *Microcystis* sp. were re-counted using a Malassez counting chamber to get the cell numbers.

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5.1. Cyanotoxins analysis

Lake water survey (quantification of intracellular MC)

For intracellular cyanotoxins analysis, the filtered biomass was extracted using 75% aqueous methanol, according to the protocols described by Cerasino et al. [52] with slight modifications. Briefly, the cultures and environmental samples were thawed to room temperature in sterile 15 mL glass vials and later conditioned with 4 mL of 75% methanol. The conditioned samples were sonicated on ice using an ultrasound probe (Sonics Vibra cell) (100% amplitude, 130 W, 20 kHz) for 3 minutes with 30-second interval pulses to aid extraction. The extracts were centrifuged at 4,000 rpm for 15 minutes (4°C) and the supernatant was collected in a clean 15 mL glass vials. To the pellet, 2 mL of 75% methanol was added for the second extraction using in an iced ultrasonic bath (Prolabo)(100% Ultrasound power at 40kHz) for 15 minutes. The second extracts were also centrifuged, and supernatants pooled together. Later, 2ml of the pooled extract was centrifuged again at 13,000 rpm for 10 minutes and part of the supernatant extracts were stored in 2 mL amber glass vials at -20°C until MS analysis. For ATX, CYN, STXs and MC analysis, cyanobacteria cultures of *Phormidium favosum* (PMC 240.05), *Raphidiopsis raciborskii* (PMC 99.03), *Aphanizomenon gracile* (PMC 638.10) and *Microcystis aeruginosa* (PMC 728.11) strains were used, respectively, as positive controls (**Table S4**).

For the characterization and quantification of intracellular MC by LC-MS/MS, the extracts were centrifuged at 13,000 rpm for 10 minutes and 500 µl of the clear supernatant were transferred into HPLC glass vials. Four µL were injected into Ultra-High-Performance Liquid Chromatography (UHPLC) chromatographic chain (Elute UHPLC-Bruker) coupled to a High-Resolution Mass Spectrophotometer (MS) system (Compact QTOF-Bruker) for identification and quantification of target cyanotoxins. The extracts were separated on a grafted-C18 stationary phase column (Acclaim RSLC polar advance 2, 2.2 µm, Thermo Fisher® 2.1 × 100 mm) along a 15-min linear-gradient (5-90%; 0.3 $\mu L.min^{-1}$ flow rate) of acidified ACN with 0.08% formic acid and ultrapure water acidified with 0.1% formic acid. In the MS, the cyanotoxin masses were analyzed between 50 and 1500 m/z in broad-band Collision Ion Dissociation (bbCID) or auto MS-MS/MS positive mode, alternating at 2 Hz the MS and MS2 modes at low and high energy respectively (mass accuracy of < 0.5 ppm).

The cyanotoxins were identified according to; i) retention time, ii) molecular mass, iii) isotopic pattern and iv) diagnostic ions. They were quantified according to the area-under-the-peak signal determined for analytical standards of STX (Cas 35554-086), ATX (Cas 64285-06-9), HTX (Cas 14926-86-1), NOD (Novakit®), CYN (Novakit®) and seven variants of MC (MC-LR,-LA,-LF,-LW,-LY,-RR and YR) (Novakit®) using the TASQ software) (Bruker®). Except for MC-RR, MC-LR and MC-YR, other MC structural variants were quantified as MC-LR equivalents calculated from the regression curves of the MC-LR analytical standard. The HTX was also identified according to i) retention time, ii) molecular mass, iii) isotopic pattern and iv) diagnostic ions previously determined by analyzing a commercial standard (Abraxis) in the same platform and under the same conditions (RT = 4.0 min: transition ion list is : m/z 180 --> 163; m/z 180 --> 145; m/z 180 --> 135; m/z 180 -->107).

Abstraction points and water treatment (quantification of intracellular MC)

Intracellular MCs were extracted according to Fastner et al. [53]. In brief, the filters carrying the algal biomass were cut into small pieces and the biomass was extracted in aqueous 75% (w/v) methanol (1.5 ml volume). The extracts were sonicated in a water bath (BANDELIN SONOREX Ultrasonics) for 10 minutes and then transferred to a shaker for 30 minutes. Subsequently to centrifugation at 13,000 rpm (10 min) the clear supernatant was transferred into new 2 ml reaction tubes which were evaporated to dryness in a vacuum concentrator at 30°C. The procedure was repeated two times to ensure efficient MC extraction.

For Liquid Chromatography- Mass Spectrometry (LC-MS) analysis, the dried extracts were resuspended using 150 µl of 100% (v/v) methanol, sonicated for 10 minutes and 150 µl of MilliQ (MQ) water was added. The clear extracts were then injected into the HPLC-DAD using a LiChrospher ® 100, octyldecyl silane (ODS), 5µm particle size, LiChroCART ® 250-4 HPLC cartridge system (Merck, Darmstadt, Germany). According to Lawton et al. [54] for chromatographic separation a linear gradient of aqueous acetonitrile (ACN) in 0.05% Trifluoroacetic acid (TFA) (30-70% ACN) for 45 minutes was used. The different variants of MC were identified by (i) retention time and the order of elution using the analytical MC standards (MC-RR, YR and LR) and (ii) protonated mass [M+H] and MS^2 and MS^3 fragmentation patterns using an iontrap (amaZon SL, Bruker Daltonics, Bremen, Germany).

The UV and MS spectra and peaks were manually integrated to obtain the peak area from which the MC concentrations were determined which were quantified as concentration equivalents of external analytical standards MC-RR, YR and LR (Cyano Biotech GmbH, Berlin). These calibration curves of the MC structural variants were pooled together, and total MC concentration was calculated from the regression curve; $y = 1626.7x + 0.0989$ ($R^2 = 0.99$); where y was the absorption (mAU) recorded at 240 nm wavelength (UV) and x was the concentration of the MC standards injected on the column.

Abstraction points and water treatment (quantification of dissolved MC using ELISA)

All water samples were transported cool and dark within 2-4 hours to the laboratory for filtration. Depending on the turbidity from the sample, 250-1000 ml were filtered through the Whatman GF/C filters (Ø 47 mm) using a low-vacuum pump. For the MB-Gaba and NG-Walukuba samples, both intracellular (filtered biomass) and dissolved MC (filtrate) were determined. For the analysis of dissolved MC in the filtrate, we directly used the Abraxis MC ELISA Kit (Product No. 520011, Biosense Laboratories, Bergen, Norway). The absorbance at 450 nm was determined using an ELISA plate reader (LEDETECT 96, SN: 1357) in duplicate within 15 minutes. Standard calibration curves were constructed using the MC-LR standard (150, 400, 750, 1000, 2000 and 5000 ng L^{-1}) from which the concentrations of total dissolved MC in the samples were calculated in the linear range (0.2-2 ng/ml of MC-LR equiv.) according to manufacturer's instructions.

Abstraction points and water treatment (quantification of dissolved MC using LC-MS)

The same filtrate samples were concentrated through C18 columns (Sep-Pak ® Vac tC18 cartridge, 1cc/100mg, 37-55µm, Waters Corporation, Vienna, Austria) using a standard Solid Phase Extraction (SPE) procedure according to Dean [55]. We tested the SPE protocol at the Research Institute for Limnology, Mondsee, using 100 ml of lake water (Lake Mondsee), tap water and Millipore water spiked with 4µL of analytical MC standards (MC-RR, MC-YR and MC-LR with concentration equivalent to 1.0 µg.L^{-1}). Briefly, C18 columns were conditioned using 4.0 ml of 80% (v/v) methanol and equilibrated using 1.0 mL of MilliQ (MQ) water. Thereafter, 500-700 mL of the filtrate were allowed to flow through the column at 3 drops/second. For a pilot test, half of the columns were eluted immediately (wet) while the other half were dried at 50°C for 48 hours. Since no significant difference in the recovery of dissolved MC was observed, subsequently, SPE columns were dried at 50°C for 48 hours and thereafter stored frozen at -20°C. SPE columns were transported cool and dry to the laboratory in Mondsee, Austria. In the laboratory, the C18 SPE columns were eluted with 1.0 ml of 80% (v/v) methanol. The eluted volume (1 ml) was concentrated and injected into the HPLC-DAD (HP1100, Agilent, Vienna, Austria) coupled to Mass Spectrometry (MS, amaZon SL, Bruker, Bremen, Germany). This protocol resulted in an overall acceptable recovery of MC-RR, MC-YR and MC-LR analytical standards (CyanoBiotech GmbH, Berlin, Germany), (**Figure S4**). During November 2016 – January 2017, SPE samples (filtrates) from the lake and the subsequent steps in water treatment were also spiked using 4 µL of the analytical MC standards with concentration equivalent to 1.0 µg.L^{-1}. All the analytical MC structural variants (MC-RR, YR and LR) were recovered from C18 SPE columns with concentrations > 1.0 µg.L^{-1} (**Table S3**).

5.1. Statistical data analysis

For cyanobacteria, the taxa were grouped into two classes of i) potential toxigenic cyanobacteria (taxa that have been already described as toxin producers using strains) and, ii) non-toxigenic cyanobacteria (if toxin synthesis has not been described from strains) [25]. The cyanobacteria biovolume composition were pooled for MB and NG and then compared using the Mann-Whitney test. Within NG the differences between sampled stations were compared using repeated-measures ANOVA while within MB a non-parametric test (Friedman Tukey-Dist test) was performed.

During the water treatment processes, the efficiency of the treatment step was estimated based on the concentration of MC along the process. In addition, cell lysis was determined based on the concentration of dissolved MC along the treatment processes. Differences in MC concentration between the abstraction points and along treatment process were tested using t-test and one-way repeated measures ANOVA and TukeyHSD post hoc. However, when necessary non-parametric tests i.e., Mann-Whitney were applied.

The differences of MC concentrations amongst the sampled sites in MB and NG were tested using a one-way ANOVA. Later, the sum of the identified MC structural variants was summarized under MC-LR equivalents in order to explore the relationship with environmental variables. We predicted the MC concentrations using Support Vector Classifier (SVC) using the SVM function in caret package in R for four categories in MC concentration: low (<1 µg Equiv. MC-LR L^{-1}), moderate (1-5 µg equiv. MC-LR L^{-1}), high (6-10 µg equiv. MC-LR L^{-1}) and very high (>10 µg equiv. MC-LR L^{-1}). The data used for the SVC were centered and scaled to reduce the variability in the datasets.

A Partial Least Square (PLS) regression model was used to explore the dependence of MC concentration on environmental factors including the cell abundance or biovolume of the two potential toxigenic cyanobacteria, *Microcystis* spp. and *Dolichospermum* sp. and other environmental factors that can cause variability in MC production such as temperature, light, water column stability, pH, nutrient sources ($NO3^-$, $NH4^+$, SRSi, SRP, and N:P ratio) and meteorological variables (mean air temperature, mean wind speed of five days (D5), total rainfall of five days (D5) and mean solar radiation of 10 days (D10) before sampling) and wind

direction on the day of sampling. Correlations between variables are shown in **Table S5**. Data in NG and MB were pooled together, and the Pearson product-moment partial correlation coefficient (r) was used to explore the correlations between MC concentrations and exploratory variables from the PLS model. All statistical tests and graphical outputs were produced using R (version 4.0.2) integrated in RStudio environment (version 1.3.1093).

Author Contributions: MO; MT; WO; RS; JFH; CQ; RK and CB conceptualized the study design. MO; MT; WO; RS; KB; RK and CB performed the experiment and collected the data. MO; MT; WO; KB; RK; BM and CB performed laboratory data and statistical analysis. MO; RS; JFH; CQ; BM; RK and CB wrote the paper.

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Supplementary Materials

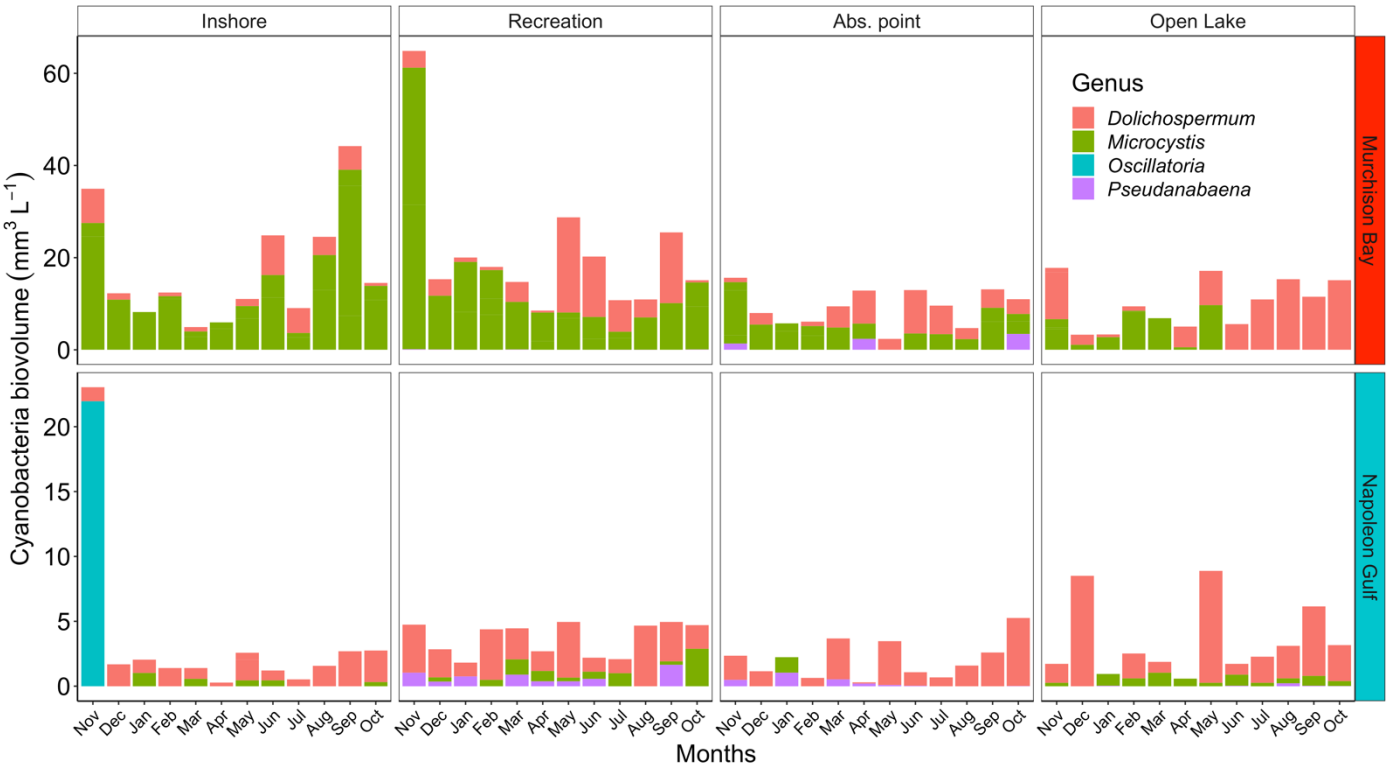

Figure S1. The temporal dynamics of the most abundant toxigenic cyanobacteria (see Figure 1) recorded from the lake survey at the sampling stations: i) inshore, ii) recreational area, iii) water treatment abstraction point (Abs. point), iv) open lake, in Murchison Bay and Napoleon Gulf. (Data collected between Nov 2017 - Oct 2018, n=120).

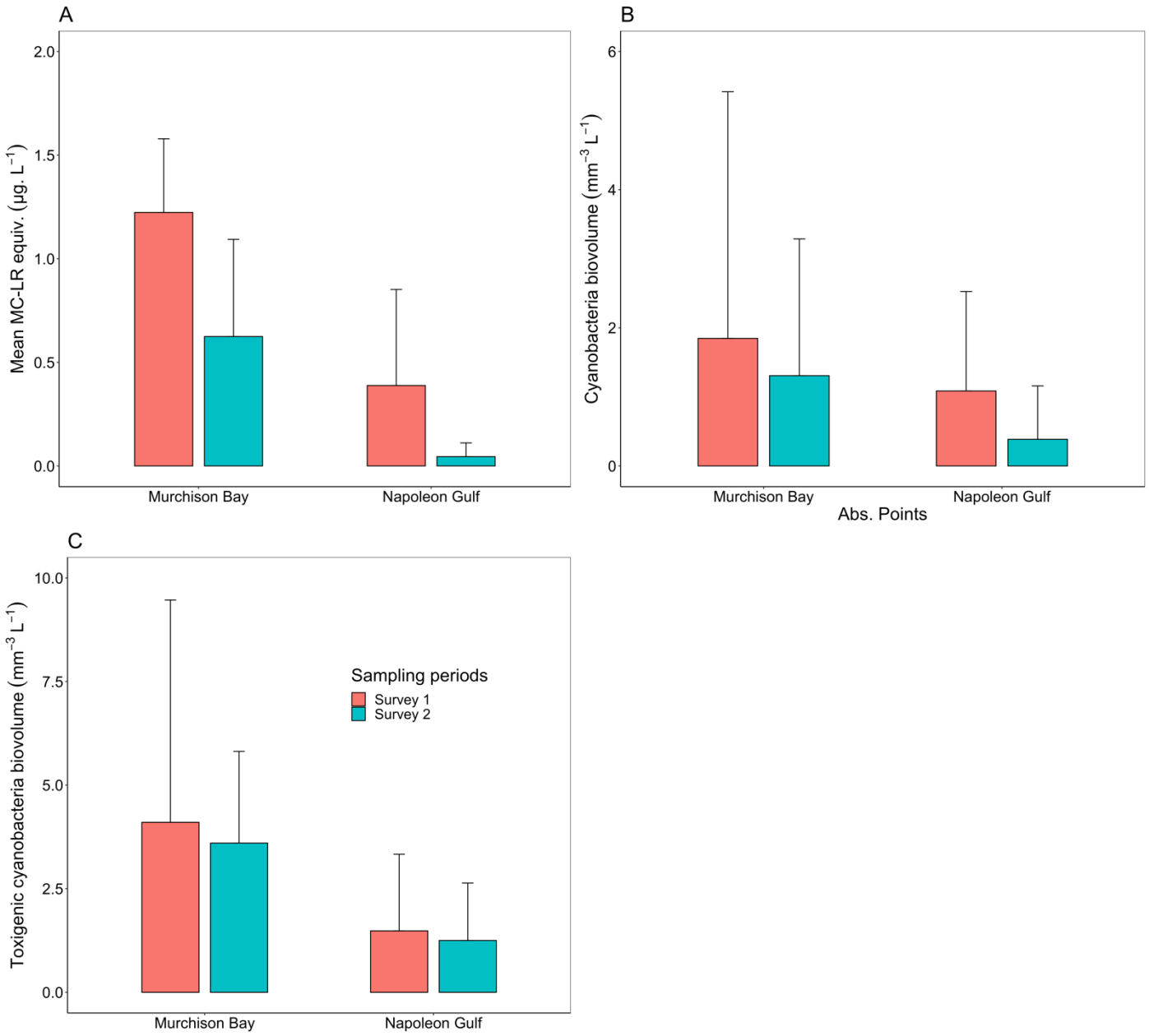

Figure S2. The mean (±SD) MC concentration (A), total cyanobacteria biovolume (B), and toxigenic cyanobacteria biovolume (C) at the water treatment abstraction point (Abs. point) in Murchison Bay and Napoleon Gulf during survey 1 (November 2016 - January 2017) and survey 2 (November 2017 - October 2018).

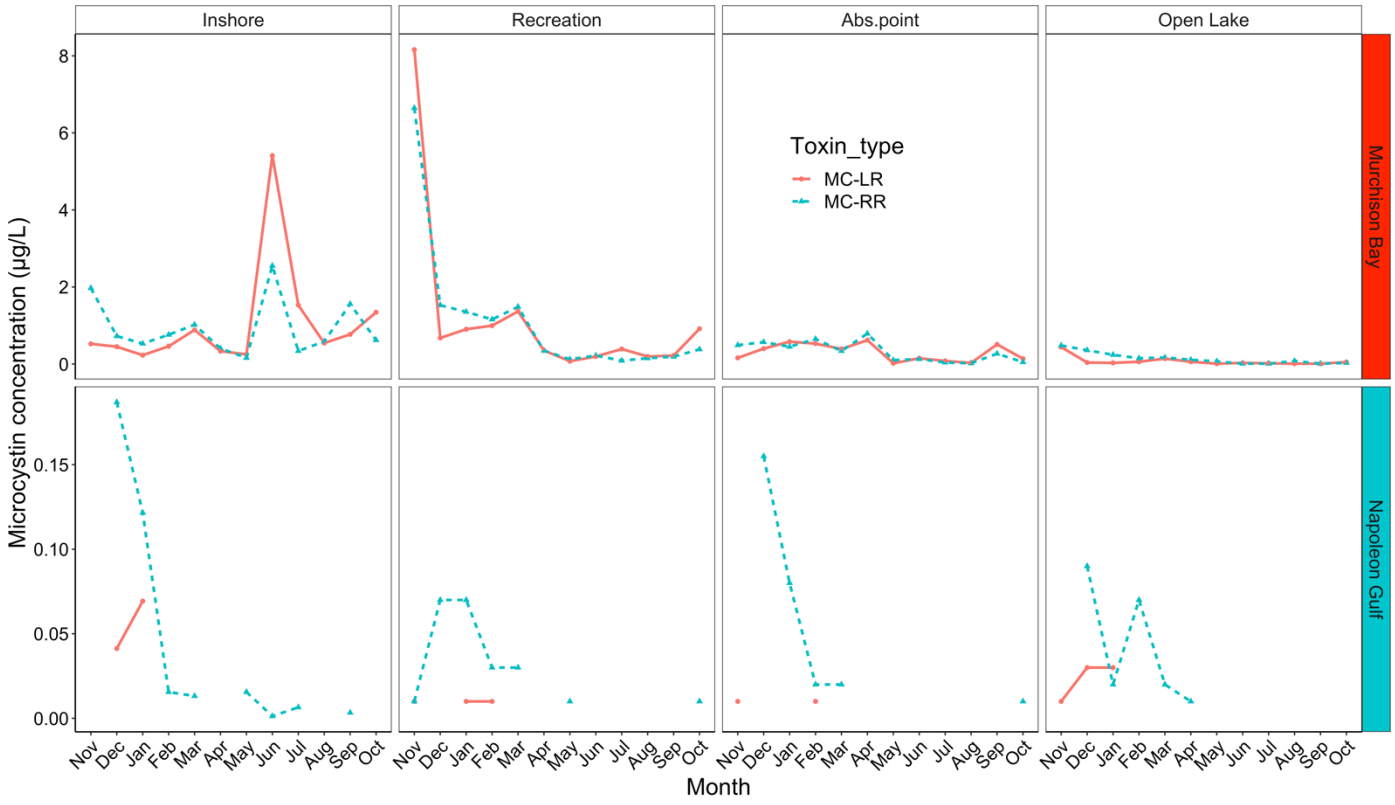

Figure S3. The temporal variation in intracellular MC concentration recorded from Murchison Bay (upper part) and Napoleon Gulf (lower part), northern Lake Victoria. Note: Abs. point= is the water treatment abstraction point (Data collected between Nov 2017 -Oct 2018, n=96). MC-YR was detected only once (0.29 µgL^{-1} in June 2018) at inshore station of Murchison Bay. Note that the scales differ between MB and NG.

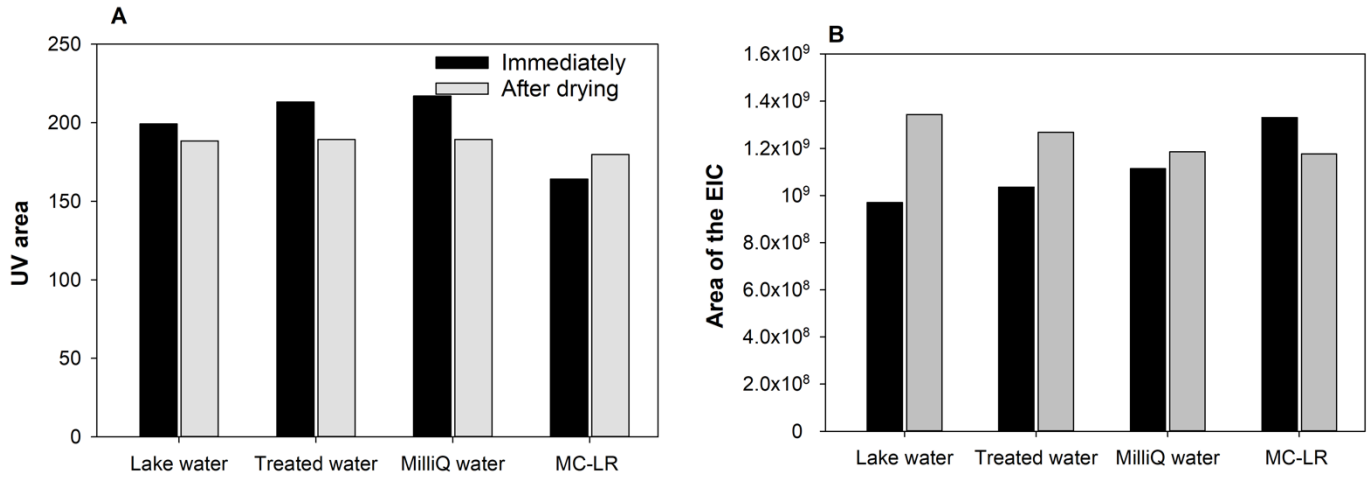

Figure S4. Recovery of MC-LR dissolved in GF/C filtered lake water (Mondsee), treated water and Millipore water using C18 SPE columns (Sep-Pak ® Vac tC18 cartridge 100mg) which have been either eluted immediately, or after drying at 50°C and stored frozen (-20°C) (24 h). The UV chromatogram area (A), and extracted ion chromatogram (EIC) areas from LC-MS analysis are shown.

Table S1. The frequency of detection (percentage) of dissolved and intracellular MC detected and identified along the water treatment process in MB-Gaba and NG-Walukuba (n=90, with 45 samples from MB-Gaba and NG-Walukuba each, i.e., 9 samples from each treatment step). Note Abs. point =Lake water, RW=Raw water, CFW=Coagulated and flocculated water, SFW=Sand filtered water, and FW=Final water) and (-) = not detected,

Dissolved MC	**Ret. time (min)**	**Mass ($M+H^+$)**	**MB-Gaba**					**NG-Walukuba**				
			Abs. point	**RW**	**CFW**	**SFW**	**FW**	**LW**	**RW**	**CFW**	**SFW**	**FW**
MC-RR	13.1-13.3	1038.5	-	-	55.6	77.8	-	-	-	-	-	-
[NMeSer^7]-MC-RY	15.7-16.1	1063.5	-	-	44.4	88.9	11.1				-	-
MC-YR	17.3-17.5	1045.5	44.4	-	44.4	66.7	-	-	-	-	-	-
MC-LR	18.6-18.9	995.5	-	-	55.6	100	-	-	-	-	-	-
[Asp^3]-MC-RY	20.8	1031.5	-	-	-	11.1	-	-	-	-		-
[MeAsp^3]-MC-RY	22.1-22-2	1045.5	11.1	22.2	77.8	77.8	-		-	-	-	
Intracellular MC			**MB-Gaba**					**NG-Walukuba**				
			Abs. point	**RW**	**CFW**	**SFW**	**FW**	**LW**	**RW**	**CFW**	**SFW**	**FW**
MC-RR	13.1-13.3	1038.5	100	33.3	-	-	-	44.4	-	-	-	-
[NMeSer^7]-MC-RY	15.7-16.1	1063.5	88.9	33.3	-	-	-	88.9	-	-	-	-
MC-YR	17.3-17.5	1045.5	100	33.3	-	-	-	66.7	-	-	-	-
MC-LR	18.6-18.9	995.5	100	33.3	-	-	-	44.4	-	-	-	-
[Asp^3]-MC-RY	20.8	1031.5	55.6	22.2	-	-	-	-	-	-	-	-
[MeAsp^3]-MC-RY	22.1-22-2	1045.5	100	33.3	-	-	-	-	-	-	-	-

Table S2. Mean (± SD) dissolved MC in lake water in MB and NG, and during the treatment processes as analysed by ELISA (n =90) and HPLC (n =90). Note Abs. point=Lake water, RW=Raw water, CFW=Coagulated and flocculated water, SFW=Sand filtered water, and FW=Final water)

Station/Site	Treatment	MC (µg/L) (mean ±SD)		MC variant
		ELISA	HPLC-MS	
MB-Gaba	Abs. point	0.26±0.08	0.85±0.53	MC-RR; MC-LR; [NMeSer^7]-MC-YR
	RW	0.46±0.17	0.13 ±0.12	[NMeSer^7]-MC-YR
	CFW	0.53±0.41	0.33 ±0.36	MC-RR; MC-LR; [NMeSer^7]-MC-YR
	SFW	1.05±0.75	0.10±0.05	MC-RR; MC-LR; [NMeSer7]-MC-YR
	FW	0.02±0.001	0.02±0.05	[NMeSer^7]-MC-YR
NG-Walukuba	Abs. point	0.13±0.02		
	RW	0.12±0.07		
	CFW	0.03±0.02		
	SFW	0.04±0.03		
	FW	0.04±0.00		

Table S3. Recovery of MC-LR, MC-RR, MC-YR added to GF/C filtered water (1.0 µg MC-L^{-1}) to control for SPE conditions during individual water treatments. MB denotes Murchison Bay and NG is Napoleon Gulf. The hyphen "-" means not spiked.

	MB-Gaba (µg/L)			NG-Walukuba (µg/L)		
Water sample	MC-RR	MC-YR	MC-LR	MC-RR	MC-YR	MC-LR
Lake water (Abstraction point)	1.60	1.63	1.45	1.43	1.75	1.91
Raw water	-	2.09	-	1.49	-	1.04
Coagulated and flocculated water	1.03	1.31	1.35	-	-	-
Sand filtered water	-	-	-	1.49	1.63	1.78
Final water	-	-	-	-	-	1.29

Table S4. List of cyanobacteria strains used as controls for cyanotoxin extraction and detection via LC-MS/MS (during field survey Nov 2017 - Oct 2018). Strains used as a reference for cyanotoxins were cultured at Muséum National d'Histoire Naturelle (MNHN) (PMC=Paris Museum Collection) or Mondsee (MU=Murchison Bay, Lake Victoria, 19G6=Lake George and NAP, Napoleon Gulf, Lake Victoria). √, means detected; –, means not detected.

Strain	Culture collection	Anatoxin-a	Homoanatoxin	Cylindrospermopsin	Saxitoxins	MC-LR, RR, YR
parent ion (M+H)		166.1226	173.1310	416.1234	300.1415	995.56/1038.57/1045.54
Fragmentation products (M+H)		149.097/ 131.086	163.12/ 145.10/135.12/ 162.13	176.12/194.13/224.13/ 274.09/301.13/318.16	282.13/179.09/ 258.12/186.10	135.08/213.08/375.19
Retention time (min)		1.2	1.8	1.7	0.7	8.8/7.3/8.7
Phormidium favosum (PMC 972.16)	MNHN	√	–	–	√	–
Phormidium favosum (PMC 974.16,)	MNHN	–	√	–	–	–
Phormidium favosum (PMC 240.04)	MNHN	√	√		–	–
Raphidiopsis raciborskii (PMC 99.03),	MNHN	–	–	√	–	–
Aphanizomenon gracile (PMC 638.10)	MNHN	–	–	–	√	
Microcystis aeruginosa (PMC 728.11);	MNHN	–	–	–	–	√
Microcystis sp. PMC 1082.18	MNHN	–	–	–	–	–
Microcystis sp. PMC 1083.18	MNHN	–	–	–	–	–
Microcystis sp. PMC 1084.18	MNHN	–	–	–	–	–
Microcystis sp. MU09	MNHN	–	–	–	–	–
Microcystis sp. MU17	Mondsee	–	–	–	–	–
Microcystis sp. 19G6	Mondsee	–	–	–	–	–
Microcystis sp. NAP07	Mondsee	–	–	–	–	√
Microcystis sp. NAP17	Mondsee	–	–	–	–	

Table S5. Pearson correlation coefficients calculated between environmental parameters recorded during field sampling (n=120) between Nov 2017-Oct 2018.

	Secchi	Sampling Depth	Temp	pH	Oxygen (mg/L)	Oxygen (%)	Turbidity	SRP	NO3	NH4	N:P	SRSi	TN	TP	Rainfall_D5	MeanSolar Radiation_D10	MeanAir Temp	MeanRelativeHumidity	MeanWind speed_D5
Secchi (m)																			
Sampling depth (m)	0.67																		
Temperature (°C)	0.09	-0.03																	
pH	0.22	0.13	0.47																
Oxygen (mg/L)	0.16	0.01	0.27	0.64															
Oxygen saturation (%)	0.15	0.00	0.33	0.65	1.00														
Turbidity (NTU)	-0.36	-0.36	0.04	-0.08	0.10	0.11													
SRP (µg/L)	-0.06	-0.02	0.14	-0.04	0.02	0.04	0.59												
NO3 (µg/L)	0.11	0.14	0.00	-0.10	0.07	0.07	0.18	0.34											
NH4 (µg/L)	-0.17	-0.11	0.08	-0.03	-0.08	-0.07	0.38	0.58	0.29										
N:P	0.03	0.13	0.03	-0.06	-0.01	-0.01	0.19	0.23	0.82	0.38									
SRSi (µg/L)	-0.21	-0.11	0.24	0.00	0.14	0.15	0.47	0.68	0.31	0.49	0.20								
TN (µg/L)	-0.12	-0.05	0.23	-0.02	0.11	0.13	0.52	0.39	0.27	0.48	0.39	0.34							
TP (µg/L)	-0.14	-0.04	0.30	0.15	0.31	0.34	0.46	0.44	0.28	0.45	0.26	0.51	0.71						
Rainfall_D5 (mm)	0.15	0.10	0.08	-0.05	0.06	0.06	0.10	0.16	0.55	0.10	0.43	0.10	0.16	0.13					
MeanSolarRadiation_D10 (W/m2)	-0.67	-0.26	-0.26	-0.17	-0.06	-0.07	0.38	0.21	0.10	0.31	0.23	0.25	0.25	0.27	-0.09				
MeanAirTemp (°C)	-0.34	-0.07	0.23	0.12	0.11	0.13	0.33	0.30	0.01	0.19	0.05	0.25	0.32	0.34	-0.17	0.59			
MeanRelativeHumidity (%)	0.21	0.08	-0.13	-0.28	-0.03	-0.05	0.06	0.10	0.19	0.12	0.19	0.00	0.08	0.06	0.10	-0.10	-0.50		
MeanWindspeed_D5 (km/s)	-0.25	-0.11	-0.46	-0.07	0.24	0.21	0.29	0.25	0.14	0.32	0.17	0.20	0.17	0.22	-0.13	0.65	0.28	0.10	
Wind direction from (°)	-0.07	0.03	0.04	0.15	0.19	0.19	0.12	0.10	0.26	0.15	0.22	0.27	0.10	0.18	0.20	0.12	0.14	-0.42	0.24