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**THE DYNAMICS OF CYANOBACTERIA AND POTENTIAL
THREATS OF CYANOTOXINS IN UGANDA: A CASE OF
MURCHISON BAY AND NAPOLEON GULF, NORTHERN LAKE
VICTORIA**

BY

MARK OLOKOTUM

**BSC (FISHERIES & AQUACULTURE),
MSC ZOOLOGY, MAKERERE UNIVERSITY,
MSC LIMNOLOGY & WETLAND MANAGEMENT, BOKU,
EGERTON & UNESCO-IHE**

**A THESIS SUBMITTED TO THE DIRECTORATE OF RESEARCH
AND GRADUATE TRAINING FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY OF MAKERERE UNIVERSITY**

September 2023

DECLARATION

I, **Mark Olokotum** declare that this thesis is original, unless otherwise stated and dully acknowledged. Apart from submissions for publications, this work has not been submitted to any university for the award of any degree.

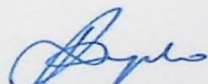
Signature:  Date: 13th / Sept / 2023

APPROVAL

This thesis entitled “**The Dynamics of Cyanobacteria and Potential Threats of Cyanotoxins in Uganda**” by **Mark Olokotum** is approved for the degree of Doctor of Philosophy (PhD) of Makerere University. It has been submitted with the approval of the following supervisors:

Ronald Semyalo (PhD)

Department of Zoology, Entomology and Fisheries Sciences (ZEFS), School of Biosciences, College of Natural Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda

Signature:  Date: 13.09.2023

William Okello (PhD)

Fish Habitat Management Programme, National Fisheries Resources Research Institute (NaFIRRI), P. O. Box 343, Jinja, Uganda

Signature:  Date: 13th September 2023

Prof. Cecile Bernard (PhD)

Cyanobacteria, Cyanotoxins and Environment (CCE), Museum National d'Histoire Naturelle (MNHN), 12 rue Buffon, Paris, France

Signature:  Date: 13 Septembre 2023

DEDICATION

The girls in my life: Violet Atugonza Olokotum, Abonyo Hailey Paige and Angom Hannah Peyton, and parents: Mr & Mrs George Egonga.

ACKNOWLEDGMENT

The PhD thesis is the culmination of five years of graduate work that started at the University of Natural Resources and Life Sciences (BOKU), Vienna, Austria. Following that, I spent over 16 months conducting extensive field work and research on Lake Victoria. Dr. William Okello and Prof. Rainer Kurmayer were the drivers behind this success. Thank you, Dr. William Okello, for introducing me to limnology and cyanobacteria research, for which I will be eternally grateful. I am beholden to Dr Jean-François Humbert and Dr Catherine Quiblier for the warm welcome and hosting me in Paris during the synopsis of this study in Spring of 2017. I do appreciate the Directors of Research of the National Fisheries Resources Research Institute (NaFIRRI); Dr Anthony Taabu-Munhyaho, and Dr Winnie Nkalubo for the support during the PhD life cycle.

I would like to thank my other supervisors Prof. Cecile Bernard, Dr William Okello and Dr Ronald Semyalo for the guidance right from developing the synopsis to data collection, analysis and write up of this thesis. I would like to also thank Dr Joseph Kyambadde and Dr Robinson Odong of Makerere University for serving diligently as part of my doctoral committee, great mentors both personally and professionally. Although Dr Marc Troussellier is not an official member of my doctoral committee, he has always provided guidance and mentorship during the study and write up.

The chapters in this thesis have benefited from the contributions of co-authors, thus I would like to acknowledge: Veronica Mitroi and Bernard Montuelle (Socio ecological analysis of the state of the knowledge on cyanobacteria), Marc Troussellier and Arthur Escalas (phytoplankton dynamics and turnover in chapter four), Benjamin Marie, Kathrin Baumann and Rainer Kurmayer (threats of toxigenic cyanobacteria and cyanotoxins); Jean-François and Catherine Quiblier (bottom-up and top-down control mesocosm planning and experimentation in Uganda). To all the reviewers for the published papers, thank you.

During this journey, I benefited from several institutions including: Center for Cyanobacteria and Cyanotoxins (CCE) of MNHN, Paris France, University of Montpellier, France, University of Bergen, Norway, National Water and Sewerage Corporation (NWSC), NaFIRRI, Uganda National Meteorological Authority (UNMA), where either data were collected, obtained, analysed, or specialized training undertaken during the study. I am indebted to my crews at Gaba KK beach (Joseph Bikanga) and Rippon pier (Muhammed Madoi) for the assistance during boat operations, Mukasa Stephen for driving me during the wee hours for the sampling campaigns, Magezi Godfrey and Sahima Hamalou for the phytoplankton

identification, Janet Naluwairo and Henry Ocaya during nutrient analyses especially the simultaneous works from the mesocosm experimentation. The community in Paris and Montpellier, France; Sandra Kim Lang, Alison Gallet, Justine Deemay, Eric Kaumme Yao, Matias Ahoutou Koffi; Claude Yepremian, Charlotte Duval, and Haietz Aloui made it easy for me at the lab and during the specialized trainings and data analysis.

My studies were made possible through financial support from the French Facility for Global Environment (Fonds Français pour l'Environnement Mondial, FFEM) under the Water Sources in Africa (WaSAf) research program. In addition, the initial financial support of the Austrian Development Agency (ADA) throughout the International Postgraduate Training in Limnology (IPGL) in Austria, Kenya, and the Netherlands. During the final stages of the study, through the training committee of National Agricultural Research Organization (NARO), the Director General of NARO granted me study leave (3 months) to complete the PhD training. I am grateful.

To my girls; my wife Violet and my daughters Paige and Peyton thanks for your support and love, patience and understanding during my long absence from home. My parents, Mr, and Mrs Egonga George, you have always supported my cause since the days of swimming in River Mubuku, then undergraduate studies in Fisheries was a calling, Thank You and I hope I have made you proud.

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LIST OF PUBLICATIONS

- Olokotum, M., Mitroi, V., Troussellier, M., Semyalo, R., Bernard, C., Montuelle, B., Okello, W., Quiblier, C., and Humbert, J.-F. (2020). A review of the socioecological causes and consequences of cyanobacterial blooms in Lake Victoria. *Harmful Algae*, 96, 101829. <https://doi.org/10.1016/j.hal.2020.10182>
- Olokotum, M., Troussellier, M., Escalas, A., Hamlaoui, S., Okello, W., Semyalo, R., Humbert, J.-F., and Bernard, C. (2021). High nutrient loading and climatic parameters influence the dominance and dissimilarity of toxigenic cyanobacteria in northern bays of Lake Victoria. *Journal of Great Lakes Research*, 47, 985-996, <https://doi.org/10.1016/j.jglr.2021.04.021>
- Olokotum, M., Humbert, J.-F., Quiblier, C., Okello, W., Semyalo, R., Troussellier, M., Marie, B., Baumann, K., Kurmayer, R., and Bernard, C. (2022). Characterization of Potential Threats from Cyanobacterial Toxins in Lake Victoria Embayments and during Water Treatment. *Toxins*, 14(10), 664. <https://doi.org/10.3390/toxins14100664>
- Olokotum, M., Humbert, J.-F., Quiblier, C., Okello, W., Semyalo, R., Marie B, Bertrand C., Bernard, C., and Troussellier, M. (2023). Differential response of planktonic communities to nutrient and fish (Nile tilapia) addition in Lake Victoria: mechanisms and implications using experimental approaches (*Manuscript in preparation*).

LIST OF ACRONYMS

ANOVA:	Analysis of Variance
ATX:	anatoxin-a
B&Gs:	Bays and Gulfs
CCE:	Cyanobacteria, Cyanotoxins and Environment
CoNAS:	College of Natural Sciences
CYN:	cylindrospermopsin
DWRM:	Directorate of Water Resources Management
ELISA:	Enzyme-linked immunosorbent assay
EDTA:	Ethylenediamine tetra acetic acid
G&Ss:	Goods and services
HTX:	homoanatoxin
KCCA:	Kampala Capital City Authority
LC-MS:	Liquid Chromatography - Mass Spectrometry
LV:	Lake Victoria
LVEMP:	Lake Victoria Environmental Management Project
MB:	Murchison Bay
MC:	microcystins
MNHN:	Museum National d'Histoire Naturelle
NaFIRRI:	National Fisheries Resources Research Institute
NARO:	National Agricultural Research Organization
NG:	Napoleon Gulf
NWSC:	National Water and Sewerage Corporation
OL:	Open Lake
PERMANOVA:	Permutational multivariate analysis of variance
PLS:	partial least squares
SDG:	Sustainable Development Goal
TDI:	Tolerable Daily Intake
UNMA:	Uganda National Meteorological Authority
WaSaf:	Water Sources in Africa
WHO:	World Health Organization
WTPs:	Water Treatment Plants
ZEFS:	Department of Zoology, Entomology and Fisheries Sciences

ABSTRACT

Lake Victoria has diverse cyanobacteria associated with varying types and concentrations of cyanotoxins. Over 31 variants of microcystins (MC) have been identified in the lake with MC-LR, MC-RR and MC-YR as the commonest. Therefore, this study determined the factors associated with cyanobacteria and cyanotoxins dynamics, and potential health risk of cyanotoxin exposure routes in Lake Victoria. For a comparative assessment, data were collected from the lake, water treatment plants (WTPs) and mesocosm experiments in Napoleon Gulf (NG) and Murchison Bay (MB) between November 2016 and October 2019. Cyanobacteria were identified based on morphological features while cyanotoxins, was based on enzyme-linked immunosorbent assay (ELISA), and Liquid Chromatography-Mass Spectrometry (LC-MS). Although the phytoplankton community was similar, their dynamics were driven by the dominant cyanobacteria, *Microcystis flos-aquae* and *M. aeruginosa* in MB and *Dolichospermum circinale* and *Planktolyngbya circumcreta* in NG. Thus, closed embayments, such as MB more susceptible to toxigenic cyanobacteria with potential cyanotoxin production. Thus, two classes of cyanotoxins were detected: MC and homoanatoxin (HTX) an analogue of anatoxin-a (ATX). Considering MC, significantly higher concentration ($5\text{--}10\text{ }\mu\text{g MC-LR equiv. L}^{-1}$) was detected in MB compared to NG. This frequently exceeded the recommended sanitary threshold of $>100,000\text{ cells}\cdot\text{mL}^{-1}$ and $1\text{ }\mu\text{g}\cdot\text{L}^{-1}$ of MC-LR for exposure via drinking water. However, the WTPs efficiently removed the cyanobacteria cells, intracellular and dissolved MC to $<1\text{ }\mu\text{g}\cdot\text{L}^{-1}$ of MC-LR. The observed MC have been related to the biovolume of *Microcystis*, influenced by solar radiation, mean wind speed (N-S direction) and turbidity. From the in-situ mesocosm experiments, continuous eutrophication will enhance the growth of *Microcystis* and consequently increase MC production and concentrations. As Nile tilapia consumed cyanobacteria, such as *Microcystis* sp. there was transfer of MC into the intestines of fish (up to $27.5\text{ }\mu\text{g/g FW of MC-LR}$) with MC detection in the liver (up to $0.48\text{ }\mu\text{g/g of MC-LR FW}$) and muscle (up to $0.3\text{ }\mu\text{g/g FW of MC-LR}$) in MB. This could increase human exposure through fish consumption. Therefore, due to the dominance of toxigenic *Microcystis* there is need for regular sensitization during bloom events and integration of *Microcystis* biovolume estimation in WTPs are recommended. Locals should desist from using contaminated lake water for domestic purpose but rather use treated water and reduce recreational activities during bloom events. From this perspective, there should be dedicated water quality and fish monitoring from water bodies with toxigenic cyanobacteria and evaluate the potential exposure risks to humans.

CHAPTER ONE: INTRODUCTION

1.1 General background to the study

Lake Victoria has been the subject of various studies, research, and innovations for example the 1927 expedition by Graham (Worthington, 1932). There will always be a knowledge gap and need to learn more about and understand this incredible environment. Since the 1950s, the lake has been subjected to human-induced perturbations, exotic species introductions, particularly Nile perch, Nile tilapia, and water hyacinth, pollution, and eutrophication, and, most recently, climate change. Particularly, the history and timing of eutrophication in the lake dates back as early as 1940s to early 1950s (Kenyan inshore areas), late 1960s to early 1970s (open lake), and from the mid-1970s to mid-1980s in Ugandan inshore areas (Stager et al., 2009). Despite the variability in this timing of eutrophication interests in studying the impacts of eutrophication in East Africa peaked in the late 1990s.

As a result, Lake Victoria serves as an excellent example of the enormous challenge that developing countries face in terms of long-term management, monitoring, and protection of their surface water resources. Eutrophication resulting from high human population growth rates (2.4 – 3.2% per annum) higher than that in the rest of the African continent (UNEP, 2006). and related activities lead to recurrent proliferations of water hyacinth and cyanobacteria blooms (Wilson et al., 2007). In the last 20 years, several surveillance programs, including two phases of the Lake Victoria Environmental Management Project (LVEMP I and II) and other research programs, have collected water quality data. However, in the early 2000s, there was a surge of interest in cyanobacteria and cyanotoxins (e.g., Ochumba & Kibaara, 1989), and by the late 2000s, there had been some breakthroughs and classifications of toxin in lake water (e.g., Krienitz et al., 2002; Sekadende et al., 2005; Okello et al., 2010a; Sitoki et al., 2012).

Lake Victoria supports human well-being with goods and services such as drinking water and inland fisheries in East Africa (Balirwa et al., 2003). However, the lake is increasingly threatened by pollution. In the Ugandan part, it has become increasingly eutrophic due to human activities since the 1950s. Consequently, the induced phytoplankton dynamics from the dominance of diatoms in the 1960s to cyanobacteria in most of the open and closed bays of the lake (Talling, 1966). The dominant cyanobacteria taxa reported in most bays of Lake Victoria are *Dolichospermum* (previously *Anabaena*), *Aphanocapsa*, *Raphidiopsis* (formerly *Cylindrospermopsis*), *Planktolyngbya*, *Merismopedia* and *Microcystis* among others (Sekadende et al., 2005; Okello & Kurmayer, 2011; Sitoki et al., 2012; Haande et al., 2011). These cyanobacteria taxa are known to produce toxins (cyanotoxins). In Lake Victoria,

Microcystis spp. has been identified as the major toxin producer (Okello et al., 2010b; Okello & Kurmayer, 2011) that might impair water quality in northern Lake Victoria. Therefore, there is an urgent need to ensure that the goods (e.g., water and fish) and services (e.g., transport and navigation) provided by Lake Victoria are safe for human consumption with proper management strategies in case of cyanotoxins production.

Cyanobacteria are prokaryotic phytoplankton members that are thought to have first colonized the earth over 2.7 billion years ago (Cavalier-Smith, 2006). Cyanobacteria can be found in the natural world as free-living cells, filaments, or colonies suspended in the water column or attached to the sediments of surface waters such as rivers and lakes (Hoek et al., 1995; Chorus, 2012). They contribute a wide range of useful contributions to human well-being (Demay et al., 2021), in addition to their significant contribution to photosynthesis and nitrogen fixation in aquatic ecosystems. For instance, *Limnospira* sp. also known as *Spirulina* sp. has been consumed by some human communities in Chad and Mexico for centuries (Carmichael, 1994) while the nitrogen-fixing species contributes to global soil and water fertility (Chorus & Bartram, 1999). The abundant growth of cyanobacteria in reservoirs and surface water, on the other hand, are sometimes harmful and degrades water quality, posing a threat to water supplies, biodiversity, and productivity.

Cyanobacteria are found in eutrophic waters all over the world and have attracted a lot of attention over the years due to their ability to synthesize and produce secondary metabolites. These secondary metabolites are either nontoxic or toxic. However, most harmful algal blooms have been linked to the production of toxic metabolites (cyanotoxins). Nonetheless, humans may be exposed to these dangerous cyanotoxins. The primary routes of exposure for these toxins are both oral and dermal, and include drinking water, contaminated fish, certain food supplements, and recreation on lakes and rivers. Although it is remarkably easy to identify acute cases of cyanotoxins exposures, those of chronic exposures are difficult to assess. For instance, fish can accumulate cyanotoxins by ingestion of toxigenic cyanobacteria that subsequently find their way up into the food chain. Therefore, in 1996, the World Health Organization (WHO) established 1.0 µg/L as a guideline on allowable concentrations of the microcystins (MC) variant MC-LR in drinking water. Furthermore, guidelines for recreational water were developed based on chlorophyll-a and the abundance of cyanobacterium *Microcystis* sp. (Table 1.1; Chorus & Bartram, 1999). Uganda, like any developing countries lacks established alert thresholds based on the WHO guidelines. This threshold should act as triggers for water quality risk assessment and monitoring programmes. While these indirect

monitoring thresholds are subject to variations, MC risk assessment has not been done in Uganda.

Table 1. 1: The WHO recreational guidelines for cyanobacteria abundance, structural microcystin variant MC-LR, and chlorophyll-a in surface waters. Source: Chorus & Bartram (1999).

Cyanobacteria (cells/mL)	Microcystin-LR ($\mu\text{g/L}$)	Chlorophyll-a ($\mu\text{g/L}$)	Probability of exposure
<20,000	<10	<10	Low
20,000-100,000	10-20	10-50	Moderate**
100,000-10,000,000	20-2,000	50-5,000	High
>10,000,000	>2,000	>5,000	Very high

**Most of the studies conducted in northern Lake Victoria reported values with moderate probability of acute health toxicity

Cyanotoxins are classified into five categories based on their effects as: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, and irritant toxins (lipopolysaccharides) (Sivonen & Jones, 1999). However, based on the chemical composition, cyanotoxins fall into three categories, *i.e.*, cyclic peptides (microcystins and nodularins), alkaloids (anatoxin-a, anatoxin-a (S), saxitoxins, aplysiatoxin, cylindrospermopsin, lyngbyatoxin-a) and lipopolysaccharides (LPSs) (Kaebernick & Neilan, 2001). Apart from the cytotoxic cylindrospermopsin (CYN) which occurs in dissolved form, the toxins are mostly inside the cyanobacterial cells and can be released in high concentrations during cell lysis (Sivonen & Jones, 1999). Microcystins (MC) are cyclic peptides with seven amino acids giving it structural heterogeneity. More than 240 variants of MC are described to date (Spoof & Catherine, 2017) and their different amino acids composition influences their toxicity. The characteristic structure of MC is the unique C20 amino acids 3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyl Deca-4, 6-dienoic acid (ADDA) linked to D-glutamate (Carmichael, 2001). Therefore, MC variants are chemically similar compounds differing in variable amino acids that also influence the toxicity of the MC. Particularly, MC-LR is formed because of the different α -amino acid found at position 2 and 4 thus L= leucine and R = arginine amino acids (**Figure 1.1A**).

The principal neurotoxins produced by cyanobacteria are anatoxin-a (ATX-a), anatoxin-a(s) (ATX-a(S)) and the saxitoxins (or PSP-toxins) which include saxitoxin, neosaxitoxin, the gonyautoxins and the C toxins. ATXs are mainly produced by strains of *Dolichospermum* (formerly *Anabaena*), but also, produced by other genera like *Aphanizomenon*, *Planktothrix*, *Raphidiopsis* (formerly *Cylindrospermopsis*), *Arthrospira*, *Cuspidothrix*, *Microcystis*, *Oscillatoria*, and *Phormidium* (Bernard et al, 2017). ATX-a(S) is one of the main ATX analogues, together with ATX-a and homo-ATX (HITX) (**Figure 1.1B**).

Over the years, ATX-a(S) has been the most potent natural neurotoxin produced by freshwater cyanobacteria (Quiblier et al., 2013). They are water-soluble and have been responsible for several incidents of dog poisoning. The chemical structure of ATXa(S) is not related to that of ATX-a because it is a unique N-hydroxyguanidine methyl phosphate ester. ATX-a(S) is an acetylcholinesterase (AChE) inhibitor with a mechanism like that of the organophosphorus (OP) insecticides (Figure 1.1B) (Colas, et al., 2021; Otero & Silver, 2022). On the other hand, ATX-a and HTX are organophosphate that cause prolonged inhibition of acetylcholinesterase (Colas, et al., 2021).

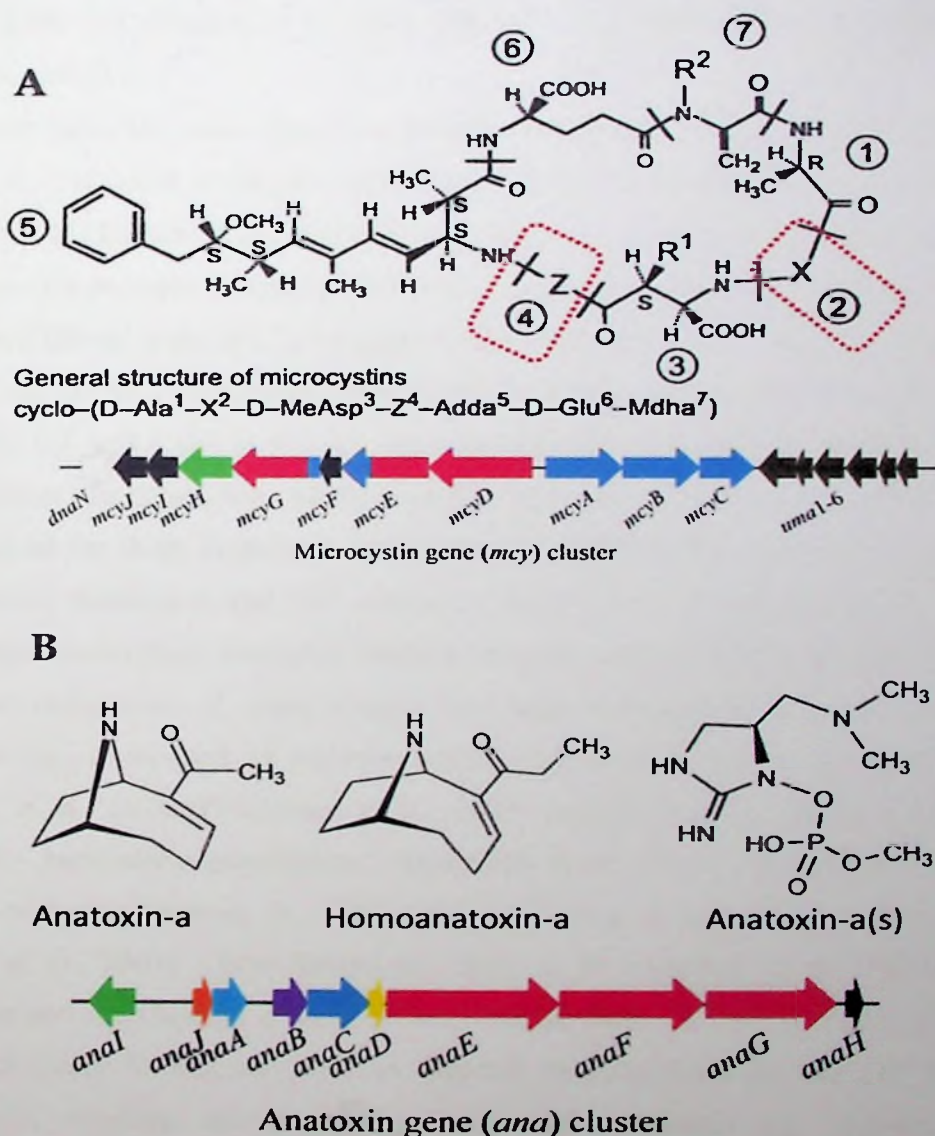


Figure 1. 1: A: The general structure of MC-LR and their biosynthesis gene clusters (*mcy*) in *Microcystis* sp. X and Z are variable amino acids. (In MC-LR, X=L-leucine [L] and Z=arginine [R]); R1 and R2 are H (demethyl microcystins) or CH₃. Source:(Carmichael, 2001; Rastogi et al., 2015). **B:** The chemical structure of anatoxins and its biosynthesis gene (*ana*) cluster in *Oscillatoria* sp. (Source: Rastogi et al., (2015)).

The structural variant MC-LR, produced by the planktonic taxa *Microcystis*, *Dolichospermum*, *Anabaenopsis* and *Planktothrix* is considered as the most toxic (Spoof & Catherine, 2017). The first report of the occurrence of cyanobacteria and MC production in Lake Victoria, was in Nyanza Gulf (Krienitz et al., 2002) after which there were increasing interests in cyanobacterial toxins in the region. The different *Microcystis* sp. such as *M. aeruginosa*, *M. botrys*, *M. flos-aquae*, and *M. wesenbergii* that have reportedly formed blooms in the lake (Okello et al., 2010a). They produced an array of microcystins such as: (MC-RR, (Asp³)-MC-RR, MC-YR, (Asp³)-MC-YR, MC-LR, MC-RY, (Asp³)-MC-RY) and [NMeSer⁷]-MC-YR (Haande et al., 2011; Okello et al., 2010b; Okello & Kurmayer, 2011; Sitoki et al., 2012).

Years later, MC were reported in Mwanza Gulf (Sekadende et al., 2005), Nyanza Gulf (Sitoki et al., 2012) and, in Murchison Bay and Napoleon Gulf (Okello et al., 2010; Okello & Kurmayer, 2011; Haande et al., 2011). These studies have indicated varying concentrations of MC in water for example in Nyanza Gulf from < 1 µg/L (Krienitz et al., 2002) up to 81 µg/L in Nyanza Gulf (Sitoki et al., 2012), 1.8 µg/L in Murchison Bay and < 1.0 µg/L in Napoleon Gulf (Okello et al., 2010). Furthermore, Semyalo et al. (2010) reported both MC in water (ranged from 0.2 to 0.7 µg/L) and in the gut, liver, and muscle tissue of Nile tilapia (*Oreochromis niloticus*) from Murchison Bay. All these studies reported fluctuating levels of MC depending on location of the study areas/bays, and season. In addition, the factors responsible for the cyanobacterial dominance and MC production have not been well elucidated, besides the concentrations in drinking water and potential bioaccumulation in the food chain not reported.

The occurrence of cyanobacteria has been sustained by specific environmental conditions e.g., i) increase in external nutrient inputs from heavily polluted watersheds (Musungu et al., 2014; Fuhrmann et al., 2015; Jovanelly et al., 2015), ii) atmospheric deposition - particularly phosphorus (Tamatah et al., 2005), iii) mixing and string of sediments (Gikuma-Njuru et al., 2005), and iv) biological processes of nitrogen fixation (Mugidde et al., 2003). These factors are likely to be escalated by the increasing water temperature and stratification in water bodies (Paerl & Otten, 2013). Therefore, harmful algal blooms are likely to increase with an increase in eutrophication and global warming, consequently, resulting into several environmental challenges e.g., decrease in light availability, bottom water anoxia (Paerl & Otten, 2013) and alteration of the trophic structure and functionality of the aquatic systems Codd et al. (2017). Based on this background, this study aims to integrate the current state of knowledge on phytoplankton and cyanobacteria,

cyanotoxins dynamics and its health threats assessment on the human health risk routes such as drinking water, fish, and recreation.

1.2 Exposure routes and potential health risks to cyanobacteria and cyanotoxins

Humans are exposed to cyanotoxin via several routes with the oral being the most important through ingestion of contaminated water and food, or accidental consumption during recreation (**Figure 1.2**). Until recently, cyanotoxins became an emerging drinking water contaminant requiring special attention due to the health concerns. Most importantly, many people use both untreated and treated water being therefore leading to exposures to different levels of cyanotoxins. This leads to both acute (short term) and chronic effects in humans exposed to cyanotoxins. Thus, comparison is made on the dynamics of cyanobacterial biomass and cyanotoxins in two contrasting ecological systems (embayments). The study also assesses the biomass-toxin relationships, integrating environmental factors and further performing mesocosms experimental set up to clarify on the environmental factors associated with cyanobacteria dominance and cyanotoxin production. Finally, the study reports on the health threat routes since humans can be directly exposed to cyanotoxins in treated and untreated water, accumulate in food fish and recreation.

The current threats to cyanobacterial toxins are on the increase mainly due to the pressures of excessive nutrient loading (eutrophication) arising from the anthropogenic activities as the main driver, supported by tropical warm climate. This increasing threats from cyanobacterial toxins required intervention that involved the characterization of these threats along the major exposure routes. This intervention has been through identification and quantification of the cyanotoxins produced in the embayments of Lake Victoria, testing the ecological status of the embayments and further project impacts of the increases in the cyanobacterial blooms and generating information to guide the water users around the lake and for drinking water production for the major cities along the shores of the lake. In **Figure 1.3**, the right panel are the drivers, threats, and consequences of the continued eutrophication while the right panel indicates the impact of continued eutrophication and the major interventions of the study and expected outcomes of the study towards sustainable utilization of the surface waters for a healthy population.

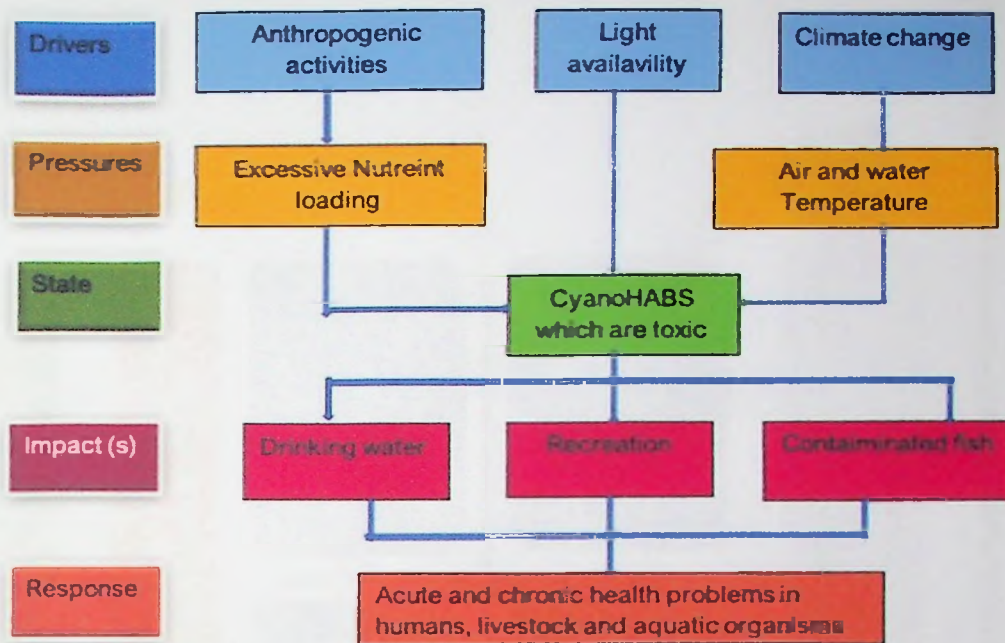


Figure 1. 2: A schematic causes of the drivers of harmful cyanobacterial blooms (CyanoHABS) and the health risks associated with the exposure routes.

1.3 Statement of the problem

Since the early 2000s, there has been increase in cyanobacterial blooms, but the dynamics and proportions of toxigenic and non-toxigenic cyanobacteria has not been well elucidated. Therefore, the potential threat of the types and concentrations of the cyanotoxins have not been assessed in the last two decades. Furthermore, the potential threats to the shoreline residents (locals), fishermen and recreationist, and water treatment plants (WTPs) have not been assessed. There is no data on the efficiencies of the National Water and Sewerage Corporation (NWSC) WTPs that heavily rely on conventional treatment processes of flocculation, rapid gravity sand filtration which may not effectively remove high concentrations of cyanotoxins.

No studies have attempted to model the factors responsible for sustaining toxigenic cyanobacteria and MC (commonest cyanotoxin) concentration in Lake Victoria to guide decision making. In the advent of increasing nutrient loading, the predicted changes in the planktonic community (phytoplankton including cyanobacteria and zooplankton), impacts on the fish community have not been well documented in Lake Victoria. Therefore, the riparian populations directly using lake water, or eating the food fish are exposed directly to different types and concentrations of cyanotoxins.

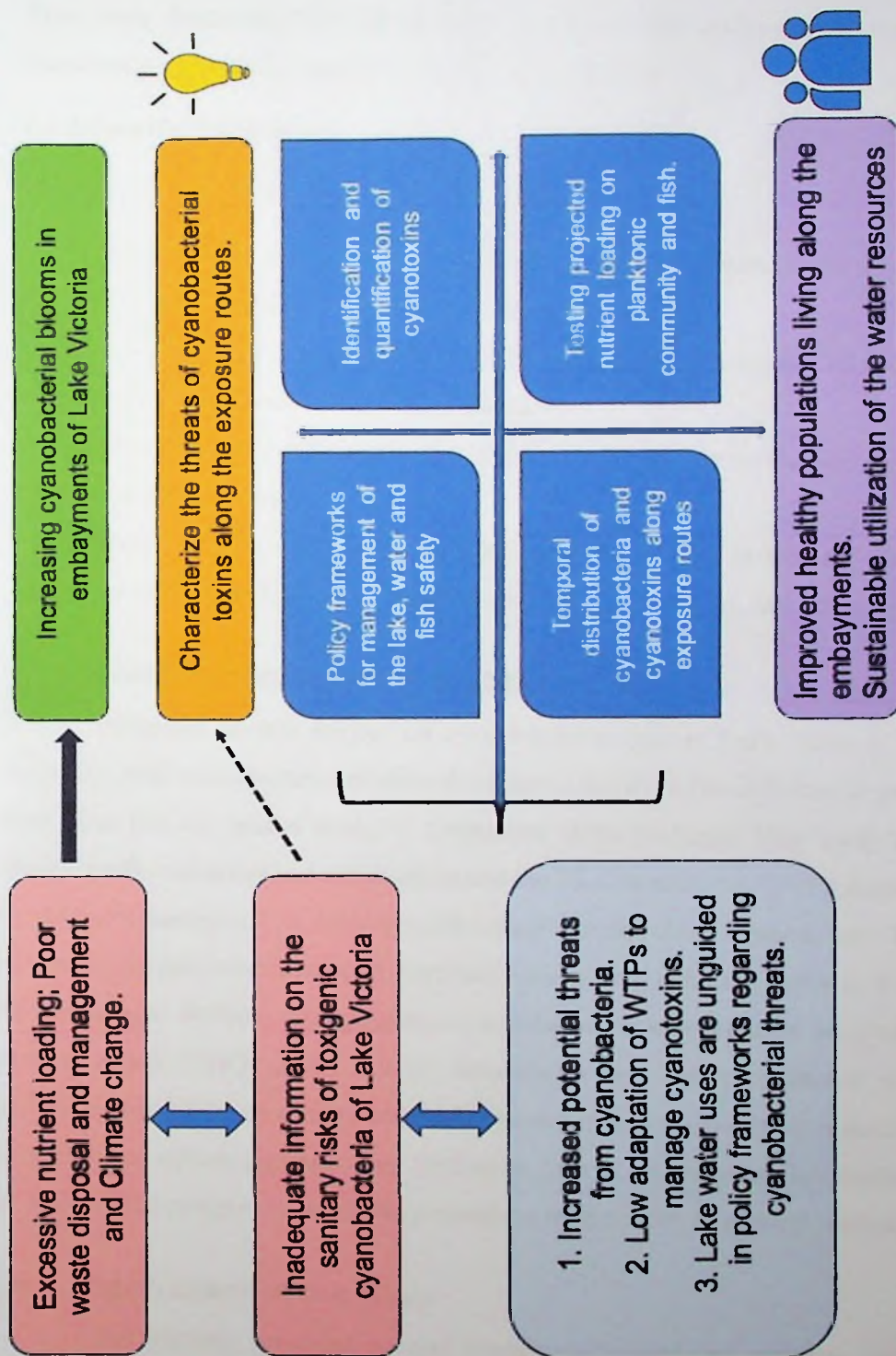


Figure 1. 3: The conceptual framework showing; the major drivers of eutrophication, its consequences (left) and intervention from this study with the projected outcomes for sustainable utilization of the surface waters of Lake Victoria (right).

1.4 Objectives

1.4.1 General Objective

This study determined the dynamics of cyanobacteria and potential threats associated with cyanotoxin exposure routes in Lake Victoria, Uganda.

1.4.2 Specific Objective

The specific objectives of the study were to:

1. Examine the temporal and spatial phytoplankton diversity and dynamics in Murchison Bay (MB) and Napoleon Gulf (NG), Lake Victoria.
2. Determine the micro conditions that govern the production of cyanotoxins within the two embayments in northern Lake Victoria.
3. Determine the efficiency of water treatment plants in the removal of cyanotoxins in potable (drinking) water.
4. Determine the response of planktonic communities to nutrient addition and potential transfer or biomagnification of cyanotoxins in the Nile tilapia *Oreochromis niloticus* L.

1.5 Justification/Importance of the study

Although several studies on cyanobacteria exist in Lake Victoria, the causes of their dynamics and consequences of their dominance are from the different bays and regions of the lake, thus this integrated study to harmonise these findings. This study identified the most severely affected areas and embayments on the lake. In addition, WTPs abstract raw water from the lake are susceptible to both the cell-bound and dissolved cyanotoxins. Therefore, the need to control the potential release of cyanotoxins in treated drinking water and clarify water safety for consumers. Without enough data, no legislative measures can be adopted to implement the recommended WHO guidelines on drinking water, and recreational activities. A better understanding of the cyanobacteria and cyanotoxin and its elimination during water treatment is required to advise management. Therefore, it will be necessary to develop monitoring and management strategies to warn the population in the event of contamination.

1.6 Significance of the study

Lake Victoria provides several ecosystems goods and services, including water and fish. Therefore, the need to control the potential release of cyanotoxins in treated drinking water and clarify water safety for consumers. This study contributes to Sustainable Development

Goal (SDGs) 6 - Clean water and sanitation, and SDG 3 - Good health and well-being. Specifically, targets 3.d: Early warning & global health risks and 6.1- Safe & clean drinking water. The study explores i) sanitary risk assessment of all cyanotoxins; ii) production and supply of safe drinking water which is cyanotoxin free or within acceptable WHO limits; and iii) develops of a dashboard tool for early warning for water safety planning.

1.7 Scope of the study

Despite the many cyanobacterial studies on Lake Victoria, the sanitary and health threats of cyanobacteria and cyanotoxins in source water, drinking water and fish is poorly understood. The scope of the study is limited to northern Lake Victoria using MB as a closed embayment and NG as an open embayment. This study integrates environmental sampling survey in the embayments for 12 months, water treatment processes for 3 months, and mesocosm environmental approaches for 7 days. In this study, microscopy and morphological features were used to identify and enumerate the cyanobacteria. For all classes of cyanotoxins, Liquid Chromatography - Mass Spectrometry (LC-MS) was used to identify and quantify the cyanotoxins, while enzyme-linked immunosorbent assay (ELISA) was used to screen the drinking water samples for MC prior to confirmatory tests using LC-MS analyses.

1.8 Guiding questions and thesis organisation

This thesis is based a literature review, monitoring survey, experimentations, and manipulations with their respective outcomes, and presented as standalone chapters. They are written in the form of manuscripts either published or in preparation for submission in peer reviewed journals. The study determined the dynamics of cyanobacteria and potential threats associated with cyanotoxin exposure routes in Lake Victoria (Uganda). After the introduction, the thesis is composed of six chapters organized following guiding questions in relation to the specific study objectives (**Figure 1.4**):

What is the knowledge on cyanobacteria and cyanotoxins in Lake Victoria? Chapter two, based on extensive literature review, the state of knowledge on cyanobacterial blooms in Lake Victoria allowed to find gaps and similarities on the dynamics of cyanobacteria, socioecological causes, and consequences of these blooms. During the review, specific aspects of levels of phytoplankton biomass (using Chlorophyll-a as a proxy), cyanobacterial taxa, diversity of the cyanotoxins, the dynamics of continued eutrophication and consequences of the blooms are integrated. This is made across the embayments and the open lake areas, but

there is threat of continued eutrophication of Lake Victoria despite the different interventions and monitoring frameworks over the past two decades. The state of art of the thesis has been published as a review, and all citations are in *Harmful Algae* 96:101829 (<https://doi.org/10.1016/j.hal.2020.101829>).

Does contrasting environmental conditions within the two embayments (MB and NG) of Lake Victoria govern the dominance of phytoplankton diversity and cyanobacteria?

Chapter three which is themed phytoplankton diversity and turnover in Lake Victoria presents the findings of the comparative examination of the temporal and spatial phytoplankton diversity and dynamics in Murchison Bay (MB) and Napoleon Gulf (NG). This chapter also partly addresses the micro conditions that govern the dominance of the different phytoplankton particularly cyanobacteria within the two embayments. It gives an indication of the consequences of the dominance of toxigenic cyanobacteria. The findings are presented as a publication with all citations in *Journal of Great Lakes Research*, 47(4), 985-996 (<https://doi.org/10.1016/j.jglr.2021.04.021>).

Do micro conditions govern the diversity and concentration of cyanotoxins within the two embayments (MB and NG) of northern Lake Victoria? In addition, do the WTPs efficiently eliminate cyanotoxins in treated water? Chapter four which is themed potential threats of toxigenic cyanobacteria and cyanotoxins in Lake Victoria presents the findings that addresses the micro conditions that govern the production of cyanotoxins within the embayments of northern Lake Victoria. It further addresses the efficiency of the water treatment plants that applies conventional water treatment processes in the removal of sestonic algae and cyanotoxins in treated water. Thus, chapter four addresses specific objectives two and three of this study. The findings are presented as a publication with all citations in *Toxins*, 14(10), 664 (<https://doi.org/10.3390/toxins14100664>).

What are the dynamics potential risks associated with bottom-up (nutrient) and top-down control using Nile tilapia *Oreochromis niloticus*? Chapter five themed bottom-up and top-down control of phytoplankton biomass addresses specific objective four that assessed the impact of Nile tilapia *Oreochromis niloticus* on the biomass and composition of cyanobacteria biomass and consequently the transfer or biomagnification of cyanotoxins in the fish. It also addresses the influence of the projected increase in nutrient loading of both major nutrient (nitrogen and phosphorus) and trace elements (EDTA and Fe) on cyanobacteria biomass in the embayments of Lake Victoria. The findings with all citations are presented as a manuscript for submission to peer reviewed journal.

The general discussion and synthesis of the results is covered in chapter six which covers the projected and vulnerability of eutrophication in the embayments of northern Lake Victoria. This chapter further assess the consequences of the increase of eutrophication, and the potential threats of acute or chronic exposures of cyanotoxins. It further gives perspectives and identifies areas for research arising from this study.

The last chapter of this study with the overall conclusions and recommendations for sustaining healthy population around the embayments of Lake Victoria.

Massive harmful cyanobacterial blooms (Cyanobacteria) in Lake Victoria

Chapter 1



Chapter 3

Characterization of toxigenic cyanobacteria
 1. Temporal and spatial phytoplankton diversity and dynamics?
 2. Conditions sustaining toxigenic cyanobacteria to produce cyanotoxins?

Chapter 4

Exposure to toxigenic cyanobacteria
 1. Diversity of cyanotoxins?
 2. Conditions sustaining cyanotoxin production?
 3. Exposure of local population?
 4. Removal of cyanotoxins in treated water?

Chapter 2

Extensive literature review
 1. Dynamics of cyanobacteria and diversity of cyanotoxins?
 2. Consequences of cyanotoxins to water quality, fish and socioeconomics?
 3. Efforts to control eutrophication and threats of cyanobacteria?

1. What are the toxigenic cyanobacteria?
 2. What cyanotoxins are being produced?
 3. What are impacts of Cyanobacteria?
 4. Exposure levels of cyanotoxins?

Chapters 6 & 7

Perspectives
 1. The future of eutrophication in Lake Victoria
 2. Consequences on the dynamics of dominant cyanobacteria and cyanotoxins
 3. Managing exposure of cyanotoxins (contaminated water and fish)?

Test the control of eutrophication
 1. Diversity of cyanobacteria
 2. The limiting factor for phytoplankton growth?
 3. Transfer of cyanotoxins in fish?

Figure 1. 4: Thematic organization and conceptual flow of the objectives of the study as guided by the research questions.

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CHAPTER TWO

STATE OF THE KNOWLEDGE ON CYANOBACTERIA AND CYANOTOXINS IN LAKE VICTORIA¹

CONTEXT

There is increasing population growth estimated at 2.4% per year around the embayments associated with land use practice such as deforestation, wetland reclamation and degradation, poor solid waste, and wastewater disposal. Thus, huge amounts of nutrient loading occur from the watershed and thus sustaining eutrophication in Lake Victoria with potential toxigenic cyanobacterial blooms. The knowledge on eutrophication, causes and consequences associated with toxigenic cyanobacteria, phytoplankton biomass, cyanobacteria and cyanotoxins, and gaps were identified during the bibliographic review. It presents the analysis of all the relevant literature and examined the phytoplankton biomass, community dominance with respect to cyanobacteria, the quantities and diversity of cyanotoxins produced in the embayments of the lake over the years, and the related or projected consequences. There has been increasing phytoplankton biomass in the embayments of Lake Victoria higher than in the open lake given the proximity of the sources of nutrients from the watershed. Generally, the interests into cyanobacterial studies gained momentum in the early 2000s and declined in the early 2010s and were no longer studied within the lake despite the health threats associated with the cyanotoxins. Generally, there has been reports of dominance of cyanobacteria in all the embayments of Lake Victoria, but the specific taxonomic dissimilarities and ecosystem functioning resulting from these cyanobacteria communities have not been studied. Furthermore, the most reported cyanotoxins from these blooms have been Microcystins (MC) and attempts to identify other toxins were only in the Kenyan part of the lake. There are other potential cyanotoxins that are produced but are not reported. The consequences of these toxigenic blooms have been identified across lake users, particularly on food (fish) and fishing, and the direct use of the lake water. Finally, the efforts to control the increasing eutrophication of the lake have also been discussed. The detailed specific review is presented below as published in *Harmful Algae* 96:101829 (<https://doi.org/10.1016/j.hal.2020.101829>)

¹ Chapter based on this publication:

Olokotum, M.; Mitroi, V.; Troussellier, M.; Semyalo, R.; Bernard, C.; Montuelle, B.; Okello, W.; Quiblier, C. and Humbert, J.-F. (2020). A review of the socioecological causes and consequences of cyanobacterial blooms in Lake Victoria. *Harmful Algae*, 96, 101829. doi: <https://doi.org/10.1016/j.hal.2020.101829>



Review of the socioecological causes and consequences of cyanobacterial blooms in Lake Victoria

Okotum^{a,b}, Veronica Mitro^c, Marc Troussellier^d, Ronald Semyalo^e, Cécile Bernard^f,
Montuelle^g, William Okello^h, Catherine Quiblier^{i,j}, Jean-François Humbert^k

^a of Zoology, Entomology and Fisheries Sciences, Makerere University, Kampala, Uganda

^b Fisheries Resources Research Institute (NaFIRRI), Jinja, Uganda

^c Institute of Ecology and Environmental Sciences of Paris (iEES), Sorbonne University, Paris, France

^d IREC, CNRS-University of Montpellier-IRD-IFREMER, Place Eugène Bataillon, Montpellier, France

^e Molécules de Communication et Adaptations des Microorganismes (MCAM), CNRS-MNHN, Muséum National d'Histoire Naturelle, Paris, France

^f CARTETEL, Thionville, France

^g Paris, France

ARTICLE INFO

ABSTRACT

Keywords:
Lake Victoria
Cyanobacterial blooms
Socioecological
analysis
of cyanobacterial blooms

Africa is experiencing high annual population growth in its major river basins. This growth has resulted in significant land use change and pollution pressures on the freshwater ecosystems. Among them, the Lake Victoria basin, with more than 42 million people, is a unique and vital resource that provides food and drinking water in East Africa. However, Lake Victoria (LV) has experienced a progressive eutrophication and substantial changes in the fish community leading to recurrent proliferation of water hyacinth and cyanobacteria. Based on an extensive literature review, we show that cyanobacterial biomasses and microcystin concentrations are higher in the bays and gulfs (B&Gs) than in the open lake (OL), with *Microcystis* and *Dolichospermum* as the dominant genera. These differences between the B&Gs and the OL are due to differences in their hydrological conditions and in the origins, type and quantities of nutrients. Using data from the literature, we describe the multiple ways in which the human population growth in the LV watershed is connected to the increasing occurrence of cyanobacterial blooms in the OL and B&Gs. We also described the consequences of cyanobacterial blooms on food resources and fishing and on direct water use and water supply of local populations, with their potential consequences on the human health. Finally, we discuss the actions that have been taken for the protection of LV. Although many projects have been implemented in the past years in order to improve the management of waste waters or to reduce deforestation and erosion, the huge challenge of the reduction of cyanobacterial blooms in LV by the control of eutrophication seems far from being achieved.

Introduction

Numerous studies have been conducted in the past 20 years with the aim of improving our knowledge of the causes and consequences of cyanobacterial blooms. Taranu et al. (2015) have shown that the increasing occurrence of cyanobacterial blooms is clearly associated with the increasing impact of human activities on freshwater ecosystems in the Anthropocene, and it is well established that the main cause of cyanobacterial blooms is the nutrient enrichment of phosphorus (P) and nitrogen (N) (O'Neil et al., 2012; Huisman et al., 2018). Recently, many papers also suggested that climate change might directly or indirectly promote cyanobacterial blooms (e.g., Moss et al., 2011;

Paerl et al., 2016; Ho et al., 2019). Blooms are also well known to have multiple impacts on the ecological functioning of freshwater ecosystems (e.g., Paerl et al., 2016; Huisman et al., 2018; Escalas et al., 2019) and on the goods and services (G&S) they provide (e.g., Dodds et al., 2009). Finally, many papers deal with the production of harmful toxins by cyanobacteria and the sanitary risks associated with them (e.g., Briand et al., 2003; Merel et al., 2013; Meriluoto et al., 2017).

Among these previous studies, few papers have focused on developing countries, with the exception of a few countries such as Brazil and China (Merel et al., 2013). In particular, the issue of cyanobacterial blooms has been poorly investigated on the African continent, as illustrated recently by Svircev et al. (2019). When looking in this review at

Corresponding author at: UMR 7245 Molécules de Communication et Adaptations des Microorganismes (MCAM), CNRS-MNHN, Muséum National d'Histoire Naturelle, Paris, France.
E-mail address: okotum@univ-montpellier.fr (V. Okotum).

<https://doi.org/10.1016/j.hal.2020.101829>

January 2020; Received in revised form 1 May 2020; Accepted 7 May 2020

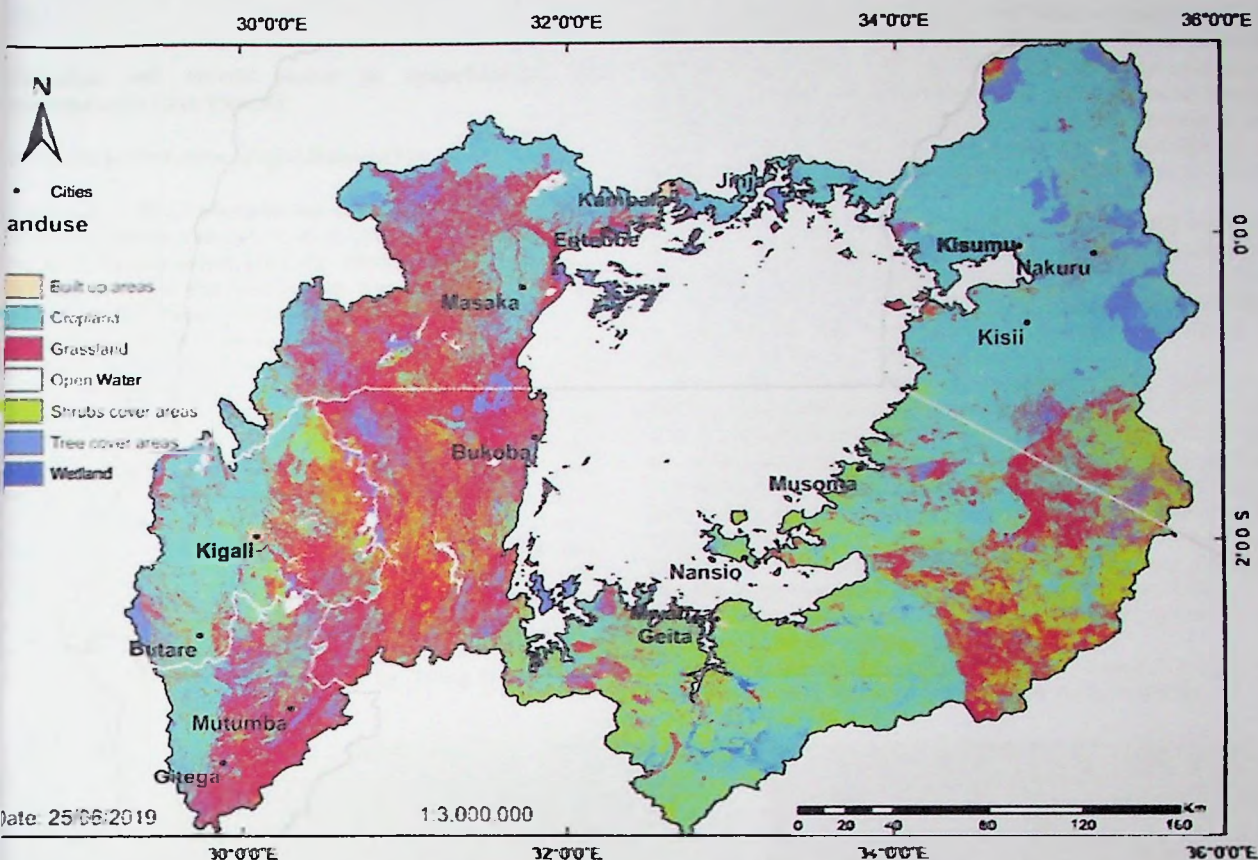


Figure 1. Lake Victoria catchment area with major land use occupation. Land use data obtained from the ESA Climate Change Initiative - Land Cover project 2017, June 2019.

distribution of cyanotoxins worldwide, it appears that data on cyanobacteria are available only for 14 of the 54 African countries and for ecosystems on the continent. Among these ecosystems, Lake Victoria is the most studied, probably because it is the second largest lake in the world and provides goods and services (G&S) to millions of people (Downing et al., 2014; El-Noshokaty, 2017). Moreover, LV has experienced rapid water quality degradation that has led to eutrophication, which is considered a major threat to the ecological health of the lake (Hecky et al., 1994; Juma et al., 2014) as it results in recurrent proliferations of aquatic weeds (e.g., water hyacinth) and cyanobacteria (Lung'ayia et al., 2000; Opande et al., 2004; Opande et al., 2014). The proliferation of water hyacinth has been more controlled since the end of the 1990s with biological, mechanical and chemical strategies (Opande et al., 2004; Wanda et al., 2015), but cyanobacteria blooms persist in LV, particularly in the bays and gulfs (Haande et al., 2011; Sitoki et al., 2012; Mbonde et al., 2015). The LV basin has one of the highest population densities in Africa, with several large cities located along the shores of the large bays and gulfs, e.g., Kampala in Uganda, Kisumu in Kenya, and Mwanza in Tanzania (Fig. 1). An understanding of the interplay of ecological and socioeconomic processes acting directly or indirectly on the lake is of interest. In this context, we address the state of knowledge on (i) the distribution of cyanobacterial blooms and cyanotoxins in LV; (ii) the natural and environmental factors and processes potentially explaining the occurrence of cyanobacterial blooms, with a particular focus on changes that have occurred in its watershed during the last decades; (iii) the consequences of these blooms on the ecosystem G&S provided by the lake and (iv) the management practices implemented with the goal of reducing nutrient loads and consequently the

cyanobacterial blooms.

2. Study site: the Lake Victoria basin

The LV catchment area has a surface area of 184,200 km² and is shared between five countries (Burundi, Kenya, Rwanda, Tanzania and Uganda). As shown in Fig. 1, the catchment is dominated by cropland and grassland, with major built-up areas (for example Kampala, Kisumu, Musoma and Mwanza) located on the shorelines of the B&Gs. LV is located 1,100 meters above sea level in East Africa and is the source of the White Nile. The lake is shared between the three countries of Kenya (6%), Tanzania (51%) and Uganda (43%). LV has a shoreline of 3,500 km with many B&Gs and a 68,000 km² surface area, making it the world's second largest freshwater lake and the largest lake in the intertropical area (Dobies et al., 2010). The maximum depth of the lake is 80 m, the average depth is 40 m, and the residence time of the water is 23 years. As shown by Talling (1966), Lake Victoria is monomictic, the overturn period occurring between May and August, while early thermal stratification occurs between September and December and persistent thermal stratification from January to April (Mugidde et al., 2005).

Depending on the areas, the general climate of the Lake Victoria basin ranges from a modified equatorial type characterized by heavy rainfall throughout the year to a semiarid type characterized by intermittent drought periods. Overall, there are two rainfall periods (long rains, March-May, and short rains, October-December) with variations ranging from 870–1,561 mm in Uganda and from 400–2,736 mm in Tanzania (Kizza et al., 2009). Water temperature recorded at the surface of the lake range from 23°C to 29°C throughout the year (Mugidde

05).

Historical and current status of cyanobacteria and cyanotoxins in Lake Victoria

Historical and current status of cyanobacterial blooms

Evolution of the LV phytoplankton community. As shown from historical data by Verschuren et al. (2002), the phytoplankton in LV has increased since the 1930s. Though there is insufficient data for this time period, it appears that the phosphorus concentrations increased (from 1.1 to 2.9 $\mu\text{mol L}^{-1}$ between the 1960s and 1990s) and that eutrophication manifested towards the late 1980s (Hecky, 1993; Hecky et al., 2010). The increasing phosphorus content led to an increase in the phytoplanktonic biomasses in the diatom community, from the dominance of *Aulacoseira* to the dominance of *Nitzschia* (Stager et al., 2009) and in the total phytoplankton community, from the dominance of green algae (Chlorophyta) and large diatoms (Bacillariophyta) to the dominance of cyanobacteria (Lehman and Branstrator, 1994; Ochumba and Kibaara, 2001; Verschuren et al., 2002; Hecky et al., 2010). This increase of cyanobacteria in phytoplankton communities is now widely found in the three countries bordering the lake (Kenya, Uganda, and Uganda), particularly in the numerous B&Gs (Fig. 2).

Changes in the phytoplankton biomasses and community compositions in B&Gs and the open lake (OL). As noted by Talling (1987), the

physicochemical conditions and microbial communities of the OL are quite different from those of the B&Gs. In the B&Gs, phytoplankton biomasses are often $>30 \mu\text{g Chl-a L}^{-1}$, with cyanobacteria often comprising more than 60% of the total phytoplanktonic biomass and frequently comprising more than 80% (Table 1). The rest of the phytoplanktonic community is mainly composed of Chlorophyta, Cryptophyta, Dinophyta and Euglenophyta (e.g., Haande et al., 2011; Sitoki et al., 2012).

In the OL, the phytoplanktonic biomass is generally less than $20 \mu\text{g Chl-a L}^{-1}$, and changes from a dominance of diatoms to a dominance of cyanobacteria are frequently found (Table 1). The works of Lung'aya et al. (2000) and Sitoki et al. (2012) in the Nyanza Gulf and the OL in Kenya suggest that these changes could be partly driven by the alternation of dry and rainy seasons.

Irrespective of the season, cyanobacteria were dominant in the B&Gs, while diatoms were clearly dominant in the OL during the dry season and represented $\leq 50\%$ of the phytoplanktonic biomass during the rainy season. In the latter case, cyanobacteria were the second most dominant group of phytoplankton. Interestingly, Mbonde et al. (2015) have shown in a comparative study performed in 16 closed and open bays that the degree of connectivity of these bays to the OL greatly impacts their phytoplankton communities. The average phytoplankton biovolumes were much higher in the closed bays than in the open bays, and the same finding was found when considering only the cyanobacterial biovolumes (Supplemental Fig. 1).

Main cyanobacterial genera found in Lake Victoria. A great diversity of cyanobacterial taxa, including both nitrogen fixing taxa such as

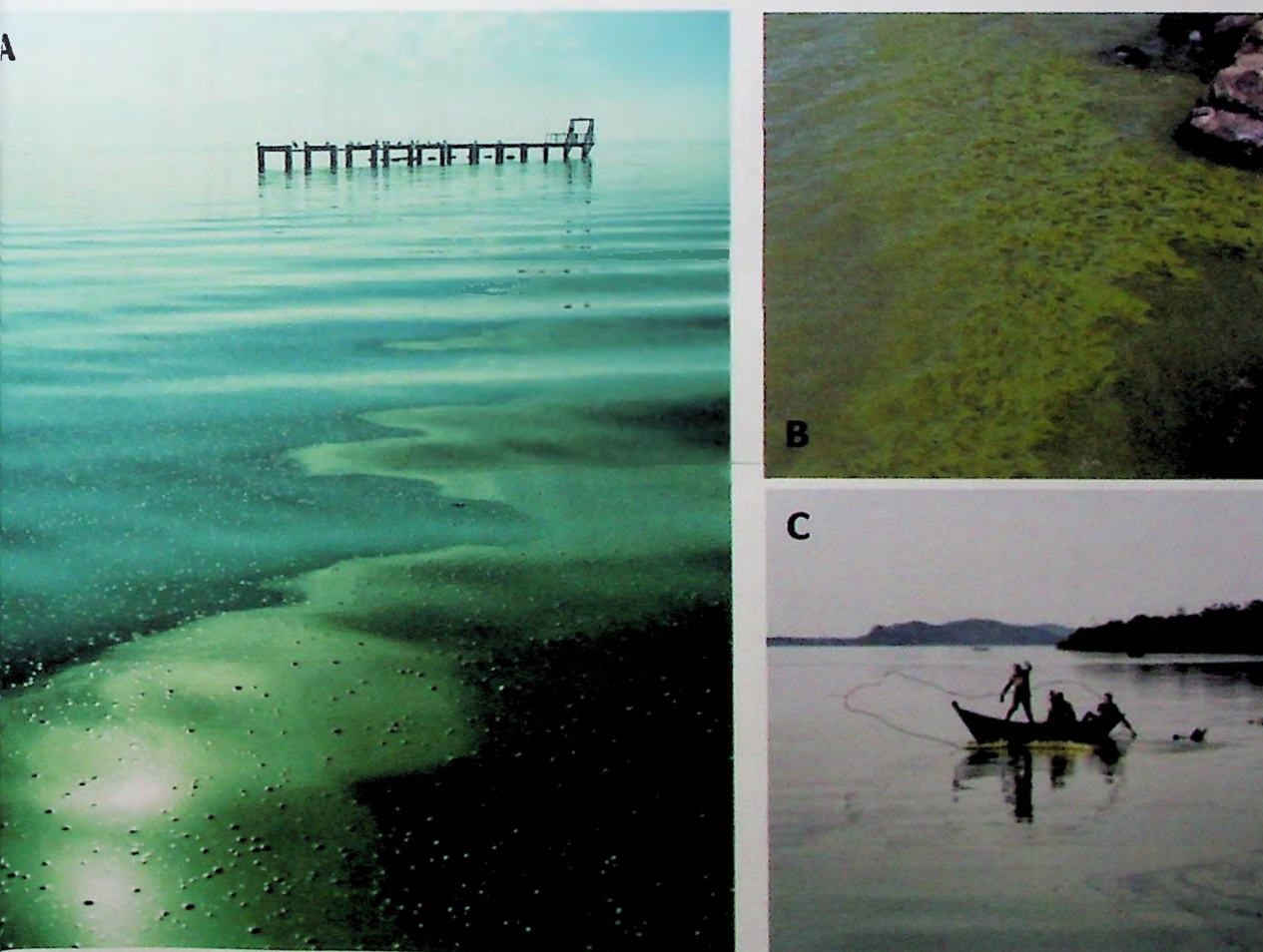


Fig. 2. Cyanobacteria blooms in Uganda, Murchison Bay (A); Kenya, Nyanza Gulf (B); Tanzania, Mwanza Gulf (C) (C Photos: J.F. Humbert, INRAE)

microcystins, not determined. N+G, Nyanza Gulf (Kenya), MB, Murchison Bay (Uganda), NG, Napoleon Gulf (Uganda), MG, Mwanza Gulf (Tanzania) (see the Supplemental Fig. 1 for the location of these sites) * *Dolichospermum* genus name is used instead of *Anabaena*.

Study location	Sampling period	Total phytoplankton biomass (Chla, wet weight or biovolume)	Dominant phytoplankton group (% of the total phytoplankton for each sampling)	Main genus	MC in water ($\mu\text{g MC-LR eq. L}^{-1}$)	MC variants	Authors
Bays and Gulfs (BGs)							
N+G	Sept 1994 and Mar 1995	Chla, min-max 9-71 $\mu\text{g L}^{-1}$	Cell abundance % Cyanobacteria > 60	Microcystis, Dolichospermum*	nd	nd	Lung'Ayia et al. (2000)
N+G	Dec 2000 to May 2002	Chla, min-max 6-31 $\mu\text{g L}^{-1}$	nd	Anabaenopsis	nd	nd	Gikuma-Njuru et al. (2005)
N+G	5 Nov 2001	Wet weight 282 mg L^{-1}	Biomass % Cyanobacteria > 95	Dolichospermum	1.1	MC-RR, MC-LR, MC-LA, MC-LF	Kemnitz et al. (2002) and Totat et al. (2003)
N+G	Mar 2005 to Mar 2006	Chla, min-max 10-45 $\mu\text{g L}^{-1}$	Mean biomass % Cyanobacteria = 72	Cyanodictyon, Aphanocapsa, Dolichospermum	nd	nd	Gikuma-Njuru et al. (2013)
N+G	Jul 2008 to Sept 2009	Biovolume, min-max 4-20 $\text{mm}^3 \text{L}^{-1}$	Biomass %, min-max Cyanobacteria 50-95	Microcystis, Dolichospermum	min-max 0-81	[Asp] ¹ MC-RR, MC-RR, [NmeSer] ² MC-YR, [Asp] ³ MC-YR, MC-LR, MC-LR	Stokki et al. (2012)
N+G	Oct 2011 to Jan 2012	Chla, min-max Integrated 10-30 $\mu\text{g L}^{-1}$ Patch 274-4382 $\mu\text{g L}^{-1}$	Biomass %, min-max Integrated 70-90 Patch 95-100	Microcystis, Planktolyngbya, Dolichospermum	min-max Integrated 2-5 Patch 80-2000 MC m/z 1052 and MC m/z 1002	MC-YR, MC-LR, two unknown MC m/z 1052 and MC m/z 1002	Simiyu et al. (2018)
NG	1994-1998	Chla, mean $\pm$ SD 71 $\pm$ 100 $\mu\text{g L}^{-1}$	nd	nd	nd	nd	Mugiddie et al. (2003)
MB	Apr 2003 to Mar 2004	Chla, min-max 15-60 $\mu\text{g L}^{-1}$	Cell abundance %, min-max Cyanobacteria 20-85 Diatoms 2-75	Microcystis, Dolichospermum, Nitzschia, Aulacoseira	nd	nd	Flaude et al. (2011)
MB	Jul 2004 to Jul 2005	Chla, min-max 28-37 $\mu\text{g L}^{-1}$	nd	nd	min-max 0.2-0.7	MC-RR, MC-YR and MC-LR	Semiyu et al. (2010)
MB, NG	May-Jun 2004; April 2008	Chla, min-max 8-23 $\mu\text{g L}^{-1}$	Biovolume % Cyanobacteria > 70	Dolichospermum, Aphanocapsa, Microcystis	(1.3-93 [g MC-LR eq. cell ⁻¹])	ID-MeAsp ¹ , MdhA ² -MC-RR, [Asp] ³ -MC-RY, [MeAsp] ⁴ -MC-RY	Okello et al. (2010b)
MB, NG	Apr-May 2007; Sept 2008 to Feb 2009	Chla, mean $\pm$ SD MB: 101 $\pm$ 48 $\mu\text{g L}^{-1}$ NG: 24 $\pm$ 18 $\mu\text{g L}^{-1}$	nd	nd	Mean $\pm$ SD MB: 7.3 $\pm$ 5.7 NG: 1.5 $\pm$ 1.3	nd	Poste et al. (2011)
MB, NG	May 2007 to Apr 2008	Chla, min-max MB: 30-98 $\mu\text{g L}^{-1}$ NG: 8-47 $\mu\text{g L}^{-1}$	Biovolume % Cyanobacteria > 70	Microcystis, Dolichospermum, Planktolyngbya	min-max MB: 0-1.6 NG: 0-0.5	[Asp] ¹ -MC-RR, MC-RR, [Asp] ² -MC-YR, MC-YR, MC-LR, [Asp] ³ -MC-RY, MC-RY	Okello and Kurnayer (2011)
MG	May to Aug 2002	Biovolume, min-max 0.6-8 $\text{mm}^3 \text{L}^{-1}$	Biovolume %, min-max Diatoms 10-90 Cyanobacteria 5-80	Nitzschia, Planktolyngbya, Aphanocapsa	0	0	Sekadende et al. (2005)
Several bays and gulf in Tanzania							
MB, NG	Sept 2005 to Oct 2007	Chla, mean $\pm$ SD 16 $\pm$ 5 $\mu\text{g L}^{-1}$	nd	Nitzschia, Cyclotella Microcystis, Dolichospermum, Lyngbya	nd	nd	Nzupula et al. (2011)
MB, NG	Sept 2008 to Feb 2009	Chla, mean $\pm$ SD MB: 9% $\pm$ 38 $\mu\text{g L}^{-1}$ NG: 25 $\pm$ 18 $\mu\text{g L}^{-1}$	Cyanobacteria	Microcystis, Dolichospermum, Planktolyngbya	Mean $\pm$ SD MB: 7.26 $\pm$ 5.73 NG: 1.75 $\pm$ 1.26	nd	Poste et al. (2013)
Several bays and gulf in Tanzania							
MG	Nov to Dec 2009	Chla, mean $\pm$ SD 25 $\pm$ 6 $\mu\text{g L}^{-1}$	Mean biovolume % $\pm$ SD Cyanobacteria 82 $\pm$ 9	Dolichospermum, Microcystis, Planktolyngbya	0-13	MC-LR, MC-RR, MC-YR, MC-AR, di-methyl-MC-RR	Mhandu et al. (2013)
MG	2010	nd	nd	nd	nd	Mainly: MC-RR, [MSer] ² MC-YR, MC-YR, [MSer] ³ MC-LR, MC-LR, MC-RA, MC-RF, MC-RY	Miles et al. (2013)
MB, NG, N+G, MG	Aug 2000 to Apr 2005	Mean Chla, min-max 12-40 $\mu\text{g L}^{-1}$	Mean biomass % Cyanobacteria 66 Diatoms 13	Microcystis, Dolichospermum, Cyclindropspermopsis/Raphidopsis, Nitzschia	nd	nd	Gikuma-Njuru et al. (2005)

(continued on next page)

Table 1 (continued)

Study location	Sampling period	Total phytoplankton biomass (Chla, wet weight or biovolume)	Dominant phytoplankton group (% of the total phytoplankton for each sampling)	Main genus	MC in water ($\mu\text{g MCLR eq. L}^{-1}$)	MC variants	Authors
Open Lake (OL)							
Kenya	Jun 1985-Aug 1986	Chla, min-max 8-78 $\mu\text{g.L}^{-1}$	nd	Microcystis, Dolichospermum, Nitzschia	nd	nd	Ochuma et Kibanza (1989)
Kenya	Sept 1994 and Mar 1995	Chla, min-max 2-21 $\mu\text{g.L}^{-1}$	Cell abundance %, min-max Cyanobacteria 20-85 Diatoms 12-80	Microcystis, Dolichospermum, Anabaenopsis, Nitzschia	nd	nd	Lung Ayia et al. (2000)
Kenya	Mar 2005 to Mar 2006	Chla, min-max 2-19 $\mu\text{g.L}^{-1}$	Mean biomass % Cyanobacteria = 54 Diatoms = 43	Aphanizomenon, Planktolyngbya, Pseudonitzschia, Dolichospermum, Cylindrocapsa, Raphidiopsis, Nitzschia	nd	nd	Gikuma-Njiru et al. (2013)
Kenya	Jul 2008 to Sept 2009	Biovolume, min-max 4-8 $\text{mm}^3.\text{L}^{-1}$	Biomass min-max Diatoms 40-75 Cyanobacteria 6-50	Synedra, Nitzschia, Microcystis, Dolichospermum	min-max 0.01-3.1	[Asp] ³ MC-RR, MC-RR, [NmeSer] ² MC-YR, [Asp] ³ MC-YR, MC-YR, MC-LR	Sitoki et al. (2012)
Uganda	1994-1998	Chla, mean $\pm$ SD 13.5 $\pm$ 5.8 $\mu\text{g.L}^{-1}$	nd	nd	nd	nd	Mugiddi et al. (2003)
Tanzania	May to Aug 2002	Biovolume, min-max 0.6-8 $\text{mm}^3.\text{L}^{-1}$	Biovolume %, min-max Diatoms 50-90 Cyanobacteria 15-50	Nitzschia, Planktolyngbya, Aphanizomenon	min-max 0-1	[Asp] ³ MC-RR	Sekadende et al. (2005)
Tanzania	Sept 2005 to Oct 2007	Chla, mean $\pm$ SD 5 $\pm$ 3 $\mu\text{g.L}^{-1}$	nd	Nitzschia, Microcystis, Dolichospermum, Lyngbya	nd	nd	Ngupula et al. (2011)
Tanzania (open bays)	Nov to Dec 2009	Chla, mean $\pm$ SD 10 $\pm$ 2 $\mu\text{g.L}^{-1}$	Mean biovolume % $\pm$ SD Cyanobacteria 44 $\pm$ 5 Diatoms 36 $\pm$ 6	Microcystis, Nitzschia	0	0	Mbrude et al. (2015)
Uganda, Kenya, Tanzania	Aug 2000 to Apr 2005	Mean Chla, min-max 4-12 $\mu\text{g.L}^{-1}$	Mean biomass % Cyanobacteria 53 Diatoms 33	Microcystis, Dolichospermum, Cylindrocapsa/Raphidiopsis, Nitzschia	nd	nd	Gikuma-Njiru et al. (2005)

Spermum, *Cylindrospermopsis*/*Raphidiopsis* or *Anabaenopsis* and nitrogen-fixing taxa such as *Microcystis*, *Planktolyngbya*, *Scenedesmus*, *Cyanodictyon* or *Aphanocapsa*, is often found in B&Gs and (Table 1). An underlying dominance of *Dolichospermum* spp. or *Microcystis* spp. cyanobacteria has been observed in the literature depending on the location, year and month of sampling considered. For example, Gikuma-Njuru et al. (2013a) observed a dominance of *Dolichospermum* and small cyanobacteria such as *Cyanodictyon* and *Aphanocapsa* between March 2005 and March 2006 in the Nyanza Gulf, while Sitoki et al. (2012) found that *Microcystis* was clearly the dominant cyanobacteria in the same area from July 2008 to October 2009. In Murchison Bay (Uganda), *Microcystis* dominance was observed by Poste et al. (2013), whereas the dominance of *Dolichospermum* was found in Murchison Bay and the Napoleon Gulf by Okello and Kurmayer (2011) and Okello et al. (2010a). Similar observations and variations between a dominance of *Dolichospermum* and a very high density cyanobacterial community were also observed by Laand et al. (2011), depending on the date and the sampling station within Murchison Bay. In Tanzania, Sekadende et al. (2003) found a dominance of cyanobacteria belonging to the *Planktolyngbya* genus in the Nyanza Gulf between May and August 2002. Several years later, Okello et al. (2015) observed a dominance of *Dolichospermum* and *Microcystis* in the different B&Gs of Tanzania, including the Mwanza Gulf between November and December 2009. There is no clear explanation for these spatial and temporal variations in the dominant cyanobacterial genera, but it is known that variations in nutrient concentrations can influence the composition of cyanobacterial communities. For example, it has been proposed that the proportions of nitrogen-fixing cyanobacteria such as *Spermum* sp. could be linked to a nitrogen limitation in the lake (Okello et al., 2003; Gikuma-Njuru and Hecky, 2005). In the same lake, many studies have shown that *Microcystis* sp. is the most common cyanobacterial species in freshwater ecosystems with high phosphorus concentrations (e.g., Poste et al., 2013).

Cyanobacterial toxin production

Microcystis and *Dolichospermum* genera are well known as the main producers of cyanotoxins of the microcystins (MC) family, but *Spermum* is also known to produce several families of cyanotoxins, including anatoxin-a (ATX) (Bernard et al., 2017). To our knowledge, all studies in LV have focused on MC, and only one study was conducted in the Nyanza Gulf (Kenya) focused on ATX (Kotut et al., 2006). Although *Dolichospermum* was dominant and *Microcystis* was present, ATX was undetectable in both environmental samples and in laboratory strains. During the same study period, the estimated MC concentration was approximately $1 \mu\text{g eqMC-LR.L}^{-1}$ (Krienitz et al., 2006). Some of these cyanobacteria are also known to produce other cyanotoxins such as the amino acid variant L-homocysteine-L-alanine (BMAA) and saxitoxins, but these cyanobacteria have not been investigated in LV.

Numerous studies have dealt with the detection of MC in LV (Table 1). The first study reporting the presence of MC was that of Krienitz et al. (2006) and was performed in the Nyanza Gulf (Table 1). After this study, MC occurrence was recorded in different parts of the lake, including the B&Gs but also in the OL in some cases (Sekadende et al., 2003; Sitoki et al., 2012) (Table 1). Highly variable concentrations of MC were found in LV, from less than $1 \mu\text{g.L}^{-1}$ in Murchison Bay (Uganda) (Okello et al., 2010) up to $81 \mu\text{g.L}^{-1}$ and $100 \mu\text{g.L}^{-1}$ in Nyanza Gulf (Kenya) (Sitoki et al., 2012; Simiyu et al., 2015). Respectively, the highest concentrations being found in scums or surface of the lake (Table 1). A positive correlation between the concentrations and *Microcystis* abundance was found by Okello et al. (2010a, b), Sitoki et al. (2012) and Mbonde et al. (2015). Finally, Okello et al. (2010b) observed during a one-year monitoring study (July - April 2008) that the proportions of the *mcyB+*

genotypes (potentially producing MC) in *Microcystis* populations varied from 3.3% to 39.2% in Murchison Bay and from 12.9% to 59.3% in the Napoleon Gulf.

So far, 240 MC variants have been described in the world (Spoof and Catherine, 2017), but only a few studies have investigated the MC variant composition in the cyanobacterial blooms from LV (Table 1). Nevertheless, up to seven different variants have been identified by Okello and Kurmayer (2011) and up to 31 variants have been identified by Miles et al. (2013). However, the most common variants detected in the lake are MC-LR, MC-RR and MC-YR (e.g., Okello and Kurmayer, 2011, in Murchison Bay and the Napoleon Gulf; Sitoki et al., 2012, in the Nyanza Gulf; Mbonde et al., 2015, in the Mwanza Gulf). The proportions of all these MC variants were highly variable depending on the sampled area. For example, the average estimated proportions of the MC-LR variant in the Murchison Bay and the Napoleon Gulf (May 2007 - April 2008) were $12.4 \pm 2\%$ and $0.5 \pm 0.3\%$, respectively, according to Okello and Kurmayer (2011) but reached $50 \pm 6\%$ in the Nyanza Gulf (July 2008 - Sept 2009) (Sitoki et al., 2012). The small standard deviations associated with these proportions seem to express low temporal variations for a given area in LV. However, higher variations are sometimes reported in the literature, as shown for example by the large standard deviation found in the mean proportion ($31 \pm 52\%$) of the MC-YR in the Nyanza Gulf (Sitoki et al. 2012).

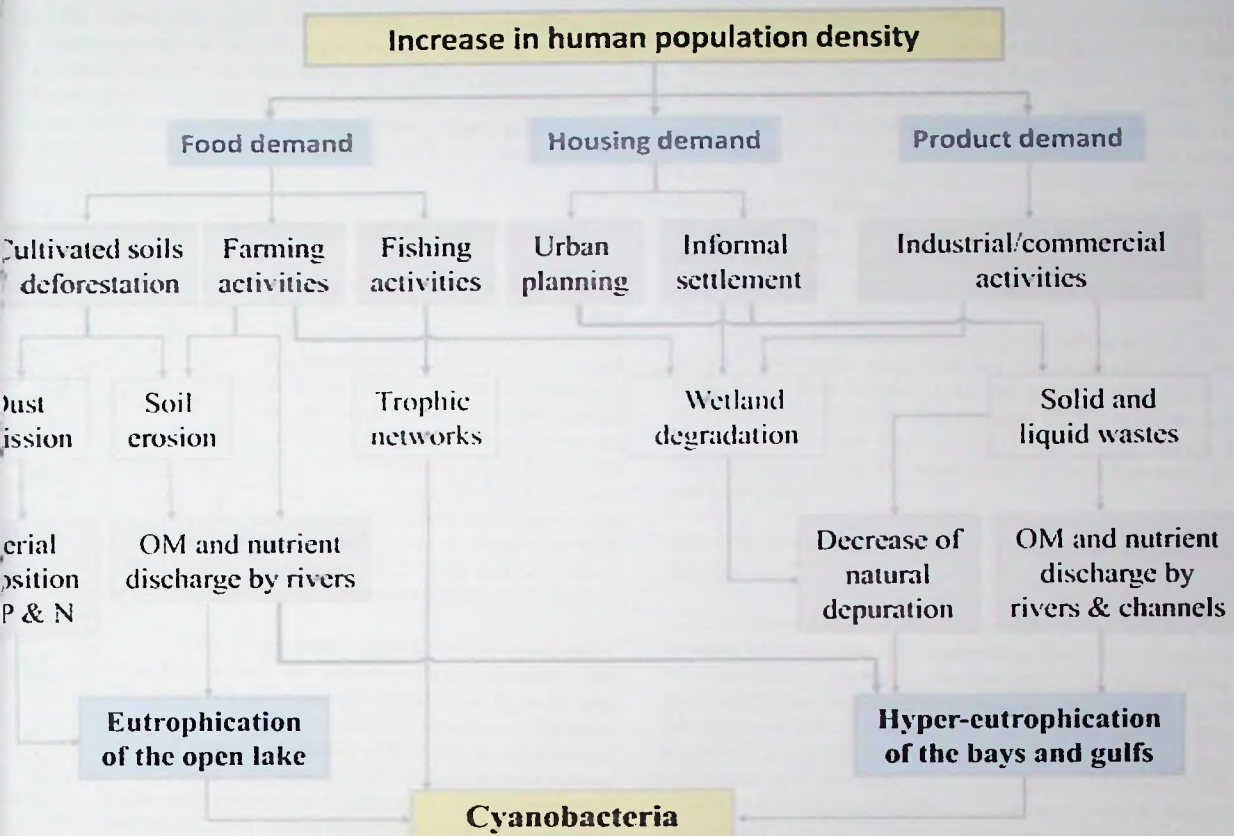
4. Linking demographic changes with the increasing occurrence of cyanobacterial blooms

From the analysis of the data available in the literature on the causes of cyanobacterial blooms in LV, we built a flow diagram (Fig. 3) describing the multiple ways in which the human population growth in the LV watershed is connected to the increasing occurrence of cyanobacterial blooms in the OL and B&Gs.

As summarized in Fig. 3, the increase in the populations density in the LV basin, such as everywhere in the world, has resulted in increasing food, housing and product demands (FAO, 2016). All these demands have many consequences on land use in the watershed, including farming and fishing activities, as well as formal and informal urbanization and industrial and commercial activities. All these processes are described in Section 4.1. Then, as developed in Section 4.2, the changes occurring in anthropogenic activities and land use have led to increasing nutrient pollution in the OL and B&Gs through three main processes: the aerial deposition of phosphorus and nitrogen, the discharge of mineral and organic nutrients by rivers and a decrease in natural purification due to wetland degradation. Finally, Section 4.3 shows why the B&Gs are more polluted than the OL and the processes leading to the decrease in water quality.

3.3. Determinism and consequences of human population growth in the LV watershed

Demographic data on the LV watershed. The African continent continues to experience a high annual population growth estimated at 2.6% per year in its major river basins (World Population Prospects, 2017). The population density in the LV basin almost doubled over the past 20 years from 27.2 million to ca. 42 million inhabitants (Bremner et al., 2013), which is considered the largest human population in the watersheds of the great lakes of the world (Dobiesz et al., 2010). This population increased by 2.4 - 3.2% annually (> 6% in some urban areas such as Kampala, Uganda), a growth rate that is higher than that observed in the rest of the African continent (UNEP, 2006). The data from the Center for International Earth Science Information Network - CIESIN - Columbia University (2018) indicates that the population density in major cities has increased from a maximum of 100 persons per km^2 to over 23,000 persons per km^2 in the last two decades. A strong and similar exponential population increase has been observed



between the increase in the human population density around Lake Victoria and the occurrence of cyanobacteria blooms in the open lake and in the bays and gulfs. P: Phosphorus; N: Nitrogen

60s in Kenya, Tanzania and Uganda (Supplemental Figure 1). On the other hand, Burundi and Rwanda, which experienced civil wars in the 1990s and do not have direct access to the lake, have seen rapid population growth.

This population growth in the LV watershed. A recent report of the International Institute for Sustainable Development (Dazé and Perrings, 2016) pointed out that migration in the Great Lakes Region is due partly to both voluntary decisions to migrate and forced migration. Concerning the voluntary migration, many people have moved towards the lake basin to take advantage of the economic opportunities in the fisheries in the basin (Awange and Obiero, 2006; Obiero et al., 2009). As reported in Mkumbo & Marshall (2015), more than 1 million people are directly or indirectly employed in fisheries, and the fisheries supports approximately 4 million people. Lake Victoria is the world's largest host for inland freshwater fisheries (Dazé and Perrings, 2016). On the other hand, forced migrations have occurred from armed conflicts and violence in Rwanda and Burundi (1993 to 2005) and in the Democratic Republic of Congo (2002 to 2005). Many of these migrants are located in rural areas (Crawford, 2016).

Impacts of population growth on land use, urbanization and activities. As established in the diagnostic analysis performed by the Lake Victoria Environmental Management Project (LVEMP), more than 80% of the inhabitants living in the LV basin are from the rural areas; in Burundi, Kenya, Rwanda and Tanzania (see also Fig. 1). In Burundi, 94% of the inhabitants living in the LV basin are urban (Lake Victoria Environmental Management Project LVEMP, 2007). This is already observed in the developing world (UNEP, 2008), the three countries with direct access to the lake

and with livelihoods that rely on the lake have also experienced rapid urbanization that has led to the development of large cities located on the B&Cs, such as Kampala (>2 million inhabitants) on Murchison Bay (Uganda), Kisumu (approximately 1 million inhabitants) on the Nyanza Gulf (Kenya) and Mwanza (>400,000 inhabitants) on the Mwanza Gulf (Tanzania) (Supplemental Fig 3).

The main consequence of increasing population densities in rural areas is a change in land use, particularly to cropland (see Fig. 1). The agricultural development involves ca. > 80% inhabitants that depend on small-scale mixed farming operations, thus, influencing the land use. As highlighted in the LVEMP report (Lake Victoria Environmental Management Project LVEMP, 2007), agricultural development includes wetland destruction and/or degradation, livestock overgrazing, bush burning, land fragmentation, and deforestation. Deforestation (for settlement and agriculture development) is spreading at an alarming rate, e.g., in Uganda between 2000 and 2015, the deforestation rate was 3.3% annually, which is higher than that in other countries of the watershed (0.2% for Burundi, 0.3% for Kenya, 1.7% for Rwanda and 0.8% for Tanzania) (FAO, 2015). This deforestation rate has increased from 1990-2015 in Uganda (from 2.0% in 1990-2000 to 3.3% in 2000-2010, reaching 5.5% in 2010-2015). In typical developing countries, both rural and urban populations depend heavily on the forest environment and products, such as building materials, crafts, firewood, charcoal, food, flavoring, and traditional medicine, for their livelihood (UNEP, 2008; Mwavu and Witkowski, 2008).

The drivers of deforestation identified in the LV watershed can be classified into two broad categories (Waiswa et al., 2015). The direct human uses of forest resources (i.e., (Geist and Lambin, 2002; Mwavu and Witkowski, 2008) include the unplanned spread of agricultural areas over forest areas, firewood and timber harvesting, clearing

and for human settlement, sand mining and brickmaking. The main drivers concern the underlying sociopolitical factors impacting land use practices, such as unclear land tenure and/or property rights, lack of forest monitoring and the noninvolvement of users in forest management, conflicts of interest, political interference, and negative attitudes about planning policies (Place and Otsuka, 2002; Mwambi and Witkowski, 2008).

Relationships between land use changes and the eutrophication of LV

As shown in Fig. 3, various processes associated with land use changes and activities have led to the eutrophication of the OL and B&Gs. Numerous studies have focused on identifying the different processes that lead to nitrogen and phosphorus pollution in LV, and several processes have been identified. These processes include (i) the charge of rivers from heavily polluted catchments (Verschuren et al., 2002; Musungu et al., 2014; Fuhrmann et al., 2015; Jovanely et al., 2015); (ii) atmospheric deposition, in particular of phosphorus (P) accounting for 55% of the total phosphorus input in LV (Tamataiah et al., 2005); (iii) the biological fixation of atmospheric dinitrogen (N_2), which seems to greatly exceed the contributions of atmospheric N deposition and river N inputs (Mugidde et al., 2003); and to a lesser extent (iv) nutrient recycling and release from the sediments (Gikuma-Njuru et al., 2010).

Two of the first nutrient pollution pathways for LV water are directly linked to human population growth and activities. First, changes in land use patterns have contributed to enhanced erosion. In Uganda and Kenya, the presence of settlements on the LV shoreline zone were associated with high soil erosion estimates of between 17 and 87 ton ha^{-1} (Isabirye et al., 2010). The rate of soil loss in the LV basin appears to depend on the land use system (tree plantation versus agriculture) (Bamutaze et al., 2017). Other human-induced disturbances, such as timber harvesting and soil compaction by cattle, also increase the rate of soil erosion (Karamage et al., 2017). Similarly, TP and TN inputs from atmospheric deposition mainly seem to result from burnt biomass, agricultural dust and industrial and domestic activities (see the review by Ssebiyonga and Mulhandiki, 2014).

Second, due to the urban human population growth, increasing quantities of solid and liquid waste originate from (i) the human population living in the large cities located near the B&Gs and (ii) industrial activities such as the activities of large manufacturers of steel, plastic materials, factories that produce batteries, soap, paint, plastics, corrugated iron sheets, or pharmaceuticals, and breweries, former copper smelters and abattoirs (Muwanga and Njiru, 2006). Moreover, as shown in Tanzania (Cockx et al., 2019), the rapid expansion of the rural population results in changing food diets, which include increasing consumption of high-sugar foods and processed foods. These changes in the demand for food have led for example to the proliferation of bottling factories in the three main cities located around the lake (Kampala, Mwanza and Kisumu). For Kampala, only ca. 8% of all wastes (domestic and industrial) are collected and treated (Matagi, 2002), and the efficiency of the sewage treatment plants is poor, with up to ca. 89% frequency of non-compliance with the national standard for BOD₅ (Lake Victoria Environmental Management Project (LVEMP) 2005). In the same way, Gikuma-Njuru et al. (2008) have shown that point source effluents due to industrial activities in the Jinja area have contributed to the increasing nutrient loads in the Napoleon Gulf.

Finally, the progressive reduction and degradation of wetlands has played a major role in the degradation of water quality. This decrease is due to housing, industrialization, infrastructure development and agriculture (Kansime and Nalubega, 1999; Fuhrmann et al., 2015). In the Kampala Metropolitan Area (Uganda), the proportion of degraded wetlands increased from 13% in 1993 to 46% by 2007 (Mwambi et al., 2007). In particular, Nakivubo wetlands experienced a 62% loss in wetland vegetation between 2002 and 2014,

mainly due to crop cultivation (Isunju, 2016), despite their high estimated economic value of between US\$ 760,000–1,300,000 (Schuijt, 2002). Similar observations have been made for the Nyanza Gulf in Kenya by Juma et al. (2014). In Tanzania, Musaniba et al. (2011), showed that an increase in the anthropogenic activities in the Musoma urban area was associated with the degradation of wetland coverage at the average rate of 6.5 $ha\ yr^{-1}$ between 2001 and 2008. Knowing that wetlands are located at the mouths of the major rivers and in the inshore areas of the lake (Okevo-Owuor et al., 2012) and that they play a major role in waste removal and water purification (Raburu et al., 2012), their degradation contributes to the decrease in water quality in the B&Gs.

The consequences of all these processes acting on the nutrient load in LV is that high concentrations in soluble elements (SRP , NO_3^- and NH_4^+) have been recorded in the past twenty years in this lake (Supplemental Table 1). The average TN:TP ratios in B&Gs (14.5) are almost double those in OL (8.1) (Mugidde et al., 2005). This suggests that there is a global N limitation in LV, which is more pronounced in the offshore area.

3.5. Why do the bays and gulfs of LV have more cyanobacteria blooms than the open lake?

Two main kinds of processes seem to be involved in the greater amount of eutrophication observed in the B&Gs compared to the OL.

First, several big towns are located on the banks of the B&Gs, such as Kampala on the Murchison Bay, Kisumu on the Nyanza Gulf and Mwanza on the Mwanza Gulf. As shown for the Murchison Bay (Uganda) (Kabenge et al., 2016; Akurut et al., 2017), the exponential deterioration of the water quality between 1990 and 2014 was largely due to increasing quantities of waste generated in Kampala City and discharged in the bay. Similar observations were made by Cornelissen et al. (2014) for the Mwanza Gulf (Tanzania). Several B&Gs also receive water from rivers carrying high nutrient loads resulting from agricultural farmland in the catchment (Shayo et al., 2011). This contribution has been shown for example for the Nyanza Gulf (Kenya) by Gikuma-Njuru et al. (2013b). As already described in section 3.2, the decrease in the natural depuration in the wetlands located in the B&Gs contributes to their hyper eutrophication.

Second, the hydrodynamic conditions experienced by the B&Gs favor cyanobacteria blooms. In the closed B&Gs (e.g., Murchison Bay, the Nyanza and Mwanza Gulfs) experiencing limited exchange with the OL, the long water residence time and the high nutrient load from their watersheds promote and sustain the development of cyanobacterial blooms. Moreover, the majority of the B&Gs are very shallow, as illustrated by the Nyanza Gulf, which has a mean depth of <10 m (<5 m in the eastern part of the gulf). Typically, at shallow depths, all water is mixed daily and there is thermal stratification during high insolation periods (Gikuma-Njuru, 2008). Consequently, the alternation of mixing and stratified periods can potentially promote the release of nutrients from the sediments, which can then sustain cyanobacteria growth (see for ex. Cao et al., 2016). In addition to the mixing and stratification that occurs at shallow water depths, the high turbidity occurring in these shallow and mixed B&Gs (Silsbe et al., 2006; Loiselle et al., 2008 & 2010) could promote the development of bloom-forming cyanobacteria such as *Microcystis* spp. or *Dolichospermum* spp., which are able to occupy the top of the water column where light is available for their growth, due to their gas vesicles (Gikuma-Njuru, 2008; Ssebiyonga et al., 2013).

3.6. Other processes involved in the increasing occurrence of cyanobacterial blooms in LV

Role of changes in the fish community. Nile perch and several tilapia species (Nile tilapia: *Oreochromis niloticus* L.; *O. leuiscosticus*; *Coptodon zillii*) were introduced into the lake in the 1950s and became dominant

utu-Ohwayo, 2004; Awange and Obiero, 2006). Consequently, Marshall (2018) discussed the possibility that the changes in the fish community composition during the 1960s could have aggravated the effects of eutrophication and the proliferations of cyanobacteria in Lake Victoria. Although Goldschmidt et al. (1993) emphasized the impact of endemic phytoplanktivorous and detritivorous haplochromines in the littoral and sublittoral areas of LV before the 1980s, these species were victims of Nile perch predation, that led to a dramatic decrease in their biomasses (Marshall, 2018). Thus, in the context of eutrophication, Batjakas et al. (1997) suggested that the replacement of endemic phytoplanktivorous species by predatory Nile perch could have facilitated the proliferation of cyanobacterial blooms. Moreover, as stated by Marshall (2018), cyanobacteria could have been favored by the decrease in the population of large phytoplankton grazers and zooplanktonic community due to their consumption by juvenile Nile perch. The other main introduced fish species (Nile tilapia, *O. niloticus*) is well-known to ingest a diversity of prey items including phytoplankton (e.g. Bwanika et al. 2006). In LV, many authors have reported that the diet of *O. niloticus* often has large proportion of phytoplankton dominated by cyanobacterial species (Semyalo et al., 2011; Rumisha and Nehemia, 2013; Jihulya, 2014). Cyanobacteria has also been shown to contribute to the bulk of the diet of Nile perch in other tropical aquatic ecosystems (Teferi et al., 2000; Kolding et al., 2003; Lu et al., 2006; Torres et al., 2016; Zakaria et al., 2019). However, new data on the quantitative predation of cyanobacteria by *O. niloticus* are needed before concluding that this species plays a significant top-down role on the dynamics of cyanobacteria. In 2005, the stock of Nile perch in LV has rapidly decreased by approximately 50%, mainly due to overfishing (Matsuishi et al., 2006; Ochi et al., 2007; Mkumbo and Marshall, 2015) but also because of degradation of the water quality (e.g. anoxia). During the same time, the biomasses of other species such as Nile tilapia, catfishes or *C. niloticus* biomasses have increased because they are less susceptible to degraded water quality (Kolding et al., 2008). The recent decrease in Nile perch stocks also favored an increase in haplochromine prey species, although their biomass and diversity remain low compared to the pre-perch era (Marshall, 2018). It is difficult to predict the consequences for cyanobacterial blooms to all these changes occurring in the communities of LV knowing that the grazing rates of both fish and zooplankton have always been low in LV, particularly since the 1980's (Marshall et al., 2012).

In addition to the changes occurring in capture fisheries, the recent expansion of cage culture farms in the B&Gs (Aura et al., 2018; Opiyo et al., 2018; Musinguzi et al., 2019) constitutes an additional threat for the lake. It is well known that aquaculture can contribute significantly to nutrient enrichment of freshwater ecosystems (Zhang et al., 2016). In Lake George (Uganda), Egessa et al. (2018) observed organic matter and nutrient enrichment in the sediment due to cage fish farming. In Lake Tanganyika (Tanzania), an increase in nutrient concentrations was observed after cage farming establishment (Kashindye et al., 2015). In Lake Naivasha, Kenya, it was reported increasing eutrophication in shallow waters due to aquaculture waste feeds (Njiru et al., 2018). These areas are all portions of LV where caged fish farming has occurred, but it has not yet begun to spread among the various B&Gs of the lake. Thus, more data are needed to study this issue and its related consequences on water quality.

Potential impact of climate change. The potential impact of climate change on eutrophication in LV and consequently on cyanobacterial blooms has been poorly investigated. Lehman et al. (1998) suggested that eutrophication of LV may have been accelerated by climate change, particularly by increased water temperature and reduced mixing, which are two processes that are known to influence the dynamics of cyanobacteria.

Recently, Tariku and Gan (2018), modeled the impacts of climate change on the extreme precipitation indices and temperature of the Lake Basin and indicated that the extreme precipitation indices

are projected to increase in the second part of this century. Similarly, Thiery et al. (2016) projected that LV will be a hotspot for heavy thunderstorm events in a future with warmer climate scenarios. These changes might have dramatic consequences on LV because extreme rain events contribute to runoff and soil erosion, which enhance the eutrophication of aquatic ecosystems (e.g., Moss et al., 2011) and consequently the occurrence and intensity of cyanobacterial blooms (e.g., Michalak et al., 2013).

5. Consequences of cyanobacterial blooms on the different uses of the lake

3.7. Consequences on food resources and fishing

Fishing is an important food resource for approximately 1.5 million people living on the lake shores as well as for the 42 million inhabitants in the watershed of LV. In this context, cyanobacterial blooms have two main impacts on the fish communities: (i) the changes in the environmental conditions of the fish due to the blooms, with potential negative impacts on the abundance and diversity of the LV fish community, and (ii) the accumulation of cyanotoxins in the fish and the associated risks for the human populations consuming those fish.

It has been shown in numerous water bodies worldwide (e.g., Padmavathi and Veeraiiah, 2009; Lehman et al., 2010) that severe cyanobacterial blooms impact fish populations, mainly through changes induced in environmental parameters such as dissolved oxygen concentrations. Indeed, severe anoxia is recorded at the bottom of deep lakes or throughout the water column in shallow lakes due to the high respiration activity of bacteria degrading organic matter produced by cyanobacteria. In LV, several studies have linked water quality degradation to eutrophication and the changes occurring in the fish communities. For instance, Kundu et al. (2017) reported significant differences in the fish community structure in the Nyanza Gulf, where severe cyanobacterial blooms are frequently observed, and just outside of the gulf where better water quality occurs. Furthermore, Kolding et al. (2008) examined the relationship between the deterioration of water quality and the decline of Nile perch in the lake. They hypothesized that the low oxygen concentrations at the bottom of the lake resulting from the degradation of phytoplanktonic organic matter may be the cause of the decline in Nile perch. However, this hypothesis is still in debate, as discussed by Mkumbo & Marshall (2015).

The second impact of cyanobacterial blooms on fish communities is associated with the potential accumulation of cyanotoxins in the fish and their subsequent potential consequences on human health. Based on laboratory and field studies in several countries, it is known that MC can accumulate in fish tissues (e.g., Jiang et al., 2012; Nchabeleng et al., 2014). In LV, three studies (Semyalo et al., 2010; Poste et al., 2011 and Simiyu et al., 2018) have shown the accumulation of MC in several fish species. The extent of MC accumulation displayed considerable variability (from 0.5 to 90 $\mu\text{g.kg}^{-1}$ wet weight) among the fish species. For the highest toxin concentrations, the estimated daily exposure to MC could exceed the tolerable daily intake (TDI) proposed by the WHO for chronic exposure from fish consumption (i.e., TDI of 0.04 $\mu\text{g MC-LR per kg body weight per day}$) (Ibelins and Chorus, 2007), especially for *Haplochromis* spp. and *Rastrineobola argentea* (Silver fish), which are consumed whole. With the growing cage aquaculture in the B&Gs of LV and the incidence of increasing nutrient loading from aquaculture, cultured fish could accumulate cyanotoxins, but no data are available. Consequently, all these data suggest that the consumption of fish from LV could significantly contribute to the chronic exposure of human populations to MC.

3.8. Consequences on direct water use and water supply

As shown in Table 1, the MC concentrations in the LV water frequently exceed 1 $\mu\text{g.L}^{-1}$, which is the threshold proposed by the WHO

and Bartram, 1999) for drinking water, and sometimes exceed 10^4 L^{-1} , which is the lowest threshold set by the sanitary authorities in the northern countries for recreational activities (Chorus et al., 2006). Consequently, as emphasized by Kotut et al. (2006), these MC concentrations in the B&Gs are now a real challenge for local water utilities. However, regular monitoring of the MC concentrations in the water and in the treated water produced by the plants located in the B&Gs is not regularly performed. Moreover, there is no information on the sanitary risks for people directly using lake water for domestic activities, including cooking and washing, or for recreational activities during bloom events.

In addition to the potential threat of cyanobacterial blooms to human populations through the consumption of raw or treated water or contaminated fish, these blooms have direct and indirect consequences on the price and quantity of drinking water produced by the treatment plants. In Uganda, Oyoo (2015) showed that cyanobacterial blooms increased the price of the water in different ways. First, a clarification was added to the oldest water treatment plant (Gaba I) to improve water quality. Second, the dosage of aluminum sulfate used in the treatment increased three times from 1993 to 2008 due to the increasing frequency of cyanobacterial blooms, and pre-chlorination was introduced to enhance coagulation and settling (Olokotum, 2017). Third, cyanobacteria blooms in Murchison Bay have forced the water authorities and institutions to establish a new water treatment plant outside of the bay at Katosi (Damba channel), which will incur additional costs due to the construction of a long pipeline to carry the drinking water to the Kampala metropolitan area.

In addition to the increasing costs of water treatment due to cyanobacterial blooms, Oyoo (2015) reported the impacts of cyanobacterial blooms on the availability of water to local populations. The increased frequency of backwashing to avoid clogging of the sand filters for water treatment at Gaba I & II in Kampala decreases the runtime of the machinery, which consequently decreases the quantity of water produced by the plants for populations. The costs of production and the quality of treated water are very important in developing countries where when the price increases and/or the availability decreases, the populations tend to use alternative water resources that are not safe for their health (Moe and Rheingans, 2006).

Finally, from all these data, Fig. 4 shows the potential health impacts of cyanobacterial blooms for human populations living around the lake. Concerning the health impacts of cyanotoxins, limited data demonstrates their real adverse effects on human populations. However, the high recorded MC concentrations and the potentially high exposure of humans to these toxins (in comparison to populations from developed countries), these impacts on human health should be seriously considered. This hypothesis is supported by Roegner et al. (2020) who stated that the health of peri-urban fisher communities in the area of Lake Tanganyika is threatened by the consumption and use of lake water contaminated with MC and other harmful algal bloom components. Furthermore, in addition to the disturbances caused by cyanobacteria, the production of drinking water, human populations can also be exposed to nontreated water from the lake or poorly treated water with exposure to fecal pathogens associated with waterborne diseases.

2.3. To reduce eutrophication and the occurrence/intensity of cyanobacterial blooms

It is well established that the eutrophication of aquatic ecosystems promotes cyanobacterial blooms, the reduction of external nutrient inputs in the lakes is the main sustainable strategy to reduce cyanobacterial blooms (e.g. Huisman et al., 2018). LV is a very complex socioecological ecosystem, and there is limited documented knowledge (and understanding) regarding the social and ecological processes and interactions involving LV. However, based on existing knowledge, we provide arguments for the eutrophication

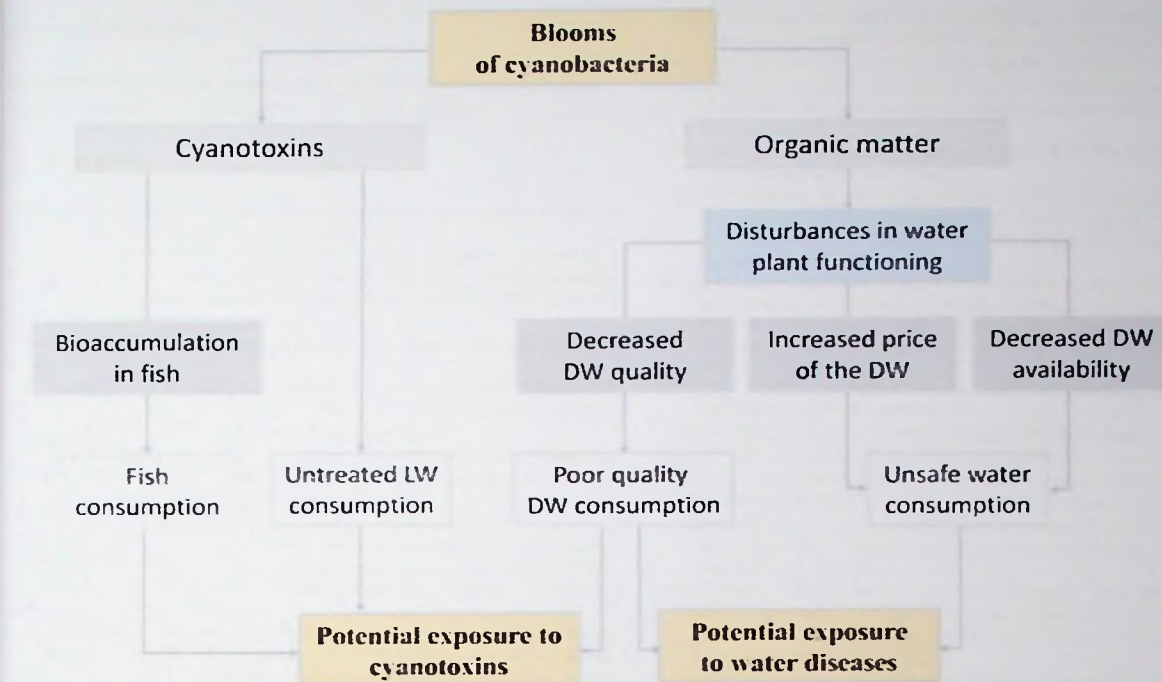
trajectory of LV and the necessity to carry out ambitious and sustainable management measures to address this major problem. The modeling approach developed by Downing et al. (2014) shows that controlling eutrophication is key to the protection and restoration of LV and its ecosystem functions and G&S. This finding is shared by many scientists as well as the local and international authorities in charge of the management of LV. Over the past 10 years, many actions have been taken to restore LV under the authorities of the East African Community (EAC), Lake Victoria Fisheries Organization (LVFO), Lake Victoria Environment Management Program (LVEMP), and Nile Basin Initiative (NBI) (Kayombo and Jorgensen, 2006).

We have attempted to differentiate the issues affecting the B&Gs and the OL resulting from population growth and human activity because (i) the B&Gs are more polluted than the OL and (ii) the main sources of pollutants affecting these areas are different. The pollution of the B&Gs is largely due to the discharge of (i) solid and liquid wastes/pollutants originating from big cities located on their shores and (ii) sediments and nutrients from the intensive agricultural farmland. Consequently, efficient wastewater collection and treatment of these domestic and industrial pollutants are critical in reducing the hyper eutrophication of the B&Gs. In Murchison Bay (Uganda), most of the inflowing nutrients are carried by the Nakivubo channel (Fuhriemann et al., 2015), comprising mainly the rainwater and the domestic and industrial wastewater of Kampala (Matagi, 2002; Kayima et al., 2008). With this goal in mind, the Kampala Capital City Authority (KCCA) launched the Green Industry Campaign in 2016 with the goal of improving wastewater treatment (KCCA, 2018). Recently, the KCCA also piloted a new action plan to improve fecal sludge collection and transport from household pit latrines, which are common in Kampala suburbs (KCCA, 2017). Finally, the construction of a new wastewater treatment plant in Kampala (Bugolobi/Nakivubo sewage plant) should increase the sewage treatment capacities of the National Water and Sewerage Corporation (NWSC), which oversees water sanitation in Uganda. Similar approaches have also been implemented for Kisumu located near the Nyanza Gulf in Kenya and for Mwanza located near the Mwanza Gulf in Tanzania in the framework of the Lake Victoria Water Sanitation Projects (LVWATSAN), which were first launched in 2004 but still continue to support actions for the improvement of solid and liquid waste management (<https://unhabitat.org/the-lake-victoria-water-and-sanitation-project/>).

All these actions are very important for the protection of the B&Gs of LV, but estimating their "real" impacts on the improvement of the water quality in the B&Gs remains difficult. Indeed, in urban areas, the wastewater collection networks are still limited, which results in the treatment of a small proportion of sewage, and with the predicted increased population growth rate (2–5% annually), sewerage production will increase.

In addition to improving wastewater collection and treatment, the protection and restoration of the numerous wetlands that are associated with the B&Gs is also important. As discussed before, many wetlands have been degraded due to the pressures linked to population growth. Consequently, several projects in the three riparian countries around LV focused on the rehabilitation of natural wetlands and the construction of artificial wetlands. These programs are aimed at securing and maintaining the hydrological and ecological integrity of wetlands (MWE, 2018). This initiative requires active involvement and participation of the local communities living in these areas, as shown in Kenya by Raburu et al. (2012) and in Uganda by Wacker et al. (2016).

Concerning the OL, it has been shown that in addition to river discharge, aerial depositions are the main source of nutrient inputs in the lake (e.g., Tamatamah et al., 2005; Kayombo and Jorgensen, 2006). Therefore, a reduction of these inputs would be associated with an integrated approach for the protection of the catchment area, including better soil cover management and improved land use patterns (Okungu and Opango, 2005; Vuai et al., 2013). To adequately protect the watershed, deforestation needs to be halted and forests need to be



potential impacts of cyanobacterial blooms on human health in Lake Victoria

ic water; DW: Drinking water

his constitutes a much greater challenge due to the com- drivers of this phenomenon (Masese et al., 2012; Waiswa). These authors also note the absolute need to in- volve local

ramework of the LVEMP I and II projects, several soil con- and afforestation projects were implemented to limit soil d the aerial deposition of nutrients in the lake. The impact of ects on the water quality of the OL has not been estimated. considering the size of the watershed and the increasing n density, soil conservation projects will require considerable efforts by the five countries before expecting "significant n the water quality of the main lake.

raction with LVEMP projects, national management plans been implemented in the countries belonging to the LV wa- Kenya for example, a master plan for the conservation and e management of water catchment areas was proposed in e Ministry of Environment and Mineral Resources (MEMR, catchment management plans have been implemented, for or the Awach Kano, leading to reduced de- forestation, water and gully erosion (Nyanchaga and Openji, 2017). In Tanzania, tchment-based actions have been implemented for the of LV in the framework of Integrated Water Resources ent and Development (IWRMD) plans (Domasa, 2019).

Conclusion

the demographic growth and the rising concentration of the h into urban areas in Africa (United Nation, 2018), we may n the next decades, eutrophication of freshwater ecosystems stated cyanobacterial blooms will continue to increase in LV n many other African lakes. Knowing that all these lakes umerous ecosystem G&S and are vital for the water and food e high microcystin concentrations recorded in water and V and the data of Roegner et al. (2020) suggest that cyano- and their toxins constitute a serious health concern for local

populations. The same is probably true in many countries of the intertropical area in Africa. Consequently, it is becoming urgent in Africa (i) to implement long term monitoring programs of cyanobacteria and cyanotoxins in lentic freshwater ecosystems, (ii) to define water policies and rules for cyanobacteria and cyanotoxins and to inform human populations about sanitary risks and (iii) to take actions for reducing the exposure of human populations to cyanotoxins knowing that among these actions, the provision of affordable and easily available treated-water is an urgent priority.

In a longer-term perspective, the battle against eutrophication will be one of the great challenge for African countries. As shown in this review, the three main components in this battle will concern (i) the wastewater treatment and management in urban areas knowing that currently available treatment methods might not be used in Africa due for example, to the lack of appropriate structure (Wang et al., 2014), (ii) the promotion of a sustainable agriculture, such as conservation agri- culture, allowing to provide foods to a growing population whilst minimizing environmental impacts (Giller et al., 2015) and (iii) the protection and restoration of wetlands, which can play a very important role in nutrient retention and reduction, even for great lakes (Watson et al., 2016).

As already experienced in developed countries, all these actions for reducing the exposure of human populations to cyanotoxins and the eutrophication will be very costly and consequently require interna- tional financial cooperation. There is also need for international colla- borative research programs involving scientists and people from local institutions working in the water domain. Finally, the involvement and participation of the local communities will be also a key factor for their successful development.

Acknowledgment

This review paper has been prepared in the framework of the WaSAf program (Sustainable Monitoring and Management of Surface Water Sources in Africa), which is funded by the French Facility for Global Environment (Fonds Français pour l'Environnement Mondial, FFEM).

Supplementary materials

Supplementary material associated with this article can be found, online version, at doi:10.1016/j.hal.2020.101829.

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CHAPTER THREE

PHYTOPLANKTON DIVERSITY AND TURNOVER IN LAKE VICTORIA²

CONTEXT

Since the 1930s, the phytoplankton production in Lake Victoria has increased but the real manifestation of eutrophication was in the late 1980s (Hecky, 1993). This increase was accompanied by shifts in the diatom community, from the dominance of *Aulacoseira* to the dominance of *Nitzschia* (Stager et al., 2009) to the dominance of cyanobacteria (Ochumba and Kibaara, 1989). Generally, the lake is characterized as “dominated by cyanobacteria” but composed of both toxigenic and non-toxigenic cyanobacteria taxa. Therefore, there is a knowledge gap in the α -diversity and β -diversity of phytoplankton species from both open and closed embayments experiencing different levels of nutrient loading. In this regard, this chapter provides a comparative assessment of the spatial-temporal dynamics of phytoplankton community and cyanobacteria in Napoleon Gulf (NG) and Murchison Bay (MB). These two embayments have large differences in the catchment population densities, are subjected to different levels of anthropogenic pressures and catchment activities such as wetland drainage, industrialization and urbanization, and wastewater management (e.g., direct discharge of waste from the Nakivubo channel into MB). Furthermore, the hydrodynamic conditions are distinct as NG is a large and open bay and has the outlet of the lake (R. Nile) while MB is a much smaller and more closed embayment. Results from the study affirms that there was high nutrient loading into MB, that caused significantly high phytoplankton biomass and affected the community diversity and turnover. For example, there was high proportions of N-fixing cyanobacteria such as *Dolichospermum* sp. in NG while in MB, there was high proportion of *Microcystis* sp. Therefore, much as the bays are dominated by cyanobacteria, different taxa are responsible the functioning of those respective bays. Consequently, there is no need for generalization of the embayments of the lake and the potential level of toxin production. The details of the dynamics of the phytoplankton community in NG and MB is presented below.

² Chapter based on this publication:

Olokotum, M.; Troussellier, M.; Escalas, A.; Hamlaoui, S.; Okello, W.; Semyalo, R.; Humbert, J.-F. and Bernard, C. (2021). High nutrient loading and climatic parameters influence the dominance and dissimilarity of toxigenic cyanobacteria in northern bays of Lake Victoria. *Journal of Great Lakes Research*, 47, 985-996, doi: <https://doi.org/10.1016/j.jglr.2021.04.021>

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr



nutrient loading and climatic parameters influence the dominance and similarity of toxigenic cyanobacteria in northern bays of Lake Victoria

Olokotum^{a,b}, Marc Troussellier^c, Arthur Escalas^c, Sahima Hamlaoui^d, William Okello^b, Ronald Semyalo^a, Jean-Humbert^e, Cécile Bernard^e

^aDepartment of Zoology, Entomology and Fisheries Sciences, Makerere University, Kampala, Uganda

^bUganda Fisheries Resources Research Institute (NaFIRRI), Jinja, Uganda

^cCE, CNRS-Univ Montpellier-IFREMER-IRD, Montpellier, France

^d245 Molécules de Communication et Adaptations des Microorganismes (MCAM), CNRS-MNHN, Muséum National d'Histoire Naturelle, Paris, France

^eInstitute of Ecology and Environmental Sciences of Paris (IEES), Sorbonne University, Paris, France

KEYWORDS

ABSTRACT

Received 11 November 2020
Received in revised form 1 April 2021
Accepted 18 May 2021
Available online 18 May 2021
Edited by R. Michael McKay

Eutrophication of Lake Victoria led to changes in its phytoplankton communities. However, different levels of eutrophication exist in the open lake and the bays, and between embayments. This study utilized spatial and temporal sampling of Napoleon Gulf and Murchison Bay, exhibiting different trophic conditions. Over one year, we investigated phytoplankton biomass, richness, diversity and dissimilarity, and related the dynamics of the dominant species to the limnological and climatic conditions. The results confirmed that Napoleon Gulf and Murchison Bay showed large differences in eutrophication status, with lower nutrient concentrations in Napoleon Gulf than in Murchison Bay, where a strong gradient was observed from inshore to offshore areas. These nutrient dynamics resulted in a 4 to 10 fold higher chlorophyll-*a* in Murchison Bay than in Napoleon Gulf. From the embayments, 135 phytoplankton taxa were recorded with no significant differences in alpha diversity. However, high dissimilarity in community structure was observed in beta diversity, mostly due to a turnover among the dominant toxigenic species. Thus, from a similar species pool, there was a shift in the dominant toxigenic cyanobacteria from *Microcystis flos-aquae* and *M. aeruginosa* in Murchison Bay, *Dolichospermum circinale* and *Planktolyngbya circumcreta* in Napoleon Gulf to *D. circinale* in the offshore stations. These cyanobacteria are toxigenic taxa with known health hazards. Using partial least square models, we showed that both climatic variables (e.g., wind, solar radiation) and levels of inorganic dissolved nutrients (e.g., SRP, NO³-, and NH⁴+) are the main drivers of differences and dominance in cyanobacteria communities in northern Lake Victoria.

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Introduction

Over years of anthropogenic pressures, Lake Victoria is now considered to be persistently eutrophicated and polluted (Downing et al., 2012; Juma et al., 2014; Mbonde et al., 2015; Nassali et al., 2020; Olokotum et al., 2020). One well-known consequence of eutrophication is the significant modification of the phytoplankton community, which is the cornerstone supporting the productivity of all aquatic ecosystems (Boynton et al., 1983). However,

ever, anthropogenic pressures are not uniformly distributed, and bays and gulfs appear more affected by eutrophication than the open lake (Ngupula et al., 2012; Olokotum et al., 2020). In addition, the trophic status of the bays and gulfs varies greatly across the lake especially for the constricted and open bays (Mbonde et al., 2015). Beyond the ecosystem services identified for the whole lake (Sterner et al., 2020), these bays and gulfs provide crucial services for the riparian populations that are likely to be impaired by eutrophication. Most of these embayments are shallow and increasingly susceptible to point source pollution and, consequently, experience changes in phytoplankton diversity and increased biomass. These planktonic communities may experience extreme increases in biomass and drastic composition shifts, which

Corresponding author.

E-mail address: cecile.bernard@mahe.fr (C. Bernard).

<https://doi.org/10.1016/j.jglr.2021.04.021>

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result in harmful algal blooms (HABs). These HABs impair the ecological functioning of these ecosystems and have both direct and indirect consequences for the local population. While many studies have separately investigated the biomass and taxonomic composition of phytoplankton community from different bays and gulfs (Olokotum et al., 2020), understanding the drivers of phytoplankton community modifications under different eutrophication pressures in Lake Victoria is still required. For that, we need to characterize not only phytoplankton communities' biomass, but also diversity, both alpha (number of species within a community) and beta (extent of species replacement along environmental gradient) diversity (Whittaker, 1972; Wilson and da, 1984). Alpha and beta diversity have been used to provide insight into habitat integrity and comparative changes along environmental gradients (Wilson and Shimida, 1984; Whittaker et al., 2006). Although phytoplankton alpha diversity is driven by local environmental conditions, beta diversity may be highly dependent on dispersal mechanisms and differences among environmental conditions of study sites (Moresco et al., 2017) which could be different between the bays and gulfs of Lake Victoria. These differences are known to be due to species replacement (turnover) and species loss or gain from one site to another (Baselga and Orme, 2014). This component of beta diversity is crucial towards understanding ecological status of bays and gulfs of Lake Victoria. Noticeable differences between these bays and gulfs, and the lake were observed as early as the 1920s (Worthington 1930), with evidence of an increase in phytoplankton biomass, as chlorophyll *a* (Chl-*a*) concentration, was recorded from the 1960s. The Kenyan part of the lake has experienced an increase in Chl-*a* concentration from 3–5 µg/L to 40–60 µg/L between the 1960s and 2000s, with an extreme value of 650 µg/L in the Nyanza Gulf (Olokotum et al., 2012). In the northern part of Lake Victoria (Uganda), all bays and gulfs showed the same pattern. For instance, in Murchison Bay, Chl-*a* concentration increased from 10–20 µg/L to 60 µg/L between the 1990s and the early 2000s (Kallqvist et al., 1996; Haande et al., 2011) and now exceeds 100 µg/L (Haande et al., 2011). Using paleolimnological data, Murchison et al. (2002) showed that the increase of phytoplankton biomass since the 1930s mirrors the growth of human population and agricultural activities within the Lake Victoria watershed. There has also been a shift in phytoplankton community composition from green algae and diatoms to cyanobacteria, especially toxin-producing ones (Olokotum et al., 2020). Eutrophic bays and gulfs such as Nyanza Gulf (Onyango et al., 2020), Tanzanian bays (Mbonde et al., 2015) and Murchison Bay in Uganda (Okello and Kurniayer 2011), have cyanobacteria as the dominant taxonomic group. Cyanobacterial blooms in the bays and gulfs may have a direct impact on humans through the contamination of water and fish consumed by local populations (Onyango et al., 2020).

Investigations of phytoplankton distribution in the 1950s (Talling 1957) found associations with thermal stratification. By 2010 based on correlations, environmental factors such as sampling depth, light attenuation, and nutrients (SRP, NH₄⁺, TN and TP) were proposed to account for the changes in community structure from diatoms to cyanobacteria (Poste et al., 2013). In this context, it is important to identify the environmental variables which have significant effects on dominant cyanobacteria species to propose actions that can efficiently limit their impact. To identify these control factors, it is necessary to obtain data from sites with different degrees of eutrophication from the closed and open embayments of Lake Victoria.

At present state, there are significant contrasts between the constricted and open embayments and the open lake, especially regarding the dominance of Cyanobacteria (Mbonde et al., 2015; Olokotum et al., 2020). Although Haande et al. (2011), provided insights into the dynamics and factors influencing Cyanobacteria in Murchison Bay, modelling approaches were not attempted. Studies by Silsbe et al., (2006), reported how spatial and temporal environmental variations have affected primary productivity from the shallow to deep waters. We investigated the spatial and temporal changes of phytoplankton community biomass and diversity (alpha and beta) in two embayments in the northern part of Lake Victoria. Murchison Bay (MB), a constricted/closed bay and Napoleon Gulf (NG) an open bay, for which previous studies have reported different eutrophication status (Silsbe et al., 2006; Poste et al., 2013), and connection with the main lake provided suitable sites for a comparative study. In these two sites, the lake receives wastewater from and supplies drinking water for the two largest cities in Uganda (Kampala and Jinja) but is also consumed directly by people living on the shores who depend on it for fishing (Outa et al., 2020) and aquaculture (Musinguzi et al., 2019). In both bays, the phytoplankton community characteristics, climatic and limnological variables were measured monthly for one year along a transect from the inshore to the open lake.

Materials and methods

Study site and sampling procedures

The Napoleon Gulf and Murchison Bay are in the northern part of Lake Victoria (Uganda) and their characteristics are summarized in Table 1. Napoleon Gulf is surrounded by Jinja City, Buikwe and Buvuma districts. The gulf receives non-point source chemical pollutants (Oguttu et al., 2008), nutrients and wastewater across the wetland systems near the source of the Nile (major drainage outlet of Lake Victoria). Murchison Bay is surrounded by the Greater Kampala Metropolitan Area (KMA) which includes the districts of Kampala and Wakiso that are highly industrialized with large human populations. This bay receives a direct discharge of

or characteristics of the study sites and their catchment.

Characteristics	Napoleon Gulf	Murchison Bay
Location	3° 15' 11.64" E, 0° 24' 59.54" N	3° 38' 51.52" E, 0° 15' 25.03" N
Sampled depth (m) (Min–Max)	5.7–18.1	1.4–18.4
Size (km ²)	125	256 (137**)
Catchment area (km ²)	187.92	593.92
Estimated population	900,000	5 million (Day) and 2 million (Night)
Major wetlands (km ²)	Kafuma (1.62) Kirinya (0.92), Masese (2.2) and Budumbuli (4.3)	Kinawataka (0.21), Nakivubo I and II (4.3 and 4.91) and Namwanje (38.3)
Wastewater plant (connections)*	Kirinya (3,611)	Bugolobi (9,734)
Drinking water plant (connections)*	Walukuba (28,881)	Gaba I, II, & III (315,897)

*National Water and Sewerage Corporation (NWSC) annual report of 2019; water connections by NWSC doesn't necessarily equal the number of households since one connection might serve 7 households. **Area of Inner Murchison Bay in parentheses.

er mainly from the KMA through the Nakivubo channel adjacent wetland systems. Thus, point source pollution is ninent in Murchison Bay than Napoleon Gulf. In addition, n Bay is a more enclosed bay and has a much more pop- ershed than Napoleon Gulf.

ampling campaign was carried out monthly between r 2017 and October 2018. At each site, water samples were from four stations (NG1–4, MB1–4) along a transect from the the offshore station located 16 km outside the Murchison Bay leon Gulf area (Fig. 1).

rological variables

eteorological data (rainfall, air temperature, wind speed and iation) were obtained from the Jinja and Kampala weather perated by the Uganda National Meteorological Authority . To determine the influence of seasons, we used the rainfall m UNMA to classify the months as wet (>60 mm total n = 6 in Napoleon Gulf Napoleon Gulf and n = 7 in Murchison dry months (<60 mm total rainfall, n = 6 in Napoleon Gulf and Murchison Bay). In addition, climatic data for the previous 5 days before sampling (D5 & D10) and day of sampling (D0) ir temperature, mean wind speed D5, total rainfall D5 and lar radiation D10) were used for modelling the biovolume ant phytoplankton species.

ical and chemical variables

each sampling operation (site x month x station), we ed several limnological variables. Water depth was ed

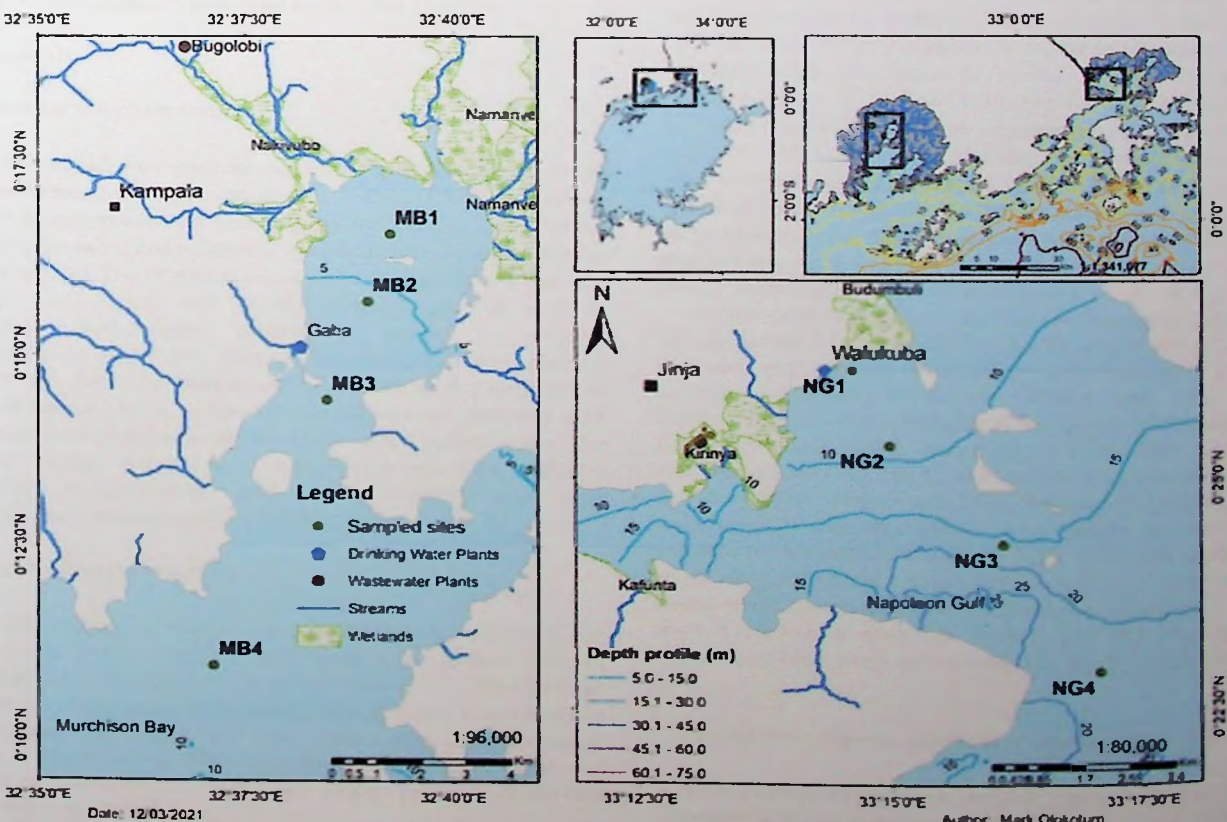
using a portable depth sounder (Hondex PS-7), and water trans- parency using a Secchi disk (diameter = 20 cm). Water temperature, pH, electrical conductivity, dissolved oxygen, turbidity, and fluorescent dissolved organic matter (fDOM) were measured in-situ using the YSI Sonde EXO2 multiprobe meter (Sonde 16B102461). Water samples for chemical analysis were collected every meter of depth at each site using a 2-L horizontal van Dorn sampler as described by Wetzel and Likens (2000a). The samples were collected in a 20 L bucket as a composite sample. From the composite sample, 2 L were taken for chlorophyll-a (Chl-a) and nutrients analyses while 20 mL were used for phytoplankton analysis.

Samples for dissolved nutrient measurement were immediately filtered using Whatman GF/C filters (Ø 47 mm). Soluble reactive phosphorus (SRP) was determined using the ammonium molyb- date method (Wetzel and Likens 2000b), nitrate (NO₃-N) by the sodium salicylate method (Müller and Wiedemann 1955), ammon- ium (NH₄-N) using the indophenol blue method (Krom 1980), and soluble reactive silica (SRSi) using the yellow molybdate- silicic acid method (Wetzel and Likens 2000b). Raw water samples were also collected in acid-washed polyethylene containers for total phosphorus (TP) and nitrogen (TN) analysis using the persulphate digestion oxidation methods described in Wetzel and Likens (2000b).

Biological variables

Chlorophyll a (Chl-a) concentration

The Chl-a concentration was measured by filtering 100–500 mL of water (Whatman GF/C filters), followed by hot 90% ethanol



Maps of Murchison Bay (MB) and Napoleon Gulf (NG) with the locations of sampling stations (MB1 to 4 and NG1 to 4) along the spatial transects. Inset are maps of northern Lake Victoria

ction (ISO-10260, 1992) of the filtered biomass and analysis with a Cary 6000 UV/vis spectrophotometer. Briefly, 10 mL of 90% ethanol was added to filtered biomass and incubated in a water bath for 2 min at 78 °C and later sonicated for 15 min to accelerate cell lysis. The extract was cooled in the dark and then filtered through the 0.2 µm mesh size membrane filter (Millex, GS). Absorbance measurement was done at 665 nm wavelength and corrected for turbidity by subtracting the absorbance measured at 750 nm wavelength. To the measured extract in the cuvette, 100 µL of 0.25 M perchloric acid was added, shaken and absorbance measured again at 665 nm and 750 nm.

Phytoplankton communities

Taxonomic identification, taxa abundance and biovolume estimation were performed following the procedure described by Laine et al. (2017). The phytoplankton samples were fixed using 1% iodine solution and 2–5 mL of sample were sedimented for 6–8 min (Möhl, 1958). Chl-*a* concentration was determined in each sample. When Chl-*a* concentration was low (20 µg/L), 5 mL were sedimented. When it was high (> 20 µg/L), 2–3 mL were sedimented. Phytoplankton units were counted under a Nikon Eclipse TS-100 inverted microscope (400X) and identified using taxonomic guides or keys based on morphological characteristics as described in Talling (1979) and Cronberg and Annadotter (2006). At least 30 microscopic fields (microscopical units) were counted to reduce errors to < 10% (as described in Wetzel and Likens (2000a)). *Microcystis* was first counted on the size of the colony and cell size. Later samples containing *Microcystis* were re-counted using a Malassez counting chamber for *Microcystis* sp. The volume of each species was estimated as geometric shapes as described by Sun and Liu (2003).

Statistical analysis

Environmental characteristics

Environmental data were centred on a Euclidean distance matrix and performing principal component analysis (PCA) to condense the information on limnological and climatic variables and the dimensions and patterns of environmental variation between the stations. The PCAs were computed in 'FactoMinerR' package (Jen et al., 2008) and 'factoextra' package was used for visualization. Permutational multivariate analysis of variance (ANOVA) using the *adonis2* function of 'vegan' package (Jen et al., 2007) was used to test the differences in environmental conditions between bays, within stations and sea-season (wet and dry). The differences between the bays and within stations were also tested using independent *t*-tests and one-way ANOVA, respectively. Mann-Whitney and Kruskal–Wallis tests were used where necessary in R software.

Phytoplankton communities

The list of phytoplankton taxa across sampling sites was tabulated, and we used Venn diagrams to interpret taxa richness relations between sites and stations along the gradient using the 'Limma' package (Ritchie et al., 2015). Indices of α -diversity were calculated using Shannon–Weiner and Pielou evenness indices using the 'vegan' package (Oksanen et al., 2007). The biovolume for each taxon was calculated and a rank-biovolume diagram created for the dominant taxa in each bay. Later, the change

in the mean biovolume of the dominant taxa along the spatial transect were evaluated. Phytoplankton biovolume data were square-root-transformed to increase the influence of rare taxa in the dissimilarity matrix. β -diversity based on the Bray–Curtis dissimilarity index was applied to the square root-transformed phytoplankton biovolume to characterize the differences in phytoplankton community between and within bays (tested using PERMANOVA). The same Bray–Curtis dissimilarity matrix was ordinated using non-metric dimensional scaling (nMDS). Further, the similarity percentage breakdown (SIMPER) analysis (Clarke, 1993) was used to assess the average contribution of individual phytoplankton taxa to biovolume-based Bray–Curtis dissimilarity between bays and within stations.

Influence of environmental variables on phytoplankton communities

Limnological and climate data, SRP, NO₃⁻, NH₄⁺, SRSi, water temperature, mean wind speed (D5) and mean solar radiation (D10), were log-transformed to ensure normal distribution before fitting partial least square (PLS) regression models to the observed biovolume of dominant taxa (*M. flos-aquae*, *M. aeruginosa*, *D. circinalis* and *P. circumcincta*).

Results

Environmental characteristics of Napoleon Gulf and Murchison Bay

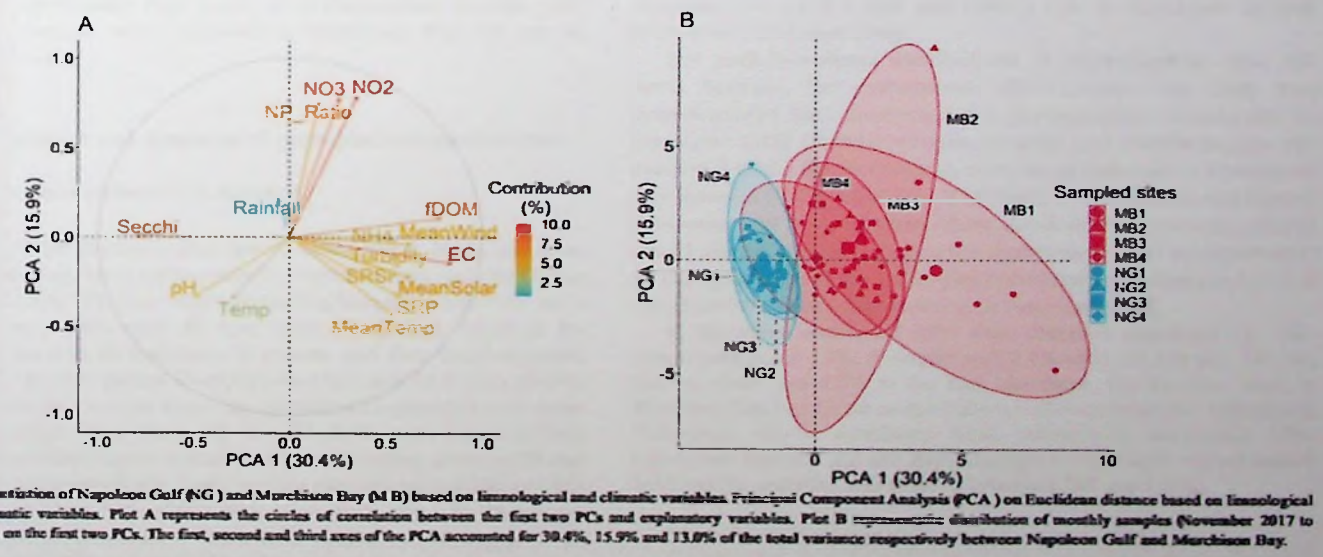
A total of 14 limnological and four climatic variables were used to characterize the environmental conditions experienced by phytoplankton communities in Napoleon Gulf and Murchison Bay (Table 2 and Electronic Supplementary Material (ESM) Table S1). The two bays differed in Secchi depth, turbidity, conductivity and fDOM (Mann–Whitney test, *p* < 0.05, ESM Table S1). Regarding limnological variables, the levels of turbidity, dissolved organic matter (fDOM), TP, SRP, NH₄⁺ and TN were significantly higher in Murchison Bay than in Napoleon Gulf (6–10 times for fDOM, 10 times for NH₄⁺, Mann–Whitney test, *p* < 0.001, ESM Table S1). We also observed correlation between variables, for instance, negative correlation between fDOM, turbidity and ratio of N:P in Napoleon Gulf, while Secchi depth and NH₄⁺ were negatively correlated in Murchison Bay (Fig. 2A). The physico-chemical conditions in the four stations of Napoleon Gulf are more homogeneous than those obtained in the Murchison Bay, with station 1 being different from stations 2 to 4. This was revealed by the lower overlap of ellipses from each station on the PCA (Fig. 2B).

Within each embayment, we observed significant differences among stations 1 to 4 (pseudo-*F* = 12.9, *P*(perm) = 0.001), and between dry and wet seasons (pseudo-*F* = 2.2, *P*(perm) = 0.018) for the following variables: conductivity, fDOM, SRP, NH₄⁺ (Table 2). Particularly, the inshore Murchison Bay station (MB1) exhibited high conductivity and fDOM values but with low SRP compared to the offshore stations (MB 2 to 4). In Napoleon Gulf, conductivity, fDOM, nutrients, NO₃⁻, NH₄⁺ and SRSi, were significantly higher in the inshore (NG1) than the offshore areas (NG2–4), while the other variables (water temperature, pH, turbidity and Secchi depth) remained stable along the spatial transect (ESM Figure S1). Generally, there is a strong environmental gradient established in Murchison Bay inferred from the levels of dissolved oxygen, pH and conductivity with significantly high levels of nutrients into the bay unlike in Napoleon Gulf.

Spatial and temporal variations of phytoplankton biomass

Total phytoplankton biovolume was significantly and positively correlated with log-transformed Chl-*a* (*R*² = 0.55, *p* < 0.0001, ESM

	MB1			MB2			MB3			MB4		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
	1.8 ± 0.3	1.4	2.5	5.4 ± 0.5	4.8	6.6	14.0 ± 2.3	9.4	18.4	12.7 ± 0.5	12	14
	0.5 ± 0.1	0.3	0.7	0.6 ± 0.1	0.4	0.8	0.9 ± 0.2	0.7	1.3	1.2 ± 0.2	0.8	1.6
	25.5 ± 0.7	24.2	26.4	25.3 ± 0.5	24.3	26	25.3 ± 0.6	24	25.9	25.2 ± 0.6	23.9	26.2
	183.4 ± 45.4	124.4	251.5	126.4 ± 9.0	112.8	146.1	121.6 ± 8.2	105.6	132.3	106.1 ± 3.9	102	116
	25.2 ± 10.6	15.7	45.8	20.9 ± 2.9	15.8	25.4	19.5 ± 3.8	12.6	27	11.8 ± 19.4	4.1	73.1
	78.9 ± 63.9	8.9	197.2	30.6 ± 18.7	9.7	73.7	14.0 ± 8.8	4.4	32.9	10.9 ± 8.6	2.1	30.9
	5.8 ± 2.4	2.7	9.6	7.2 ± 1.6	4.7	10.2	5.9 ± 1.2	4	7.7	7.2 ± 0.6	6.3	8.2
	8.2 ± 0.4	7.6	8.9	8.6 ± 0.5	8	9.4	8.3 ± 0.3	7.8	8.8	8.6 ± 0.3	8.1	9.1
	151.7 ± 150.8	18	521.9	80.3 ± 69.9	18.2	264.4	50.4 ± 37.1	14.4	139.3	59.2 ± 74.3	10.4	275.6
	73.6 ± 107.8	7.1	380.3	7.3 ± 3.8	0.9	15.8	6.4 ± 2.9	3.4	13.3	7.1 ± 9.6	0.9	36.7
	3,008.6 ± 170.5	153.1	21,633.60	1,036.2 ± 877.9	170.5	3,294.60	1,020.0 ± 1104.0	162.6	3,955.60	950.4 ± 930.1	149.1	3,108.10
	88.4 ± 100.9	19	380.4	146.8 ± 258.4	8.5	940.1	84.1 ± 67.4	7.3	231.4	64.7 ± 55.1	9.7	170.1
	337.1 ± 370.5	0.7	1,206.90	26.9 ± 37.7	0.5	133.4	32.5 ± 46.6	0.4	163.9	18.4 ± 27.6	0	81.4
	1,089.8 ± 922.7	282	3,081.90	483.0 ± 243.0	139.5	932.1	385.3 ± 185.7	112.4	685.6	235.9 ± 196.1	9	596.9
	NG1			NG2			NG3			NG4		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
	6.4 ± 0.5	5.7	7.2	9.2 ± 0.6	8	9.8	13.1 ± 1.2	11.4	15.8	18.1 ± 1.3	16.7	21.5
	1.3 ± 0.3	0.9	1.8	1.3 ± 0.2	1	1.7	1.4 ± 0.2	1.1	1.7	1.6 ± 0.2	1.3	2
	26.3 ± 0.6	25.4	27.3	26.0 ± 0.6	25.2	27	25.8 ± 0.5	24.9	26.7	25.7 ± 0.5	24.8	26.6
	105.3 ± 4.7	99.8	115.7	102.8 ± 4.2	97.9	112.2	101.0 ± 2.8	97	106	100.5 ± 3.1	96	105.5
	-0.2 ± 0.6	-1.3	1	-0.5 ± 0.5	-1.6	0.4	-0.6 ± 0.5	-1.6	-0.2	-0.8 ± 0.4	-1.5	0
	15.0 ± 15.9	0.7	58.1	12.7 ± 17.3	0.5	61.2	12.2 ± 13.9	0.2	40	6.9 ± 6.9	0	17.3
	7.3 ± 1.0	5.5	8.8	7.4 ± 0.8	6.4	8.8	7.1 ± 0.7	5.8	8.1	7.0 ± 0.8	4.9	7.9
	8.6 ± 0.3	8.4	9.4	8.8 ± 0.2	8.4	9.3	8.8 ± 0.3	8.2	9.3	8.7 ± 0.3	8	9.2
	37.9 ± 36.6	8.9	145.1	44.0 ± 24.9	8.2	168.4	40.6 ± 31.4	10.1	128.1	36.9 ± 22.8	8.6	94
	4.5 ± 1.9	0.6	8	5.2 ± 2.3	0.7	9.5	6.4 ± 3.1	0.7	11	8.8 ± 5.3	0.7	18.2
	712.1 ± 474.7	129.7	1,664.70	380.8 ± 150.6	134.1	633.6	530.8 ± 321	148.5	1,210.20	570.3 ± 352.4	154.1	1,122.60
	28.6 ± 22.0	2.1	68.5	41.6 ± 43.5	1.9	153.9	50.4 ± 46.0	1.1	170.2	121.0 ± 262.2	2.1	947.4
	6.9 ± 8.8	1.2	32.7	8.1 ± 10.4	0.6	31	7.8 ± 16.7	0.4	59.1	11.6 ± 21.1	0.5	76.4
	302.9 ± 194.2	99.9	635.4	308.2 ± 210.1	81.9	717.8	327.1 ± 194.5	80.2	629.9	368.8 ± 262.6	73.4	903.9



2), thus Chl-a was used as a proxy of total phytoplankton. These Chl-a concentrations were higher (Mann-Whitney test, $p < 0.001$) in Murchison Bay (28.1 to 1087 $\mu\text{g/L}$) than in Napoleon Gulf (50.4 $\mu\text{g/L}$). No significant differences in Chl-a were found in sampling stations of Napoleon Gulf (Kruskal-Wallis test, $p = 0.15$) while in Murchison Bay mean Chl-a decreased from the inshore (MB1) to offshore areas (MB4, Kruskal-Wallis test, $p < 0.001$, Fig. 3A). As shown by Fig. 3B, the temporal changes in Chl-a were stronger for Murchison Bay than Napoleon Gulf, notably for the two inshore stations (MB1, MB2). The highest Chl-a values were measured in December for Napoleon Gulf (50.4 $\mu\text{g/L}$) and September for Murchison Bay (1087.1 $\mu\text{g/L}$). In

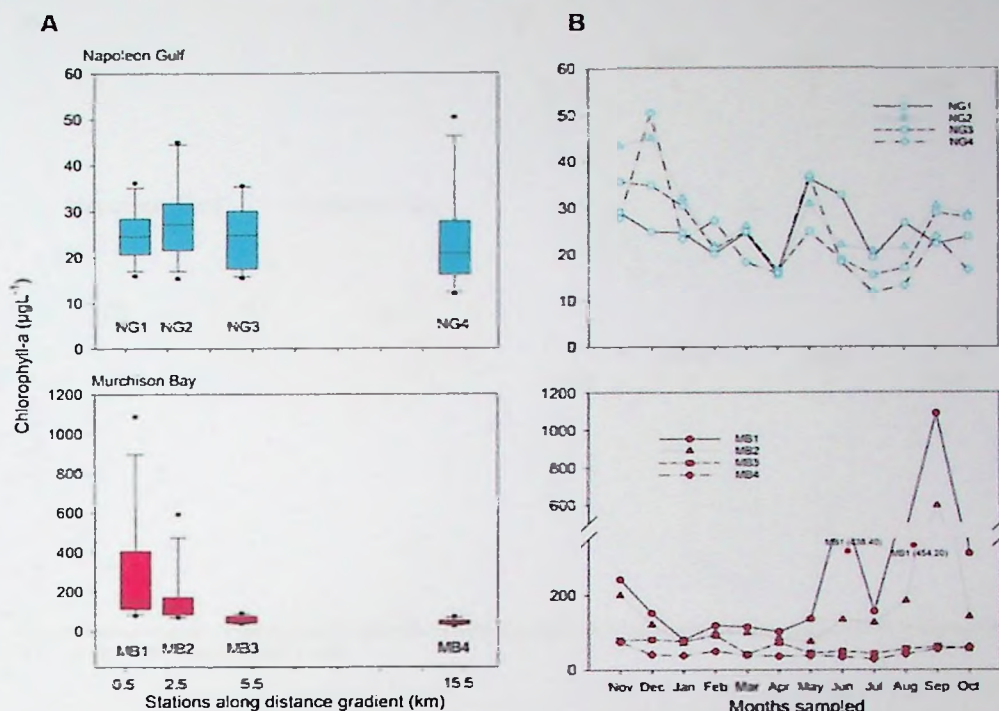


Fig. 4. Spatial and temporal variation in Chlorophyll-a. Plot A: Spatial variation along the inshore-offshore distance between stations 1 to 4 in Napoleon Gulf (NG 1 to 4) and Murchison Bay (MB 1 to 4). Plot B: Temporal variation between November 2017 and October 2018.

significantly high levels of phytoplankton biomass with reference were observed in Murchison Bay but not in Napoleon Gulf.

Patterns and dynamics of phytoplankton communities

Phytoplankton richness and diversity

135 phytoplankton species belonging to 49 genera were identified during the sampling in both Napoleon Gulf and Murchison Bay (Table S2). The Chlorophyta/Euglenophyta were the most abundant with over 62 taxa from 21 genera, followed by Bacillariophyta with 44 taxa from 13 genera, and then Dinophyta from 11 genera. Dinophyta had two taxa from two genera, two species from one genus and Chrysophyta one taxon only. From these 135 taxa, 71 (53%) were found in both Napoleon Gulf and Murchison Bay exhibited 29 and 24 taxa respectively, leading to a total richness of 100 and 106 taxa in Napoleon Gulf and Murchison Bay, respectively (Fig. 4A). The ranges of phytoplankton richness were similar in both bays, 14–32 taxa in Napoleon Gulf and Murchison Bay, respectively. Similarity of taxa were shared across all stations in each embayment: the highest number of station-specific taxa was observed in the first two stations, NG1 and MB1 (Fig. 4B). Phytoplankton diversity was not significantly different between the two bays (Mann-Whitney test, $p = 0.091$, $H' = 2.12 \pm 0.26$ in Napoleon Gulf and 1.43 ± 0.28 in Murchison Bay). During the sampling, there was no significant difference in diversity between stations in Napoleon Gulf (Mann-Whitney test, $p = 0.944$) while significant differences were found in Murchison Bay (min-max; 1.29–2.54, Mann-Whitney test, $p = 0.048$). The average

evenness was 0.672 ± 0.09 and 0.685 ± 0.07 in Murchison Bay and Napoleon Gulf, respectively.

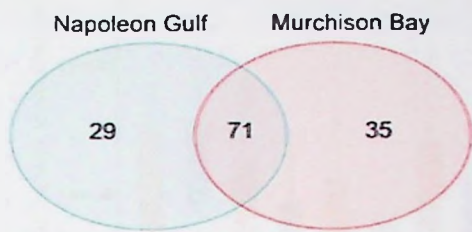
The rank-biovolume distributions of phytoplankton taxa differed between the embayments (ESM Figure S3). Only two cyanobacterial taxa dominated the phytoplankton community in Napoleon Gulf (*Dolichospermum circinale* and *Planktolyngbya circumcreta*) while the biovolumes were more balanced in Murchison Bay between the top four taxa: *D. circinale*, *P. circumcreta*, *Microcystis flos-aquae* and *M. aeruginosa*. These dominant species were among the 71 shared species between the embayments and were present at all the sampled stations. Other phytoplankton taxa were rare and had very low biovolume. For instance, in Napoleon Gulf,

M. flos-aquae contributed 4.8% while *Nitzschia acicularis* var. *acicularis* contributed 4.5%, *P. simplex* with 2.4% while the rest contributing between 0.008 and 1.5% to the total biovolume. On the other hand, in Murchison Bay, besides the co-dominance of *Microcystis* species, *Chlorophyta* *Pediastrum simplex* contributed 6.2%, *Selenastrum bibraianum* 1.9%, *Cosmarium habitat* 1.2% and Bacillariophyta *N. acicularis* var. *acicularis* 2.0% and the remaining taxa made up between 0.003 and 1.43%.

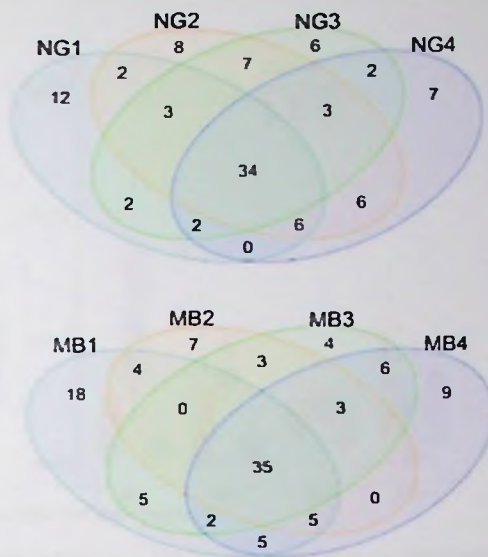
Dissimilarity between phytoplankton communities

The structure of phytoplankton community biovolume distributions differed significantly between Napoleon Gulf and Murchison Bay (PERMANOVA test, $p = 0.001$), with the two bays clustering separately on the nMDS ordination (Fig. 5A). There was a significant station effect in Napoleon Gulf and Murchison Bay (PERMANOVA test, $p = 0.002$); however, no effect of season on community structure was found (PERMANOVA test, $p = 0.086$). The estimated dissimilarity in the structure of the communities between Napoleon Gulf and Murchison Bay was 72.6% and mainly driven by 25 taxa (ESM Table S3). The average contribution of individual taxa to this dissimilarity was highly variable and mostly due

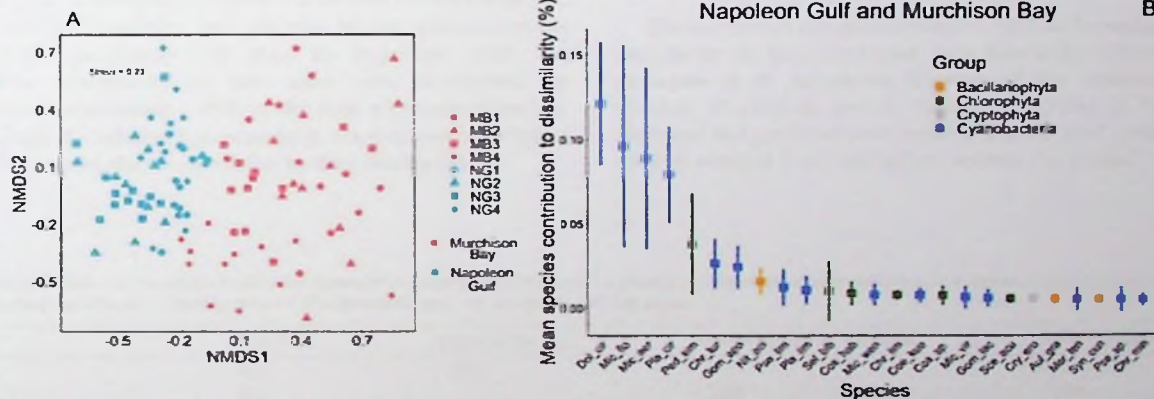
A



B



ifferences of phytoplankton communities in Napoleon Gulf and Murchison Bay. Plot A: Total phytoplankton richness within and between bays. Plot B: Station-specific richness and overlap among in Napoleon Gulf (NG 1 to 4) and Murchison Bay (MB 1 to 4).



ifferences in structure of the phytoplankton communities and contributing taxa. Plot A: nMDS ordination of the communities performed on Bray-Curtis dissimilarities matrix using relative strices (stress = 0.23). Plot B: Average taxa contribution to dissimilarity between phytoplankton communities from the two bays. The values are averaged across all pairs of communities. mes of the phytoplankton taxa are in ESM Table S3.

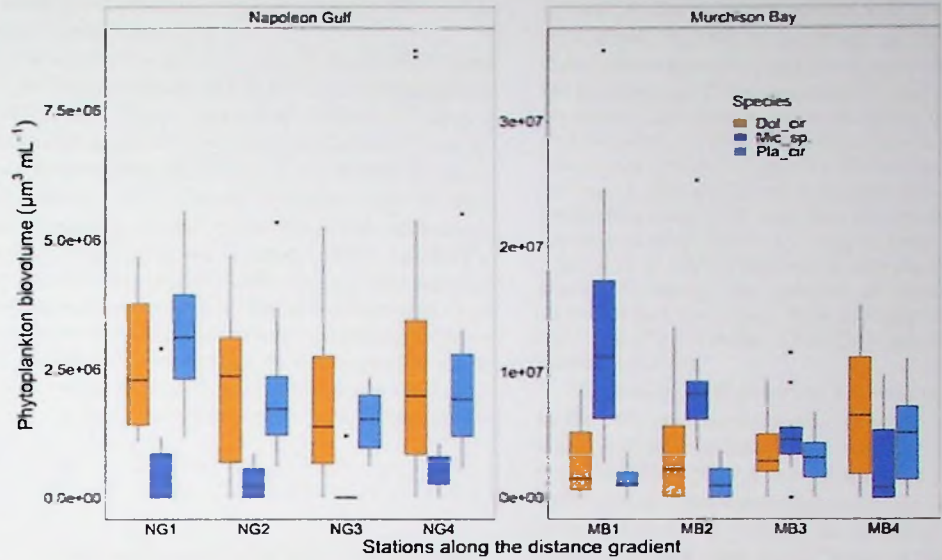
nobacterial taxa (Fig. 5B). The cyanobacteria *D. circinale*, *uginosa*, *M. flos-aquae* and *P. circumcreta* contributed the most imilarity between Napoleon Gulf and Murchison Bay communities, by the chlorophyte *Pediastrum simplex*, two cyanobacteria *cus turgidus* and *Gomphosheria aponina*) and the bacillariophyte *acicularis*. The dissimilarity among stations of Napoleon Gulf, NG1 1.9–65.9%) was driven by the Cyanobacteria *D. circinale*, *M. flos-* *P. circumcreta* followed by the Bacillariophyta *N. acicularis* and *xacteria Pseudanabaena sp., Merismopedia tenuissima* (ESM Table chison Bay, the dissimilarity among stations MB1 to MB4 (57.9– is driven by *M. flos-aquae*, *M. aeruginosa*, *D. circinale*, and *C.* ESM Table S3, Figure S4).

-temporal dynamics of the dominant taxa

g the study period, phytoplankton biovolume in Napoleon $(10^6 \pm 3.1 \times 10^6 \mu\text{m}^3/\text{mL})$ was lower compared to Murch- $(2.4 \times 10^7 \pm 1.3 \times 10^7 \mu\text{m}^3/\text{mL})$, Mann-Whitney test,

$p < 0.001$). Cyanobacteria contributed > 60% and > 90% of the total biovolume in Napoleon Gulf and Murchison Bay, respectively. The contribution of other groups (Chlorophyta/Euglenophyta, Bacillar- iophyta, and Dinophyta) was generally low with occasional dinoflagellates in the inshore areas of Napoleon Gulf (ESM Figure S5). Temporal variation in these groups was more prominent towards the offshore stations (ESM Figure S6). The relative phytoplankton species contribution was dominated by the cyanobacterium *D. circinale* (21.3%), followed by *P. circumcreta* (15.4%), *M. flos-aquae* (13.3%), *M. aeruginosa* (11.8%), the Chlorophyta *Pediastrum simplex* (5.3%) and the Bacillariophyta *Nitzschia acicularis*.var. *acicularis* (2.6%) in both Napoleon Gulf and Murchison Bay.

Only two taxa appeared co-dominant in the phytoplankton communities in Napoleon Gulf (*D. circinale* and *P. circumcreta*), fol-lowed by two taxa from the genus *Microcystis* (*M. flos-aquae* and *M. aeruginosa*). This was true in all the stations from Napoleon Gulf(Fig. 6). In Murchison Bay, these four taxa were co-dominant; while *D. circinale* and *P. circumcreta* exhibited an equivalent biovolume in all stations, the biovolume of *Microcystis* spp. decreased from the



patial changes in median biovolume of the dominant species along the distance gradient in Napoleon Gulf (Left panel) and Murchison Bay (Right panel) between November 2017 and 2018. Note: Dol_cir = *Dolichospermum circinale*, Pla_cir = *Plankolyngbya circumcreta* and Mic_sp = *Microcystis aeruginosa* + *Microcystis flos-aquae*.

to the open lake. Further, the balance between dominant taxa was similar in the offshore stations from both embayments (i.e., NG4 and MB4). Generally, and despite higher phytoplankton biomass in Murchison Bay than in Napoleon Gulf, the phytoplankton community in both sites were dominated by cyanobacteria, contributing > 70% of the total phytoplankton biovolume, although the relative Cyanobacteria composition differed in the embayments, they were similar in their offshore areas.

Factors influencing the dynamics of dominant taxa biovolumes

Environmental variables explained >64% of the variance (R^2) of PLS models for the three dominant taxa biovolume (*Microcystis* spp. (*M. flos-aquae* & *M. aeruginosa*; because of the coherence of the two species), *D. circinale* and *P. circumcreta*) (Table 3). Comparison of observed and predicted data revealed that the good performance of the models resulted from taking into account the pooled

Table 3
Partial coefficients from the global Partial Least Square (PLS) Regression models for Chl-a biomass and biovolume of phytoplankton taxa in Napoleon Gulf and Murchison Bay. Note: Only explanatory variables are included (p value < 0.05), *Microcystis* spp. = *M. aeruginosa* + *M. flos-aquae*.

	Log Chl-a	<i>Microcystis</i> spp.	<i>Dolichospermum circinale</i>	<i>Plankolyngbya circumcreta</i>
Intercept	0.14	0.11		
Station	0.11		0.13	0.09
Water Temp	0.23	0.18	0.18	0.16
Wind_D5	0.17	0.14		
Solar radiation_10	0.20	0.12	0.18	0.19
		0.23	0.29	0.30
		0.33		0.31
	0.62	0.65	0.78	0.73

Table 4
Partial parameter coefficients from the PLS models for Chl-a biomass and biovolume of phytoplankton taxa in the separate bays of Napoleon Gulf (NG1-4) and Murchison Bay (MB1-4). Note: Only significant (p value < 0.05), *Microcystis* spp. = *M. aeruginosa* + *M. flos-aquae*.

	Napoleon Gulf (NG)		Murchison Bay (MB)			
	Log Chl-a	<i>Dolichospermum circinale</i>	Log Chl-a	<i>Dolichospermum circinale</i>	<i>Plankolyngbya circumcreta</i>	<i>Microcystis</i> spp.
Intercept	0.14		0.24			0.18
Station			0.30			
Water Temp			0.30	0.21	0.17	0.24
Wind_D5	0.22	0.18	0.21		0.20	0.18
Solar radiation_10	0.21	0.19		0.29		0.19
	0.19	0.18				
	0.44	0.30	0.54	0.36	0.37	0.38

from the two contrasted embayments (ESM Figure S7; *ve* vs predicted biovolumes).

Climate variables (mean wind and solar radiation) were major predictors for the biovolume of the three dominant species. Nutrients (SRP, NO_3^- , NH_4^+ and SRSi) seemed to play a secondary role in the relative abundance of these species (Table 4). *Microcystis* and *P. circumcincta* biovolumes were explained by combinations of variables, with climatic variables such as solar radiation and wind appearing as the most important controlling factors, before dissolved inorganic forms of nitrogen (NO_3^- and NH_4^+). *Microcystis* spp. model was slightly different, as SRP nutrient controlling the biovolumes of dominant taxa. Rainfall did not affect any of these variables and the water temperature had a negative influence on the biovolume of the three dominant taxa. In summary, wind and radiation played a major role as nutrient conditions were secondary dynamics of the dominant taxa Napoleon Gulf and Murchison

tion

Limnological characteristics of Napoleon Gulf and Murchison Bay

This study assessed the spatio-temporal dynamics in phytoplankton communities in two embayments along a transect from the inner bay to open lake. Here we identified the most important predictors of the phytoplankton community structure of the dominant taxa in Napoleon Gulf and Murchison Bay.

As expected, large differences in limnological characteristics were observed between the two bays, especially regarding the concentration of inorganic nutrients which were much higher in Murchison Bay than in Napoleon Gulf. These higher concentrations in Murchison Bay originated from the newly expanded Nakivubo wetland which collects part of the runoff and wastewater from Jinja (Luyiga et al., 2015; Akurut et al., 2017). The sampling station located near the channel showed the highest concentrations of nutrients, which decreased along the sampling transect. No such source of wastewater was in Napoleon Gulf sampling area, as the Jinja city wastewater is discharged through wetlands at the edge of the gulf and near the source of the Nile. Consequently, we did not observe a concentration gradient from the inner to the outer part of the gulf in Napoleon Gulf. There are also various small-scale rivers located near the shores of the two bays that discharge lower highly contaminated flows in surface water of the bays (Ogutu et al., 2018).

According to Mbonde et al. (2015), Murchison Bay can be considered as a closed bay with only a low level of water exchange with open lake relatively to its size, while Napoleon Gulf can be considered as an open bay with higher water renewal. As a consequence, the nutrient load in Napoleon Gulf from the watershed can be efficiently diluted by open lake water than that discharged in Murchison Bay, especially as Napoleon Gulf is located at the edge of Lake Victoria (Poste et al., 2013).

Previous studies such as Kabenge et al. (2016) have reported large increases in nutrient concentrations in the inner part of Murchison Bay during recent decades, rising as high 580 $\mu\text{g/L}$ in 2011 (Kabenge et al., 2016) and 380 $\mu\text{g/L}$ in 2017–2018 (this study). However, PO_4 is readily available, its concentration depends on the Chl-a biomass. TP is a better proxy of anthropogenic P input from agriculture. The maximum TP in Murchison Bay increased from 50 $\mu\text{g/L}$ in the early 1990s (Källqvist et al., 1996) to 105–122 $\mu\text{g/L}$ in mid-2004 (Okello et al., 2010), 550 $\mu\text{g/L}$ by 2003 (Luyiga et al., 2015), and 550–1500 $\mu\text{g/L}$ ($31 \pm 27.8 \mu\text{g/L}$) by 2010 (Haande et al., 2011). According to Kabenge et al. (2016), this increase in TP results from population

growth in the Kampala urban and peri-urban areas, from 755,000 inhabitants in 1990 to 2,117,000 in 2011 and to 2,843,000 in 2017 (1,680,600 in the city) (Uganda Bureau of Statistics, 2017a).

On the contrary, PO_4 concentration did not increase as dramatically in Napoleon Gulf. Case in point, PO_4 was 0.08 mM (7.59 mg/L) (Lehman and Branstrator 1994), 54.8–68 mg/L in between 2000 and 2002 (Gikuma Njiru et al., 2005), 3–7 mg/L (Okello et al., 2010), Poste et al. (2013) and 18.2 mg/L (this study). We have also observed a mild increase in the TP within Napoleon Gulf over the years from $49.3 \pm 5.5 \mu\text{g/L}$ (Okello and Kurmayer, 2010), to $60 \pm 16.2 \mu\text{g/L}$ (Poste et al., 2013) and $190 \pm 2 \mu\text{g/L}$ (Egessa et al., 2018); but not in this study (39.8 ± 33.2 , max TP = 164 mg/L). Similarly, the number of inhabitants in the Jinja district increased at a lower rate than in Kampala: 289,476 in 1991, 387,573 in 2002, and 471,242 in 2014 (288,500 in Jinja city) (Uganda Bureau of Statistics, 2017b).

Maximum phytoplankton biomass (expressed as Chl-a) recorded in the two embayments also showed large differences as expected and was higher in Murchison Bay ($137.4 \pm 181.2 \mu\text{g Chl-a/L}$) than in Napoleon Gulf ($25.4 \pm 8.3 \mu\text{g Chl-a/L}$). Both spatial and temporal variations of Chl-a concentration were lower in Napoleon Gulf than in Murchison Bay where, in its inner part, Chl-a values ranged between 80 and 1000 $\mu\text{g/L}$. These shallow stations were subject to regular mixing that disrupts physical stratification and enhances nutrient availability but were also susceptible to the loading effect from Nakivubo channel during the rainy season.

Data from Uganda National Meteorological Authority (UNMA) indicated the increase in Chl-a in May was associated with the rainfall events of April (80 mm) and synchronised rainfall events of September (41.7 mm). Furthermore, the period between May and September was characterized by relatively high wind speed $4.9 - 8.1 \text{ m/s}$ despite the solar radiation being within the same range throughout the study period. In a spatial context, previous studies showed that Chl-a within Murchison Bay has increased since the 1990s ($<20 \mu\text{g/L}$, Källqvist et al., 1996), ranging from 20 to 60 $\mu\text{g/L}$ in the early 2000s (Haande et al., 2011) and currently between 28 and 1087 $\mu\text{g/L}$ with a mean $\pm \text{SD}$ value of $137 \pm 181.2 \mu\text{g/L}$ (this study).

In Napoleon Gulf, the range of Chl-a values observed in this study (11.8–50.4, mean $\pm \text{SD} = 25.4 \pm 8.3 \mu\text{g/L}$) corresponds to the ones reported in the 2000–2010 period (10–55 $\mu\text{g/L}$) (Silsbeet et al., 2006; North, 2008; Okello et al., 2010; Okello and Kurmayer, 2011). Therefore, using OECD (1982) classification, our TP and Chl-a data led to the classification of Murchison Bay as a hypereutrophic site while Napoleon Gulf is considered meso-trophic/eutrophic, as reported by Poste et al. (2013). The significantly greater increase in nutrient levels in Murchison Bay compared to Napoleon Gulf also suggests that the general pollution of Lake Victoria, which is directly associated with the population growth and activities of the major cities, seems to be increasingly stable.

Phytoplankton diversity and potential toxicity

Despite the large differences in limnological characteristics, no significant differences were observed between Napoleon Gulf and Murchison Bay in terms of phytoplankton richness and diversity. These richness values appeared as slightly higher than the one previously reported, with 80 taxa identified in Murchison Bay between 2003 and 2004 by Haande et al. (2011). However, the sampling effort was lower in these previous studies than the one realized in the present study.

Consequently, we were unable to conclusively account for the increase in the number of phytoplanktonic taxa in these two bays, even in the hypereutrophic Murchison Bay. This implies that the

bays contain enough environmental niches to maintain many species. Regarding the composition of the phytoplankton community, we found that around 53% of the taxa were shared by the two bays. This suggests that despite the large difference in the shape of these embayments to the lake, there is strong connectivity between the different bays and gulfs of Lake Victoria, allowing for colonization by similar planktonic communities (Borics et al., 2020). According to a study that modelled the currents in the lake (Mweya et al., 2016), the water flow alternates between gyres that promote water mixing in the open lake with a general northward flow. These patterns are expected to push the open lake waters in the direction of the northern shores towards our study sites, increasing the flow of planktonic organisms through their connectivity with the parts of the lakes.

In Murchison Bay and Napoleon Gulf, the dominant taxa are cyanobacteria, and this group represents one-third of all the taxa identified in this study. This dominance of cyanobacteria was already reported by many researchers about the embayments of Lake Victoria (Okotum et al., 2021). Among these taxa, *Microcystis*, *Dolichospermum*, and *Planktolyngbya* were potentially able to produce toxins in Murchison Bay and Napoleon Gulf as earlier reported (Okello et al., 2010; Maunde et al., 2011; Okello and Kurmayer, 2011) and were found in high abundance in this study. When potentially toxigenic cyanobacterial cells are present at > 100,000 cells/mL in drinking water, the World Health Organization (WHO) considers this the upper threshold beyond which serious health problems are likely (Poste et al., 2019). The WHO also warns that recreational activities in water with this level of potentially toxigenic cyanobacteria can cause acute and long-term health problems (WHO, 2020). We applied the WHO guidelines to our dataset and >20% of the samples (22/96) exceeded the WHO threshold especially in the wet season especially in Murchison Bay (15/96 with 15 samples during the wet season), Napoleon Gulf (4/96 with 3 samples in the wet season), and *Microcystis* (13/96 with 13 samples in the wet season). All of these samples were from Murchison Bay where fishers and locals living along the shores of the embayments directly drink the lake water. The WHO guideline for cyanobacteria is 1 µg/L of MC-LR, equivalent to ~ 5,000 cells/mL of cyanobacteria producing toxins (Chorus and Welker 2021). This is the case for fishers and the local population drinking relatively small amounts of water contaminated by toxigenic cyanobacteria could be at risk (Roegner et al., 2020). Thus, there is need for an in-depth study to assess the risks associated with cyanotoxins in these two bays and to identify exposure routes. In consequence of their substantial biomass and potential to produce toxins, the dominant cyanobacteria could pose severe environmental and sanitary problems in the two bays. To forecast and limit the development of these proliferations, it is necessary to identify the factors that trigger them (Roussio et al., 2020).

Partial least squares modelling of dominant cyanobacteria taxa

Previous studies addressed the potential controlling factors of the temporal dynamics of phytoplankton communities in Lake Victoria (Poste et al., 2015; Mchumi et al., 2019). However, to the best of our knowledge, none of them simultaneously modelled the physical and climatic variables to assess their linkages with the dynamics of dominant taxa. Here, we applied the partial least squares modelling approach to the biomass of each dominant taxon (*Microcystis* spp., *D. circinale*, and *P. circumcreta*) expressed in mg/L. We benefited from the large range of environmental and plankton variability evident in the two bays, which increased the accuracy of our statistical models. PLS modelling clearly explained a large proportion of the variance (R^2) of the bio-

volume of the dominant cyanobacteria taxa: *Microcystis* spp. at 64.6%, < *P. circumcreta* at 73.4%, < *D. circinale* at 77.6%.

Nutrient concentration (SRP, NO_3^- , NH_4^+ and SRSi) and climatic variables (mean wind and solar radiation) had a significant positive influence on the biomass of the three dominant taxa, which validates the combining these two types of drivers. Among nutrients, NH_4^+ had the strongest influence on all dominant taxa, while SRP levels significantly affected *Microcystis* spp. biomass only. Thus, our results confirm that P and N concentrations are important drivers of cyanobacteria biomass and dynamics, as reported in an extensive review of models forecasting cyanobacterial blooms in freshwater lakes (Roussio et al., 2020). However, consistently detectable dissolved nutrients at all sampling stations, suggested that neither N nor P was a limiting factor in primary production of phytoplankton in the two bays, as reported by Poste et al. (2013). In Napoleon Gulf where nutrient concentration was the lowest, the two dominant taxa, *Dolichospermum* and *Planktolyngbya*, have developed physiological adaptations to cope with nitrogen limitation: heterocyst differentiation for *Dolichospermum* and high surface/volume ratio for *Planktolyngbya*.

Mean solar radiation positively influenced biovolume of the three dominant taxa, supporting the hypothesis that under conditions of very high biomass, light can become a limiting factor (Poste et al., 2013). An increase in solar radiation may temporarily suppress this limitation and allow a biomass increase of the taxa located in the upper part of the water column. If this is the case, those cyanobacteria taxa able to regulate their buoyancy such as *Microcystis* and *Dolichospermum* (Reynolds et al., 1987) would be favoured where they were dominant, *Microcystis* in Murchison Bay and *Dolichospermum* in Napoleon Gulf. Colony-forming genera such as *Microcystis* and filamentous genera such as *Dolichospermum* can use their intracellular aerotopes, to regulate their buoyancy and access light or nutrients resources in the upper water column (Bormans et al., 1999; Brookes et al., 1999). *Planktolyngbya* that lack gas vacuoles, forms thin filaments to compensate for sinking away from the nutrient-rich water column (Komárek 2003). Under laboratory conditions, isolated strains of *Planktolyngbya circumcreta* from Lake Victoria have shown chromatic adaption under red or green light (data not shown). It has also been reported that *Planktolyngbya* (Pseudanabaenaceae) is a shade-adapted genus (Reynolds 2002). We hypothesized that the coexistence *P. circumcreta* with *Dolichospermum* in Napoleon Gulf can be attributed to this cellular adaptation of the ability to synthesize phycoerythrin under certain light conditions (Kehoe 2010).

Among climatic variables, wind speed was significantly and positively related to phytoplankton biomass. There are several ways that wind could directly or indirectly influence the phytoplankton community. We suggest that wind increases water turbulence, and this might move phytoplankton from the bottom or intermediate depths to the surface of the water column. Such vertical movements would increase phytoplankton exposure to different light regimens that may provide the best compromise between light deficiency, light stress and the response to the dynamics of mixing (Bormans et al., 1999). One intriguing result was the observation of a negative relationship with low variance between temperature and dominant taxabiomass. This is in direct contradiction to the literature which is full of examples of positive relationships between phytoplankton biomass and temperature, this latter being the most recurrent predictor in cyanobacteria models (Roussio et al., 2020). This unexpected negative relationship may arise from a non-causal covariation and also the very low variance in temperature between the embayments. In Murchison Bay, where the phytoplankton biomass is greater, self-shading due to high cell concentration may limit light penetration, including infrared wavelengths. This results in 'colder' water than in Napoleon Gulf where phytoplankton biomass is lower.

the modelling approach used here provided interesting insights into the drivers of dominant taxa biomass in Lake Victoria, one also consider predictive modelling using easily accessible data. For instance, Silsbee et al. (2006) showed that in Lake Victoria, Secchi depth measurements were good predictors of Chl-a concentration. If one could correlate the Secchi depth to dominant taxa biomass, it would offer a very simple tool to get high-frequency monitoring of cyanobacterial blooms. Such an inexpensive and easy to implement approach might be implemented by citizen science networks like the one reported by Mitron et al. (2020) in a freshwater lagoon in the French coast and would complement the monitoring of water agencies by the scientific community.

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Napoleon Gulf and Murchison Bay exhibited different levels of cyanobacterial biomass that influenced planktonic communities. The phytoplankton community comprises similar species, but the dynamics and assemblages in the phytoplankton assemblages of northern Lake Victoria are driven by the dominant cyanobacteria, *Microcystis aeruginosa* and *M. aeruginosa* in Murchison Bay and *Dolichospermum* and *Planktolyngbya circumcreta* in Napoleon Gulf. Consequently, the water quality differences and phytoplankton community composition of the two bays makes Murchison Bay more susceptible to harmful cyanobacterial blooms with potential cyanotoxin production. The influence of climatic variables such as mean wind speed and solar radiation in combination with nutrients under increasing anthropogenic pressures might increase incidences of potential toxin production. This scenario requires future investigation into the mechanisms of cyanotoxin production to assess specific health risks in the waterbodies. In addition, it would be interesting to explore alternative approaches for nutrient limitation of phytoplankton community dynamics and functioning in the two bays.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the crews at Gaba KK beach and Rippon pier for the assistance during sampling and from the National Fisheries Resources Research Institute (NAFIRRI) analytical lab during sample preparation and analysis. We thank Uganda National Meteorological Authority (NMA) for availing the climate data used during subsequent modelling and analyses. Research funding is supported by the French Ministry for Global Environment (Fonds Français pour l'Environnement Mondial, FFEM) research program Water Sources in Africa (WaSA) for M. lokotum PhD from Makerere University (2017–2020).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jglr.2021.04.021>.

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CHAPTER FOUR

POTENTIAL THREATS OF TOXIGENIC CYANOBACTERIA AND CYANOTOXINS IN LAKE VICTORIA³

CONTEXT

The previous chapters showed high proportions and dominance of distinct cyanobacteria. Specifically, dynamics and dissimilarities of cyanobacteria community was driven by *Microcystis flos-aquae* and *M. aeruginosa* in MB and *Dolichospermum circinale* and *Planktolyngbya circumcreta* in NG. This makes closed bays such as MB more susceptible to toxigenic cyanobacteria with potential cyanotoxin production. This resulted in the detection of only two classes of cyanotoxins: MC and homoanatoxin (HTX) an analogue of ATX. Considering MC, significantly higher concentration was detected in MB thus exposes fishers and local communities to cyanotoxins. This exceeds both the $1 \mu\text{g.L}^{-1}$ threshold for drinking water, and lowest sanitary threshold for recreational activities. Thus, these become a challenge for the beach owners and for water treatment plans (WTPs) using surface water from Lake Victoria. In general, this study provides the first assessment of intracellular and dissolved MC in treated water. From this study, the high cell densities of toxigenic *Microcystis* identified and high MC concentrations (intracellular and dissolved MC) measured were efficiently removed by WTPs. Nonetheless, the increase of MC-producing *Microcystis* suggest a higher probability of exceeding the WHO lifetime guideline values, hence requires regular monitoring by the concerned authorities. The concentration of soluble (SRP, NO_3^- , NH_4^+ , SRSi) and climatic variables (mean wind and solar radiation) influenced the spatial-temporal dynamics of toxigenic cyanobacteria. A further analysis on the observed MC concentrations were attributed to the biovolume of *Microcystis*, with the influence of solar radiation, mean wind speed responsible for water turbulence. This implies that solar radiation may temporarily suppress this limitation and allow a biomass increase of the taxa located in the upper part of the water column of the most buoyant taxa such as *Microcystis* and *Dolichospermum*. The details of the chapter on the potential threats of toxigenic cyanobacteria, micro conditions influencing the MC concentration and the efficiency of the WTPs in removal of toxigenic cyanobacteria, intracellular and dissolved toxins from treated water are presented below.

³ Chapter based on this publication:

Olokokari, M., Humbert, J.-F., Quiblier, C., Okello, W., Semyalo, R., Troussellier, M., Marie, B., Baumann, K., Kurmayer, R., and Bernard, C. (2022). Characterization of Potential Threats from Cyanobacterial Toxins in Lake Victoria Embayments and during Water Treatment. *Toxins*, 14(10), 664. <https://www.mdpi.com/2072-6651/14/10/664>

Characterization of Potential Threats from Cyanobacterial Toxins in Lake Victoria Embayments and during Water Treatment

Okotum ^{1,2,3}, Jean-François Humbert ⁴, Catherine Quiblier ^{5,6}, William Okello ², Ronald Semyalo ¹, Marc Troussellier ⁷, Benjamin Marie ⁵, Kathrin Rainer Kurmayer ^{3,*} and Cécile Bernard ^{5,*}

¹ Department of Zoology, Entomology and Fisheries Sciences, Makerere University, Kampala 7062, Uganda

² National Fisheries Resources Research Institute (NaFIRRI), Jinja 343, Uganda

³ Research Department for Limnology, University of Innsbruck, 5310 Mondsee, Austria

⁴ Institute of Ecology and Environmental Sciences of Paris, INRAE-Sorbonne University, 75005 Paris, France

⁵ UMR Molecules of Communication and Adaptations of Microorganisms (MCAM) CNRS-MNHN, Muséum National d'Histoire Naturelle, 75005 Paris, France

⁶ Department of Ecology, Campus Grands Moulins, Université de Paris, 75013 Paris, France

⁷ UMR Marine Biodiversity Exploitation and Conservation (MARBEC), CNRS-Université de Montpellier-IFREMER-IRD, CEDEX 5, 34095 Montpellier, France

* Correspondence: rainer.kurmayer@uibk.ac.at (R.K.); cecile.bernard@mnhn.fr (C.B.); Tel.: +43-512-5075-0242 (R.K.); +33-140793183 (C.B.)

† Current address: Department of Surface Waters-Research and Management, Swiss Federal Institute for Aquatic Science and Technology (EAWAG), 6047 Kastanienbaum, Switzerland.

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Okotum, M.; Humbert, C.; Okello, W.; Semyalo, C.; Quiblier, M.; Marie, B.;

Kurmayer, R.; Bernard, C. Characterization of Potential Threats from Cyanobacterial Toxins in Lake Victoria Embayments and during Water Treatment. *Toxins* **2022**, *14*, 664. <https://doi.org/10.3390/toxins14100664>

22 July 2022

20 September 2022

23 September 2022

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Abstract: Africa's water needs are often supported by eutrophic water bodies 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, fourteen were considered potentially toxigenic (i.e., from the genera *Dolichospermum*, *Microcystis*, *Oscillatoria*, *Pseudanabaena* and *Raphidiopsis*). A higher concentration (ranging from 5 to 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 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 routes; *Microcystis*; *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], not least because of the health threats associated with cyanotoxins [5].

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 composition, cyanobacteria and cyanotoxins in the open lake and the embayments (for review, see Ref [8]). Most of the studies on cyanotoxins in Lake Victoria have detected microcystins (MC) produced by dominant cyanobacteria, 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 anatoxin-a (ATX), which was undetectable in both environmental samples and isolated strains. More recently, very low concentrations of cylindrospermopsin (CYN) and nodularin (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 $<1 \mu\text{g MC-LR equiv. L}^{-1}$ [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) $\mu\text{g MC-LR equiv. L}^{-1}$ 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 the Langmuir spirals [12]. In general, high spatial variability has been observed, and MC concentrations did not exceed 5 $\mu\text{g MC-LR equiv. L}^{-1}$ in most of the embayments, i.e., $<2 \mu\text{g MC-LR equiv. L}^{-1}$ in the Napoleon Gulf and Murchison Bay [8,13] and $<4 \mu\text{g MC-LR equiv. L}^{-1}$ in closed bays in Tanzania [8,14]. Therefore, these studies have shown that the Lake Victoria riparian populations (lake shore residents, fishermen, recreationists and all people connected to treated water) are potentially exposed to variable concentrations of toxigenic cyanobacteria and MC.

The embayments of Lake Victoria serve as the source of water and abstraction for most water treatment facilities [8]. Therefore, the location and technology of the water intake, as well as the efficiency of clarification through flocculation, 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]. Therefore, lakeside residents, fishermen and recreationists are potentially exposed to direct consumption of toxigenic cyanobacteria and intracellular toxins, while those connected to treated water could be exposed to dissolved cyanotoxins. 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 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, the 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

(November 2017–October 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 (November 2016–January 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 conducted 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. Afterward, the potential risks associated with the toxigenic cyanobacteria and their toxins for human populations using raw and treated water from these two embayments were discussed.

2. Results

To investigate the dynamics of cyanobacteria and cyanotoxins concentrations within the two embayments of MB and NG, the temporal–spatial variability was monitored 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) in the open lake (Figure 1).

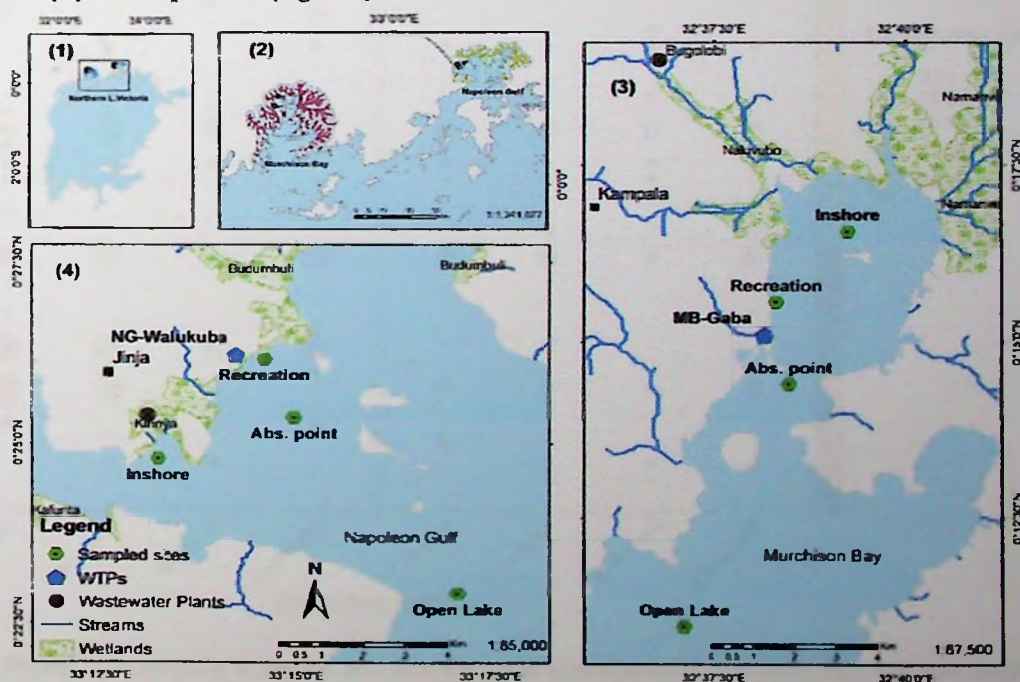


Figure 1. Location of the two study areas in Murchison Bay (MB) and Napoleon Gulf (NG) (1) and close up with tributaries and wetlands (2) and the location of sampling sites (Inshore, Recreation, WTP abstraction points (Abs. point) and open lake) in MB (3) and NG (4). The sampling depths in MB were as follows: Inshore = 1.4–2.5 m (1.84 ± 0.34), Recreation = 2–2.7 m (2.3 ± 0.3), WTP 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), WTP 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).

2.1. Toxigenic vs. Non-Toxigenic Cyanobacteria Diversity and Biovolume

In the embayments: From the lake survey, 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-toxicogenic (27 taxa from MB and 26 from NG; Figure 2). Comparing the two embayments, a higher cyanobacterial biovolume was observed in MB (0.9×10^{-4} to $45.0 \text{ mm}^3 \text{ L}^{-1}$; mean $\pm$ SD = $2 \pm 4.2 \text{ mm}^3 \text{ L}^{-1}$) than NG (0.4×10^{-4} to $22 \text{ mm}^3 \text{ L}^{-1}$; mean $\pm$ SD = $0.5 \pm 1.4 \text{ mm}^3 \text{ L}^{-1}$; Mann-Whitney test, $p < 0.0001$). In MB, higher cyanobacterial biovolumes were recorded from inshore samplings than at the WTP abstraction point and open lake stations (Friedman test, $X^2 = 4987.8$, $p < 0.001$ 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 measures ANOVA, $F = 0.618$, $p = 0.603$; Figure 2).

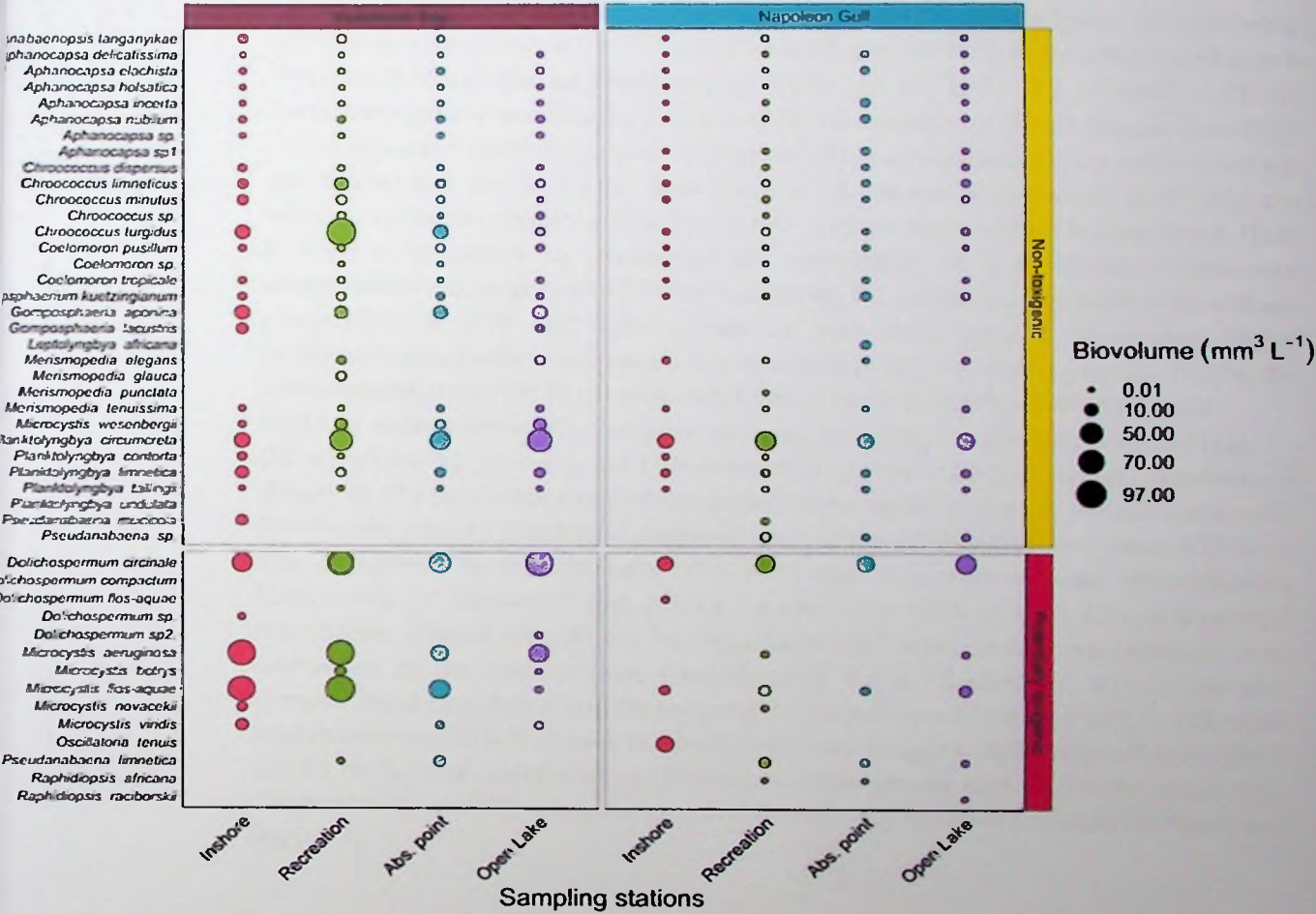


Figure 2. Potentially toxigenic and non-toxicogenic cyanobacteria recorded from the lake survey at sampling sites: (i) inshore, (ii) recreational area, (iii) WTP abstraction point (Abs. point), (iv) open lake, in Murchison Bay and Napoleon Gulf. Among the 46 cyanobacteria species, 14 were considered potentially toxigenic. Circle sizes are proportional to the total biovolume ($\text{mm}^3 \text{ L}^{-1}$) of each taxon (data collected between November 2017 and October 2018, $n = 120$).

In MB, the most dominant toxigenic cyanobacteria belonged to the genus *Microcystis* with *M. aeruginosa* ($0.5\text{--}31.4 \text{ mm}^3 \text{ L}^{-1}$, mean $\pm$ SD = $7.3 \pm 6.7 \text{ mm}^3 \text{ L}^{-1}$; 18.7% of the total cyanobacteria biovolume) and *M. flos-aquae* ($0.4\text{--}30 \text{ mm}^3 \text{ L}^{-1}$, mean $\pm$ SD = $6.6 \pm 6.7 \text{ mm}^3 \text{ L}^{-1}$; 17.9% of the total cyanobacteria biovolume). In MB, the highest biovolume of toxigenic cyanobacteria was recorded from the inshore and recreational stations ($0.6\text{--}28.2$ and $1.2\text{--}29.8 \text{ mm}^3 \text{ L}^{-1}$, respectively). The non-toxicogenic cyanobacteria made the most significant

contribution to the cyanobacterial biovolume (56.4%), ranging from 8.9×10^{-5} to $45 \text{ mm}^3 \text{ L}^{-1}$, mean $\pm$ SD = $1 \pm 3 \text{ mm}^3 \text{ L}^{-1}$, dominated by *Chroococcus turgidus* in the recreational area (Figure 2). The 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*, ranging from 0.1 to $8.6 \text{ mm}^3 \text{ L}^{-1}$, mean $\pm$ SD = $2.3 \pm 1.9 \text{ mm}^3 \text{ L}^{-1}$ (36% of the total cyanobacteria biovolume), followed by *M. flos-aquae* with 0.3– $2.9 \text{ mm}^3 \text{ L}^{-1}$, $0.7 \pm 0.6 \text{ mm}^3 \text{ L}^{-1}$ (6.1% of the total cyanobacteria biovolume). The toxigenic cyanobacterium *Oscillatoria tenuis* ($22 \text{ mm}^3 \text{ L}^{-1}$) was detected once with a high abundance 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; $0.3 \pm 0.7 \text{ mm}^3 \text{ L}^{-1}$ (47% of the total cyanobacteria biovolume), dominated by *Planktolyngbya circumcreta* (0.5 to 5.5; $2 \pm 1.3 \text{ mm}^3 \text{ L}^{-1}$; 72% of the non-toxigenic cyanobacteria biovolume) in the recreational station (Figures 2 and S1).

During water treatment: Since our survey on MC concentrations during water treatment (see below) was performed for three months (November 2016–January 2017) only, the seasonal variation in cyanobacteria biovolume in raw water could not be considered. Thus, it became important to investigate the seasonality of cyanobacteria community composition for a longer period. When comparing the toxigenic cyanobacteria biovolume composition at WTP abstraction points for both study periods, on average higher (toxigenic) cyanobacteria biovolume was recorded (*t*-test, $p = 0.04$; Figure S2). During the water treatment survey, 15 cyanobacterial taxa were found in the abstracted water (and raw water entering the treatment process), including 3 potentially toxigenic taxa (*M. aeruginosa*, *M. flos-aquae* and *Dolichospermum* sp.) and 12 non-toxigenic cyanobacteria (Figure 3). The taxonomic composition recorded during the two study periods was similar (see Figures 2 and 3). The depth-integrated water at the WTP abstraction point of MB-Gaba was dominated by cyanobacteria ($15.9 \pm 3.5 \text{ mm}^3 \text{ L}^{-1}$; >90% of total phytoplankton biovolume), i.e. *Microcystis* spp. ($12.3 \pm 2.6 \text{ mm}^3 \text{ L}^{-1}$), >70% of the total cyanobacterial biovolume), (Figure 3A). At the NG-Walukuba abstraction point, cyanobacteria were dominated by the non-toxigenic *Planktolyngbya* ($4.5 \pm 1.3 \text{ mm}^3 \text{ L}^{-1}$; 46% of the total cyanobacterial biovolume) and the toxigenic *Microcystis* spp. ($4.4 \pm 1.7 \text{ mm}^3 \text{ L}^{-1}$; 44% of the total cyanobacterial biovolume), followed by the non-toxigenic *Aphanocapsa* ($0.8 \pm 0.2 \text{ mm}^3 \text{ L}^{-1}$; 8% of the total cyanobacterial biovolume). Other genera, such as *Dolichospermum* sp., *Chroococcus* sp. and *Merismopedia* sp., contributed < 1% of the cyanobacterial biovolume (Figure 3).

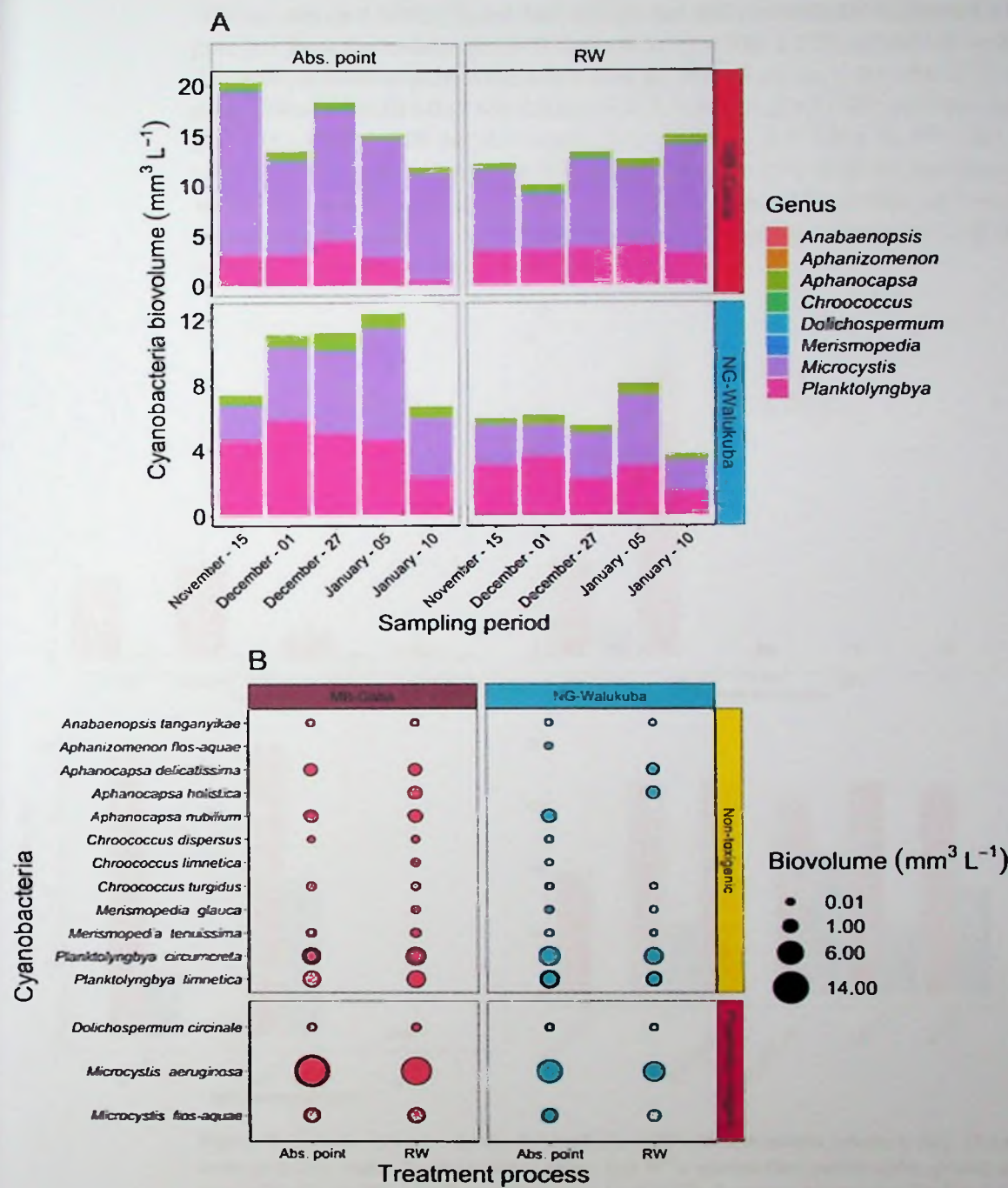


Figure 3. Cyanobacterial biovolume and composition at the WTP Abs. point and in raw water (RW) from MB-Gaba and NG-Walukuba water treatment plants. (A) Temporal dynamics of cyanobacteria genera; (B) Proportion of potentially toxicogenic and non-toxicogenic cyanobacteria (data collected between November 2016 and January 2017, $n = 90$).

2.2. Cyanotoxins Occurrence in the Lake Victoria Embayments and during Water Treatment

In the embayments: During the field survey, there was no detection of ATX, CYN or STX in all environmental samples ($n = 120$), but there was a detection of homoanatoxin-a (HTX) at the inshore station in MB (at low HTX concentrations, $<0.04 \text{ HTX L}^{-1}$) in 2 samples, while 99 samples (82.5%, $n = 120$) were found to contain MC. The most frequent MC congeners in MB and NG included MC-RR and MC-LR (Figure 4). The total concentrations of MC were significantly higher among samples obtained from MB than those from NG (Mann-

Whitney test, $p < 0.001$; Figure 4A). In MB, the MC concentrations showed a decreasing gradient from the inshore (0.15–11.7; mean $\pm$ SD = $2.01 \pm 2.37 \mu\text{g MC-LR equiv. L}^{-1}$) and recreational stations (0.18–14.8; $2.34 \pm 4.03 \mu\text{g MC-LR equiv. L}^{-1}$) to the WTP abstraction point (0.04–1.41; $0.62 \pm 0.47 \text{ MC-LR equiv. L}^{-1}$; ANOVA, $p = 0.0128$) and open lake stations (0.02–0.91; $0.22 \pm 0.25 \text{ MC-LR equiv. L}^{-1}$, ANOVA, $p < 0.05$). In NG, the mean MC concentrations were rather low ($<0.2 \mu\text{g MC-LR equiv. L}^{-1}$), with no significant difference across the sampling stations (ANOVA, $p = 0.904$; Figure 4A). In MB, MCs were detected throughout the year, but in NG, MCs mostly occurred during the first half of the study period, i.e., November 2017–April 2018 (Figure S3).

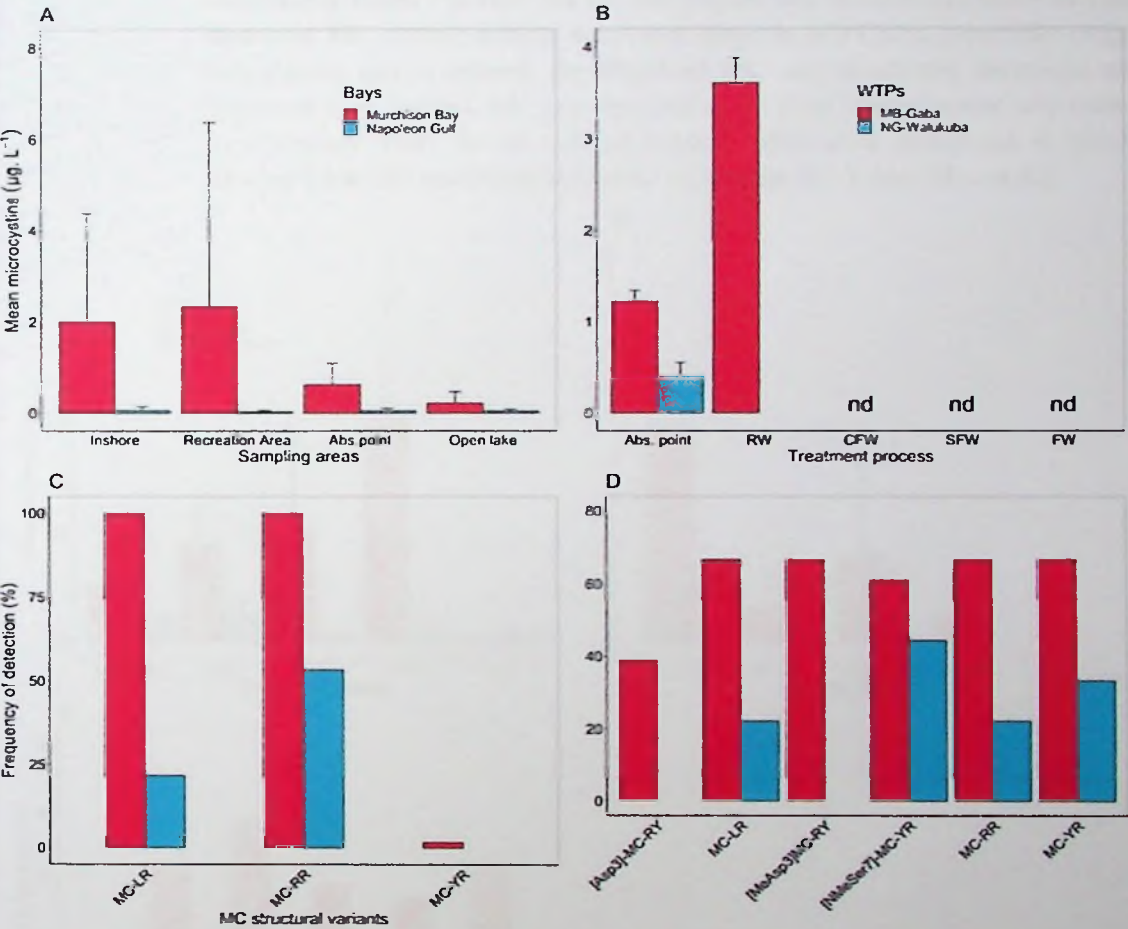


Figure 4. Spatial variation in the intracellular MC concentrations (mean $\pm$ SD), (A) at (i) inshore stations in the bays, (ii) recreational areas, (iii) WTP abstraction points (Abs. point) and (iv) open lake in Murchison Bay (MB) and Napoleon Gulf (NG), (between November 2017 and October 2018, $n = 120$), (B) during the water treatment from lake water to final water in MB-Gaba and NG-Walukuba (between November 2016 and January 2017, $n = 90$). Note the different scales in the y-axis. (C) Relative frequency of the structural variants of MC detected during the lake survey and (D) from the WTP abstraction points and raw water entering the WTPs ($n = 18$), from MB-Gaba and NG-Walukuba. Abbreviations: Abs. point = WTP abstraction point, RW = Raw water, CFW = Coagulated and flocculated water, SFW = Sand filtered water, FW = Final water, nd = not detected.

During water treatment: During the water treatment survey, there was no analysis for ATX (HTX), CYN and STX. In total, 21 samples (23.3%, $n = 90$) were found positive for MC. For the MB-Gaba abstraction point and raw water, six MC congeners, including MC-RR, MC-YR, MC-LR, [Asp³]-MC-RY, [MeAsp³]-MC-RY and [NMeSer⁷]-MC-YR, were regularly detected. In NG-Walukuba, four congeners were detected at the abstraction

point, including MC-LR, MC-RR, MC-YR and [NMeSer⁷]-MC-YR (Figure 4D). For the WTP survey, both intracellular and dissolved MCs were differentiated. In general, intracellular MC concentrations at the two WTP abstraction points were found comparable to MC concentrations observed inshore (Figure 4B), i.e., the mean concentrations of intracellular MCs were $1.22 \pm 0.36 \mu\text{g MC-LR equiv. L}^{-1}$ at MB-Gaba and $0.36 \pm 0.46 \mu\text{g MC-LR equiv. L}^{-1}$ at NG-Walukuba. For MB-Gaba only, the MC concentration increased to $3.61 \pm 0.48 \mu\text{g MC-LR equiv. L}^{-1}$ in raw water (Figure 4B). Accordingly, the concentrations of dissolved MCs, as determined via ELISA, were significantly higher in MB-Gaba than in NG-Walukuba (Figure 5A). However, for NG-Walukuba, the dissolved MCs were consistently below the LOD for LC-MS (Figure 5B). In addition, there was an increase in dissolved MC during certain treatment steps in MB-Gaba, especially coagulation and flocculation, but in general, the dissolved MC was drastically decreased until the final treatment step. Indeed, MC was detected in the final treated water only once ($0.14 \mu\text{g L}^{-1}$ on 4 January 2017). In the diluted fraction, MCs were composed of MC-LR, MC-YR, [MeAsp³]-MC-RY and [NMeSer⁷]-MC-YR (Figure 5C, Tables S1 and S2).

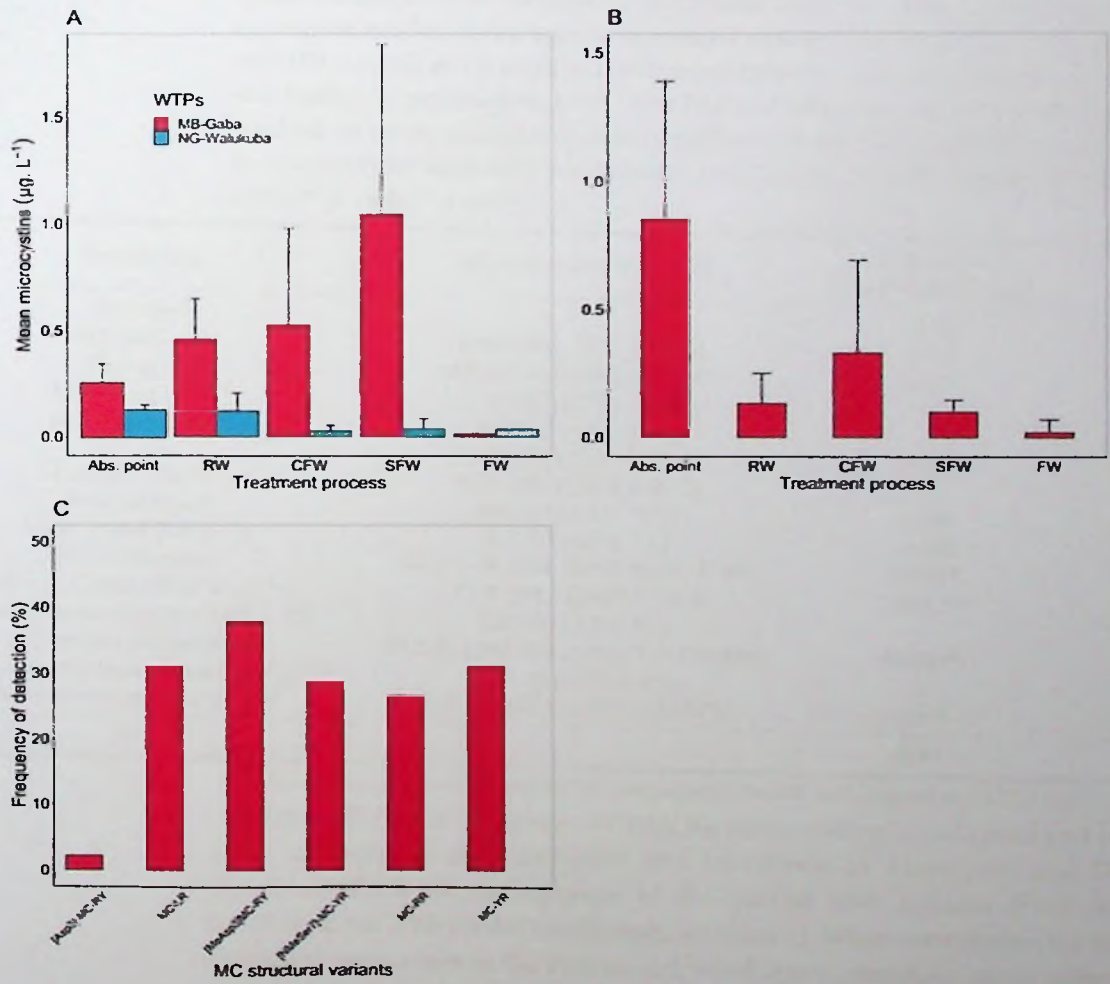


Figure 5. Concentration of dissolved MC (mean $\pm$ SD) as detected by ELISA (A) and LC-MS (B) from WTP abstraction points in MB and NG, and during the treatment process in MB-Gaba and NG-Walukuba. (C) Relative frequency of the structural variants of MC detected in the dissolved water from MB-Gaba ($n = 45$). Note: there was no detection of dissolved MC in NG-Walukuba. Abbreviations: Abs. point = WTP abstraction point, 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 to 197 NTU, and the dissolved N:P ratio ranged from 0.16 to 149.1 (mean 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, during the sampling days varied from no rain (dry season) 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 field survey period (November 2017–October 2018), 30.4% of the sampling days had an average wind speed of 4.3 km h⁻¹ in the N-S direction, followed by 26.1% of sampling days with a wind speed of 3.5 km h⁻¹ in the E-W direction and 21.7% days with wind of 5.4 km h⁻¹ in the S-SE direction. The average wind speed ranged from 3.1 to 8.1 with a mean of 4.5 ± 1.1 km h⁻¹, while the mean solar radiation (for the 10 days prior to each sampling date, D10) was 291 ± 81 Wm² day⁻¹.

Table 1. Standardized parameter coefficients from the global partial least square (PLS) regression models using log₁₀ transformed variables to predict MC concentration in NG and MB, as well as correlation coefficients between MC concentration and environmental and biological parameters. Data from NG and MB were pooled together, and the Pearson product-moment partial correlation coefficient was used. Significant variables are marked in bold, statistically significant coefficients are indicated by asterisks: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Parameters	Min–Max (Mean $\pm$ SD)	Regression Coefficient (Log ₁₀)	Correlation Coefficient
Intercept		–2.044	
SRP ($\mu\text{g L}^{-1}$)	0.56–380.3 (13.4 ± 38.8)	0.055	0.31
NH ₄ ($\mu\text{g L}^{-1}$)	0.01–1206.8 (49.3 ± 150.9)	0.041	0.32
NO ₃ [–] ($\mu\text{g L}^{-1}$) #	1.11–947.4 (73.5 ± 127)	–	0.02
NO ₂ [–] ($\mu\text{g L}^{-1}$) #	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 h ^{–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
<i>Microcystis</i> biovolume (mm ³ L ^{–1})	0.27–69 (7.3 ± 9.4)	–	0.62
<i>Microcystis</i> cell number	39,000–1,061,206 ($106,277 \pm 148,826$)	0.031 **	0.57
<i>Dolichospermum</i> biovolume (mm ³ L ^{–1})	0.07–20.6 (3.9 ± 4.1)	–	0.22
<i>Dolichospermum</i> cell number	645–182,627 ($33,822 \pm 34,975$)	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.

Taking all the data together ($n = 120$), the parameters of 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 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% of the variance in MC concentrations (Table 1). Furthermore, there was a significant correlation between *Microcystis* in MB and MC concentration (abundance data: $r = 0.57$, $p < 0.001$; biovolume data: $r = 0.62$, $p < 0.001$), but no significant correlation between *Dolichospermum* (abundance or biovolume) and MC concentration.

The decision support tree (DST), which is a guiding tool for management actions during suspected harmful blooms, was applied to provide an alert level framework for the health

risk through MC concentrations as well as the need to monitor MC concentrations. As a precautionary tool, in the DST, the 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 6). When *Microcystis* abundance was $>200,000$ cells mL⁻¹ (or equivalent of 13.7 mm³ L⁻¹), there was a 100% probability of observing MC. When *Microcystis* abundance was between 58,000 (the equivalent of 3.9 mm³ L⁻¹) and 200,000 cells mL⁻¹, the MC concentrations were significantly related to the mean solar radiation (>248 Wm² day⁻¹) and mean wind speed (<4.8 km h⁻¹) in the N-S direction. Additionally, when *Microcystis* abundance was $<58,000$ cells mL⁻¹, the mean solar radiation intensity for the 10 days prior to sampling (D10), turbidity (>17.4 NTU) and abundance of *Dolichospermum* were correlated with low concentrations of MC (Figure 6).

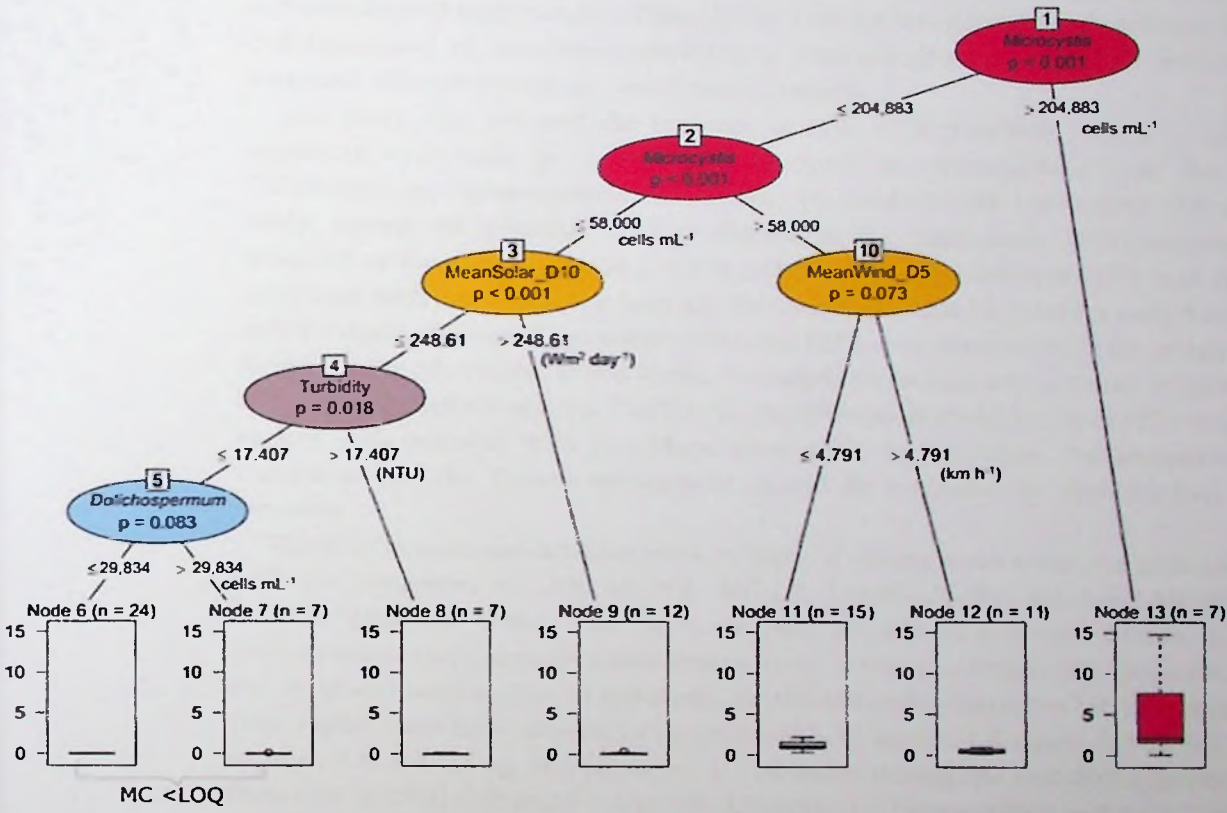


Figure 6. Decision support tree for prediction of MC concentrations in lake water from Murchison Bay and Napoleon Gulf, northern Lake Victoria. Note: LOQ for MC = 0.04 $\mu\text{g L}^{-1}$; MeanWind_D5 = the mean wind speed for the five days (km h⁻¹) (N-S direction) and MeanSolar_10 = mean solar irradiance for the ten days prior to sampling (W m² day⁻¹). (Data collected between November 2017 and October 2018, $n = 120$). Colors: Red = 0.08–14.8 $\mu\text{g MC-LR equiv. L}^{-1}$, Yellow = LOQ–4.5 $\mu\text{g MC-LR equiv. L}^{-1}$, Brown and Blue = LOQ–0.91 $\mu\text{g MC-LR equiv. L}^{-1}$.

3. Discussion

3.1. Toxigenic Cyanobacteria and Cyanotoxins in the Lake Victoria Embayments

The predominant toxigenic cyanobacteria in the two embayments of northern Lake Victoria during the study period 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* biovolume recorded between April 2003 and March 2004 formed 0.3 ± 0.1 mm³ L⁻¹, representing 20.1% of the total cyanobacteria biovolume (1.6 ± 0.7 mm³ L⁻¹) in

inner MB [20]. In another study between May and June 2004, the *Microcystis* biovolume ranged from 3.1 to

23.2 mm³ L⁻¹ [21], and in 2017–2018 (this study), it varied between 0.5 and 69 mm³ L⁻¹ (10.5 ± 10.6 mm³ L⁻¹). Considering these data, they suggest that there has been an increase in *Microcystis* biomass (six-fold) in MB over the last two decades.

Analogously, the range of *Dolichospermum* biovolume in the mid-2000s ranged from 8.6 to 15 mm³ L⁻¹ [21], but during this study, it varied between 0.4 and 20.6 mm³ L⁻¹. In NG, the *Microcystis* biovolume varied between 3.1 and 23.2 mm³ L⁻¹ in the mid-2000s and 0.18 and 31.4 mm³ L⁻¹ (this study), but the *Dolichospermum* biovolume declined from 9.8–83 mm³ L⁻¹ to 0.1–8.63 mm³ L⁻¹. This suggests that either during the mid-2000s, a *Dolichospermum* bloom event occurred in NG, or the dominance of *Dolichospermum* shifted to *Planktolyngbya* more recently. This shift between the two genera, i.e., heterocyte-forming *Dolichospermum* vs. non-heterocyte-forming *Planktolyngbya*, could reflect the 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 known to be produced by cyanobacteria from the genera *Dolichospermum*, *Aphanizomenon*, *Oscillatoria*, *Phormidium* and *Planktothrix* [22]. In this study, among the potential HTX producers, at the same time, *Dolichospermum* was observed in the depth integrated water sample; thus, the detected HTX was possibly associated with this genus. For instance, the *Dolichospermum* biovolumes were 8.6 and 5.1 mm³ L⁻¹ during June and September 2018 when HTX were detected from the inshore areas in MB. On the other hand, in this study, *Oscillatoria tenuis* was detected once in November 2017 but with high abundance. *Oscillatoria* can either produce ATX only or HTX as a major variant with traces of ATX (see Mann et al. [23]). In the future, the suggested HTX occurrence in Lake Victoria embayment should be evaluated by cyanobacteria strain isolation.

The main cyanotoxins detected in lake water and during water treatment included MCs with the congeners MC-RR, MC-YR, MC-LR, [Asp³]-MC-RY, [MeAsp³]-MC-RY and [NMeSer⁷]-MC-YR, which have been recorded already in 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) [24]; 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 mm³ 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 [13,21,25]. 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 [26].

The concentrations of the MC detected in Lake Victoria have varied over the past two decades. In general, the concentrations of MC in the water column (depth-integrated samples) have been found relatively low (≤2 µg L⁻¹, e.g., in Ref [8]). However, during bloom events and in surface sums, high concentrations of 81 µg L⁻¹ [27] and >2 mg L⁻¹ [12] have been reported in Nyanza Gulf (Kenya). This implies that higher concentrations of MC in the lake can occur, as well as the consequences for humans and fish. In Africa, MCs have been detected in 21 of the 55 countries, where in the south, the concentrations have ranged between <1 and <20 µg L⁻¹ in Zimbabwe (and up to 43 µg L⁻¹ in Lake Krugersdrift in South Africa [28]). Similarly, in the west and north African countries, MC concentrations

ranged from $3.2 \mu\text{g L}^{-1}$ (Ghana), $5.8 \mu\text{g L}^{-1}$ (Nigeria) and up to $28.9 \mu\text{g L}^{-1}$ (Algeria) [28]. This can affect direct water use and even wildlife, as reported from the Kruger National Park, when MC concentrations exceeded $> 2 \text{ mg L}^{-1}$ [29]. Consequently, the reported MC concentrations can have variable consequences for humans, livestock and wildlife on the African continent.

3.2. 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 [30], and the concentrations and forms of N and P [31,32]. 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. [33] 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 the 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 [34]. Earlier studies by Roegner et al. [35] did not find 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, which provided a stepwise increase in MC concentration (Figure 6), it can be inferred that MC concentration was increasing at lower wind speed ($<4.8 \text{ km h}^{-1}$) 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 [36] and physical density of the *Microcystis* cells [37]. During the study period, the most frequent wind direction 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 of being 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 favored by eutrophic and polymictic conditions because *Microcystis* forms buoyant colonies that can float up quickly and use the light for photosynthesis more efficiently than other non-buoyant taxa, such as diatoms [38]. For example, in Nyanza Gulf of Lake Victoria (Kenya), the growth of the chain-forming diatom *Aulacoseira granulata* was found positively related to transparency (Secchi depth), while the growth of *Microcystis* sp. was found negatively related [39]. Marshall et al. [40] predicted an overall increase in temperature by 2°C in northern Lake Victoria by 2055, which will also cause increased physical stratification of the main basin and possibly favor the growth of buoyant cyanobacteria, such as *Microcystis*.

In addition, an earlier field survey from MB and NG and other Ugandan water bodies [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 [41]. Under high light intensity, the higher level of MC was related to the higher transcription levels of both *mcyB* and *mcyD* genes [42]. In this study, neither the genetic basis nor genetic regulation of MC biosynthesis was tested, but in addition to *Microcystis* biovolume, accordingly, the effect 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.

3.3. The Fate of MC during Water Treatment

In general, WTPs are susceptible to cyanotoxin contamination, but the data on cyanotoxin occurrence in treated water produced in the tropics are scanty [4,43]. Earlier studies in Lake Victoria focused on intracellular toxins [8,34], but fewer considered 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 [43] reported that WTPs in general, using coagulation, clarification and rapid sand filtration for surface water treatment, are effective at removing cyanobacterial cells but only partially effective at removing free (dissolved) 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 in raw water ($47.6 \pm 7.7 \mu\text{g L}^{-1}$ in Gaba III and $12.3 \pm 5.4 \mu\text{g L}^{-1}$ in Walukuba) declined by $>90\%$ in final water (i.e., $2.7 \pm 1.8 \mu\text{g L}^{-1}$ in Gaba and $2.3 \pm 0.4 \mu\text{g L}^{-1}$ in Walukuba), suggesting that sestonic algae and cyanobacterial cells were effectively removed. In addition, our data showed that intracellular MCs were detected in 62.6% and 33.3% of the raw water samples from MB-Gaba III and NG-Walukuba, respectively, but were no longer detected in final water.

On the other hand, rapid sand filtration may be more susceptible to cell lysis [4,43]. Therefore, the water authorities in charge of conventional WTPs might face a challenge arising from dissolved MC. In this study, when raw water MC concentration was $0.46 \pm 0.19 \mu\text{g MC-LR equiv. L}^{-1}$, we observed dissolved MC during rapid sand filtration. This is in agreement with studies in Finland, where Tarczyska et al. [44] found that, on average, $0.8 \mu\text{g L}^{-1}$ of MC was detected in treated water, suggesting that pre-chlorination, coagulation and rapid sand filtration were ineffective in removing dissolved MC. Similarly, Lahti et al. [45] 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. [46] 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 was slow sand filtration or filtration through granular activated carbon [47]. However, both methods are less common in water treatment in east Africa and the global south.

For MB-Gaba, the 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). It is possible that a higher cyanobacteria biomass in the abstracted raw water caused a higher dissolved MC concentration. Nevertheless, the total dissolved MC concentration in treated water was always found below the lifetime guideline value for exposure via drinking water (WHO 2021, i.e., $<1.0 \mu\text{g MC-LR equiv. L}^{-1}$). 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 the potential health threats over longer periods. This will be in addition to the daily

measurements of pH, conductivity, water color, turbidity, total suspended solids, and residual chlorine, and *E. coli* measurements for treated water safety for the riparian population.

3.4. 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 [34]. 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 MCs were released into the water and were composed of five or six structural congeners. In addition to the globally most frequent MC-RR, -YR and -LR variants, the rare [NMeSer⁷]-MC-YR congener was found frequently. 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 [48]. Thus, in the future, if an increased concentration of free MC is recorded in final water, investigations on the potential chlorination degradation by-products would be required as well.

In general, MC-LR was found to be the most potent toxin, followed by MC-YR and MCRR, i.e., when administered intraperitoneally to mice (e.g., Ref [49]). When administered orally to mice, fewer indications of acute toxicity have been found for two MC-RR related structural variants (e.g., Chernoff et al. [50]). To our knowledge, the toxicity equivalents for the more frequently detected [NMeSer⁷]-MC-YR and MC-RY variants during the WTP survey were not determined. However, since MC-LR was recorded frequently during both the field survey and the WTP survey, the resulting toxicity is considered relevant. It should be noted that, in the absence of oral toxicity data for other MC structural variants, it is recommended that the guideline values are applied to total MCs, based on the worst case assumption of the congeners having similar toxicity [4]. In general, the average intracellular MC concentrations observed inshore exceeded the lifetime (1.0 µg MC-LR equiv. L⁻¹) guideline value in MB but not in NG. Therefore, to guarantee the elimination of MC from treated water, the actual toxin concentrations in the source water should be monitored. Early warning parameters, such as *Microcystis* 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*, $859,690 \pm 582,749$ cells mL⁻¹ and *M. aeruginosa*, $792,609 \pm 418,230$ cells mL⁻¹) exceeded the frequently cited alert level of 100,000 cells mL⁻¹ when assessing health risk and regular monitoring is recommended [43]. A general increase in MC-producing *Microcystis* in MB would suggest that the probability of exceeding the WHO lifetime guideline value of 24 µg MC-LR equiv. L⁻¹ in recreational water (untreated water) will increase as well. Thus, the monitoring of *Microcystis* cell abundance could be used as an early warning tool both for recreational services providers and WTP operators [51]. 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 treatment process, are recommended [52]. These measures

could involve warning signs and the closure or discontinuation of swimming or bathing. So far, these measures have been effective in developed countries, such as the Netherlands [52], 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 the dynamics of MC in lake water used for water treatment in Murchison Bay and Napoleon Gulf in Lake Victoria. Although MCs were efficiently reduced by both WTPs, the high abundance of toxigenic cyanobacteria within the lake water and the possible occurrence of the cyanotoxin MC can 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 system, especially during cyanobacterial bloom events. The increase in MC concentration was 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 using lake water for domestic purpose (cooking, drinking, washing) but rather using treated water and reducing 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 of toxigenic cyanobacteria [19] (Figure 1). MB is a relatively 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 outflow from 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 processed by the WTPs.

Depending on the location, the sampling depth ranged from 1.5 to 18 m (Figure 1). At the various sampling stations, water samples were depth integrated from every 1 m depth using a 2 L horizontal van Dorn sampler. The samples were collected in a 20 L bucket as a composite sample to represent the whole water column. From the depth-integrated sample, 2 L were taken 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 performed 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 1). Although both plants use the conventional water treatment methods 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 $4100 \text{ m}^3 \text{ h}^{-1}$ from 1.5 km offshore at a depth of 8 m, while NG-Walukuba abstracts about $880 \text{ m}^3 \text{ h}^{-1}$ from 600 m offshore at a depth of 4 m. In addition, the MB-Gaba plant performs pre-chlorination of the raw water and pH adjustment of the final treated water by using calcium carbonate (CaCO_3). Sampling was performed to monitor the intracellular and dissolved MC

concentration during different steps of the water treatment: (i) in the lake water at the WTP 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). In general, the abstraction pipes are protected with a mesh to prevent the uptake of coarse matter. After abstraction, the water is pumped to the WTP for treatment, usually within a short retention time (the pumped water is considered as raw water used for water treatment).

processes between November 2016 and January 2017. MB: Murchison Bay, NG: Napoteon Gunt ($n = 70$, 47 samples for each WTP). The number of consumers is based on the Uganda National Household Survey (2019/2020) assuming an average of 4.5 persons per household.

Physical-Chemical Parameters during the Treatment Process								
Capacity (m ³ .day ⁻¹)	Piped Water Connection	Demand (m ³ /day)	Treatment Steps	Depth-Integrated Lake Water (at WTP Abstraction Point)	Raw Water (Entry Point of the WTP)	After Flocculation	After Sand Filtration	Finished Water
MB-Gaba								
80,000, but supplementing Gaba I and II water treatment plants	315,897 connections supplying between 1,421,536 and 2 million people in Kampala (including lake shore residents)	300,000	Pre-chlorination, coagulation, clarification, rapid sand filtration, pH correction and chlorination	pH	7.6 ± 0.1	7.2 ± 0.3	7.2 ± 0.3	7.0 ± 0.2
				Conductivity (µS cm ⁻¹)	118 ± 2.1	129 ± 5.5	129 ± 5.9	131 ± 5.3
				Temperature (°C)	26.1 ± 0.3	26.0 ± 0.4	26.0 ± 0.3	26.4 ± 0.4
				Dissolved oxygen (mg L ⁻¹)	5.0 ± 0.7	7.1 ± 0.1	7.4 ± 0.1	7.1 ± 0.1
				Dissolved oxygen (%)	71.0 ± 9.6	102 ± 1.7	104 ± 1.2	101 ± 1.2
NG-Walukuba								
50,000, but producing only 30,100	28,881 connections supplying between 129,964 and 500,000 people (including the neighboring districts of Iganga, Bulikwe and Mayuge)	30,000	Coagulation, clarification, rapid sand filtration and chlorination	pH	7.8 ± 0.2	7.5 ± 0.4	7.4 ± 0.2	7.6 ± 0.2
				Conductivity (µS cm ⁻¹)	102 ± 1.2	105 ± 6.8	103 ± 2.8	109 ± 4.3
				Temperature (°C)	27.1 ± 0.3	27.3 ± 0.4	27.0 ± 0.4	27.2 ± 0.2
				Dissolved oxygen (mg L ⁻¹)	6.6 ± 1.0	3.9 ± 0.6	3.2 ± 0.8	6.4 ± 0.6
				Dissolved oxygen (%)	94.3 ± 15.1	56.7 ± 9.5	47 ± 11.8	92.1 ± 8.9

5.2. 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 were acquired from <https://www.timeanddate.com> (retrieved on 12 January 2022).

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 ($\text{NO}_3\text{-N}$), nitrite ($\text{NO}_2\text{-N}$), ammonium ($\text{NH}_4\text{-N}$), soluble reactive silica (SRSi), total phosphorus and total nitrogen were measured at the analytical laboratory of the National Fisheries Resources Research Institute (NaFIRRI), following standard operating procedures, as described in Olokotum et al. [19].

For microscopical analysis, 20 mL of the water samples were fixed using Lugol's solution [53], stored away from light. Later, 2–5 mL of the fixed water samples were sedimented for 6–12 h [53] before taxa identification and enumeration. Cyanobacteria taxonomic identification and taxa abundance and biovolume estimation were performed based on geometric shapes described in Sun and Liu [54]. The cyanobacteria species were identified using a taxonomic guide and keys as described in Talling [55], Komárek and Anagnostidis [56–58], and Cronberg and Annadotter [59]. 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 ($R^2 = 0.55$, $p < 0.001$) [19]. When Chl-a concentration was low ($\leq 20 \mu\text{g L}^{-1}$), 5 mL was sedimented. When it was high ($\geq 20 \mu\text{g L}^{-1}$), 2–3 mL was 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 estimate the *Microcystis* cell numbers.

5.3. Cyanotoxins Analysis

Field 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. [60], 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 min with 30 s interval pulses to aid extraction. The extracts were centrifuged at 4,000 rpm for 15 min (4°C), and the supernatant was collected in clean 15 mL glass vials. An amount of 2 mL of 75% methanol was added to the pellet for the second extraction using an iced ultrasonic bath (Prolabo) (100% ultrasound power at 40 kHz) for 15 min. The second extracts were also centrifuged and supernatants pooled together. Later, 2 mL of the pooled extract was centrifuged at 13,000 rpm for 10 min, and part of the supernatant extracts was stored in 2 mL amber glass vials at -20°C until MS analysis. For ATX, CYN, STXs and MC analysis, cyanobacteria strain cultures of *Phormidium favosum* (PMC 240.05), *Raphidiopsis raciborskii* (PMC 99.03), *Aphanizomenon gracile* (PMC 638.10) and *Microcystis aeruginosa* (PMC 728.11), respectively, were used 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 min, and 500 μL of the clear supernatant

was transferred into HPLC glass vials. Four microliters were injected into an 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 the target cyanotoxins. The extracts were separated on a grafted-C18 stationary phase column (Acclaim RSLC polar advance 2, 2.2 μm , Thermo Fisher[®], Waltham, MA, USA, 2.1 $\times$ 100 mm) along a 15 min linear gradient (5–90%; 0.3 $\mu\text{L min}^{-1}$ flow rate) of ACN containing 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 v1.3 (Bruker[®], Bremen, Germany). Except for MC-RR, MC-LR and MC-YR, the other MC structural variants were quantified as MC-LR equivalents calculated from the regression curves of the MC-LR analytical standard. The LOD and LOQ for the MC standard were 0.02 $\mu\text{g L}^{-1}$. 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 m/z 180 $\rightarrow$ 163; m/z 180 $\rightarrow$ 145; m/z 180 $\rightarrow$ 135; m/z 180 $\rightarrow$ 107).

5.4. Abstraction Points and Water Treatment (Quantification of Intracellular MC)

Intracellular MCs were extracted according to Fastner et al. [61]. In brief, the filters carrying the algal biomass were cut into small pieces, and the algal 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 min and then transferred to a shaker for 30 min. Subsequent 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 min, 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), 5 μm particle size, LiChroCART[®] 250-4 HPLC cartridge system (Merck, Darmstadt, Germany). According to Lawton et al. [62], for chromatographic separation, a linear gradient of aqueous acetonitrile (ACN) in 0.05% Trifluoroacetic acid (TFA) (30–70% ACN) for 45 min was used. The different variants of MCs 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 MS² and MS³ fragmentation patterns using an iontrap (amaZon SL, Bruker Daltonics, Bremen, Germany). The LOQ for the MC standards were 0.05 μg injected. The globally rarer structural variants of MC [Asp³]-MC-RY, [MeAsp³]-MC-RY and [NMeSer⁷]-MC-YR were described from the same habitats previously [21]. Representative LC-MS chromatograms are presented as Supplementary Material (Figure S5).

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, Germany). These calibration curves of the MC structural variants were pooled together, and the 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 in the column.

5.5. Abstraction Points and Water Treatment (Quantification of Dissolved MC Using ELISA)

All water samples were transported cool and dark within 2–4 h to the laboratory for filtration. Depending on the turbidity from the sample, 250–1000 mL was filtered through the Whatman GF/C filters ($\varnothing$ 47 mm) using a low vacuum pump. For the MB-Gaba and NG-Walukuba samples, both intracellular (filtered biomass) and dissolved MCs (filtrate) were determined. For the analysis of dissolved MCs 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 min. 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 the manufacturer's instructions.

5.6. Abstraction Points and Water Treatment (Quantification of Dissolved MC Using LC-MS)

The same GF/C filtrated samples were concentrated through C18 columns (Sep-Pak® Vac tC18 cartridge, 1 cc/100 mg, 37–55 μ m, Waters Corporation, Vienna, Austria) using a standard solid phase extraction (SPE) procedure according to Dean [63]. 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 4 μ L of analytical MC standards (MC-RR, MC-YR and MC-LR with MC-LR concentration equivalent to 1.0 μ g 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 was 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 h. Since no significant difference in the recovery of dissolved MC was observed, subsequently, SPE columns were dried at 50 °C for 48 h 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 the WTP survey (November 2016 to January 2017), SPE samples (filtrates) from the lake and the subsequent steps in water treatment were also spiked using 4 μ L of the analytical MC standards with MC-LR concentration equivalent to 1.0 μ g L⁻¹. All the analytical MC structural variants (MCRR, YR and LR) were recovered from C18 SPE columns with concentrations > 1.0 μ g L⁻¹ (Table S3).

5.7. Statistical Data Analysis

For cyanobacteria, the taxa were grouped into two classes of (i) (potentially) toxigenic cyanobacteria (taxa that were already described as toxin producers using strains) and (ii) non-toxigenic cyanobacteria (if toxin synthesis was not described from strains) [25]. The data on cyanobacteria biovolume composition were pooled for MB and NG and then

compared using the Mann-Whitney test. Within NG, the differences between the sampled stations were compared using repeated-measures ANOVA, while within MB, a non-parametric test (Friedman test) was performed at 95% confidence interval.

During the water treatment processes, the efficiency of the treatment step was estimated based on the concentration of MC during the process. In addition, cell lysis was determined based on the concentration of dissolved MC during the treatment processes. Differences in MC concentration between the WTP abstraction points, raw water and during the treatment process were tested using the 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 among 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 as MC-LR equivalents in order to explore the relationship with environmental variables. We predicted the MC concentrations using a support vector classifier (SVC) using the SVM function in caret package in R for four categories of MC concentration: low ($<1 \mu\text{g MC-LR equiv. L}^{-1}$), moderate ($1\text{--}5 \mu\text{g MC-LR equiv. L}^{-1}$), high ($6\text{--}10 \mu\text{g MC-LR equiv. L}^{-1}$) and very high ($>10 \mu\text{g MC-LR equiv. L}^{-1}$). 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 concentrations on environmental factors, including the cell abundance or biovolume of the two 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, nutrients (NO_3^- , NH_4^+ , SRSi , SRP and N:P ratio) and meteorological variables (mean air temperature, mean wind speed for the five days (D5), total rainfall for the five days (D5) and mean solar radiation for the ten days (D10) prior to sampling) and wind direction on the day of sampling. Correlations between the variables are shown in Table S5. Data in NG and MB were pooled together, and the Pearson product-moment partial correlation coefficient 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).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/toxins14100664/s1>, 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) WTP abstraction point (Abs. point), (iv) open lake, in Murchison Bay and Napoleon Gulf. (Data collected between November 2017 and October 2018, $n = 120$); Figure S2: The mean ($\pm\text{SD}$) MC concentration (A), total cyanobacteria biovolume (B) and toxigenic cyanobacteria biovolume (C) at the WTP abstraction point (Abs. point) in Murchison Bay and Napoleon Gulf during WTP survey 1 (November 2016–January 2017) and lake survey 2 (November 2017–October 2018); Figure S3: The temporal variation in intracellular MC concentration (MC-LR equiv.) recorded from Murchison Bay (upper part) and Napoleon Gulf (lower part). Note: Abs. point = WTP abstraction point (Data collected between November 2017 and October 2018, $n = 96$). MC-YR was detected only once ($0.29 \mu\text{g MC-LR equiv. L}^{-1}$ 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 100 mg) 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 (B) from LC-MS analysis are shown; Figure S5: LC-MS chromatograms (UV absorption at 240nm) for particulate and dissolved MC structural variants recorded from water samples and during treatment steps including abstraction, raw water, flocculation, sand filtration, and finished water. For

clarity MS base peak spectra were omitted. Elution of indicated MC structural variants was confirmed through ion extraction, i.e. MC-RR [M+H]⁺ 1038.5, MeSerMC-YR [M+H]⁺ 1063.5, MC-YR [M+H]⁺ 1045.5, MC-LR [M+H]⁺ 995.5, DM-MC-RY [M+H]⁺ 1031.5, MC-RY [M+H]⁺ 1045.5. (A) Murchison Bay (Gaba III WTP), from 22 November 2016. (B) Napoleon Gulf (Walukuba WTP), from 20 December 2016, (C) Chromatographic and fragmentation patterns of the common MC analogues MC-LR, -RR and -YR recorded during the field survey (November 2017–October 2018); Table S1: The frequency of detection (percentage) of dissolved and intracellular MC detected and identified during 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). Abbreviations: Abs. point = depth-integrated lake water at WTP abstraction point, RW = Raw water (entering water treatment), CFW = Coagulated and flocculated water, SFW = Sand filtered water and FW = Final water and (-) = not detected; Table S2: Mean (±SD) dissolved MC in lake water in MB and NG, and during the treatment processes, as analyzed by ELISA (*n* = 90) and HPLC (*n* = 90). Abbreviations: Abs. point = depth-integrated lake water at WTP abstraction point, RW = Raw water (entering water treatment), CFW = Coagulated and flocculated water, SFW = Sand filtered water and FW = Final water; Table S3: Recovery of MC-LR, MC-RR, MC-YR added to GF/C filtered water (1.0 µg MC-LR equiv. 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; Table S4: List of cyanobacteria strains used as controls for cyanotoxin extraction and detection via LC-MS/MS (during field survey, November 2017–October 2018). Strains used as a reference for cyanotoxins were cultured at Muséum National d’Histoire Naturelle (MNHN) (PMC = Paris Museum Collection) or at Research Department for Limnology in Mondsee (MU = Murchison Bay, Lake Victoria, 19G6 = Lake George and NAP = Napoleon Gulf, Lake Victoria), √ means detected; –, means not detected; Table S5: Pearson correlation coefficients calculated between environmental parameters recorded during field sampling (*n* = 120) between November 2017 and October 2018.

Author Contributions: M.O., M.T., W.O., R.S., J.-F.H., C.Q., R.K. and C.B. conceptualized the study design. M.O., M.T., W.O., R.S., K.B., R.K. and C.B. performed the experiments and collected the data. M.O., M.T., W.O., K.B., R.K., B.M. and C.B. performed laboratory data and statistical analysis. M.O., R.S., J.-F.H., C.Q., B.M., R.K. and C.B. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Austrian Development Agency (ADA) through International Postgraduate Training in Limnology (IPGL), the Austrian Science Fund (FWF) P24070, the Fonds Français pour l’Environnement Mondial (FFEM), Research Program WaSAf.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in Supplementary Material (Figures S1–S5; Tables S1–S5). The field survey data presented in this study are available on request from the corresponding author (C.B.). The WTP survey data presented in this study are available on request from the corresponding author (R.K.).

Acknowledgments: We thank the crews at Gaba KK beach and Rippon pier for the assistance during field sampling and at the NaFIRRI analytical lab during sample preparation and analysis. We thank Gerold Winkler and the team (University of Natural Resources, Vienna) for administration support and organizing access to the water treatment plants in Kampala and Jinja, and the management of National Water and Sewerage Corporation (NWSC) for granting access and assistance during sampling at the WTPs. Special thanks to Babu and Christopher Kanyesigye (Manager, Research and Development- NWSC, Kampala) for coordinating the sampling in the two WTPs. The meteorological data used for modeling were obtained from the Uganda National Meteorological Authority (UNMA). Benard Simiyu retrieved and compiled the wind direction data. The authors are grateful to three anonymous reviewers for their valuable comments during the reviewing process.

Conflicts of Interest: The authors declare no conflict of interest.

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CHAPTER FIVE

COMMUNITY RESPONSE TO AND POTENTIAL IMPACTS OF EUTROPHICATION⁴

CONTEXT

With the predicted population growth and increase in nutrient inputs, the potential impact of this pollution on the planktonic community functioning of the lake is at stake. This study explored the response of both phytoplankton and zooplankton dynamics and functioning in the embayments using mesocosm experimental approaches. Furthermore, determine the transfer, or accumulation of MC in Nile tilapia during grazing (top- down control) since it is well-known to ingest a diversity of prey items, including cyanobacterial (Semyalo et al, 2011; Umisha and Nehemia, 2013; Jihulya, 2014). Earlier studies by Poste et al. (2011) that reported high concentrations of MC in fish used ELISA, however, this could be due to non-specific binding or due to bioassay reaction with the MC conjugates/congeners (Ibelings et al., 2014). Therefore, the determination of fractions of bound, free, and conjugate toxins are required for fish especially Nile tilapia which is a delicacy and a major aquaculture species for cage fish farming in Lake Victoria. Results from the NG mesocosm indicated that phytoplankton biomasses and the dominance of cyanobacteria are limited by combination of N and P. The addition of Nile tilapia during experimentation also increased in phytoplankton biomass rather than biomass reduction. This suggests that grazing effect was largely offset by an increase in net phytoplankton growth. This can result from selective grazing on zooplankton rather than phytoplankton by fish, and (ii) nutrient enrichment from fish excretion. In the hypertrophic MB, there was no evidence of nutrient limitation but rather light is the likely limiting factor. Indeed, the buoyant *Microcystis*, known to occupy the surface of water column was the dominant taxa and occasionally exceeded 1,000,000 cells/mL and MC concentrations exceeded $\mu\text{g MC-LR Equi L}^{-1}$ (thus there is chances of oral exposure). Given that the oral exposure has been important, there is need to estimate the potential contribution of MC from fish tissue. This will contribute to data and information to assess the extent of chronic exposure to MC from fish consumption. . The detail of this assessment is presented below, as a manuscript being prepared for submission to a peer reviewed journal.

Chapter based on this manuscript in preparation:

Okotum, M.; Humbert, J.-F.; Quiblier, C.; Okello, W.; Semyalo, R.; Marie B; Bertrand C.; Bernard, C; and Roussellier, M. (2023) Differential response of planktonic communities to nutrient and fish (Nile tilapia) addition Lake Victoria: mechanisms and implications using experimental approaches.

Abstract

Planktonic communities are altered by eutrophication with the predominance of toxigenic cyanobacterial blooms, which impact on the overall ecosystem functioning. Therefore, an in-situ mesocosm experiment was conducted in September 2019 at Napoleon Gulf (NG) and Murchison Bay (MB), Lake Victoria, to examine the response of planktonic communities to the addition of nutrients and fish. The results show that the addition of N+P greatly promoted phytoplankton biomass but also increased the growth of cyanobacteria. Co-limitation was not detected in NG but was instead seen in MB, and the trace elements had no effect on plankton biomass in MB but did in NG. Changes in the phytoplankton and zooplankton community's composition, diversity, and biovolume/density were observed. The addition of fish had a significant impact on the composition of the zooplankton community and density of *Lecane* sp. and *Brachionus* sp. There was a decrease in the composition of green algae, however the addition of fish had no effect on the composition of cyanobacteria. The dominance of cyanobacteria resulted into higher microcystin (MC) production and its transfer into the intestines (0.001-27.5 µg/g FW of MC-LR) of fish in MB than NG (0.005- 0.44 µg/g FW of MC-LR), and some liver (0.002-0.48 µg/g of MC-LR FW) and muscle (0.006-0.3 µg/g FW of MC-LR) in MB. In NG, there was no evidence of MC accumulation in the fish muscle. However, the prevalence of cyanobacteria linked to elevated MC will further enhance the possible health risks from consuming entire fish from Lake Victoria.

Keywords: *Mesocosms; Bottom-up and top-down control; Microcystin transfer; Secondary limitation*

Introduction

Inland lakes which support a large range of ecosystem services for humanity (Sterner et al. 2020) have been subjected to growing anthropogenic stresses (Jenny et al. 2020), especially in Africa (Cohen et al. 2019). Among the African Great Lakes (AGL), Lake Victoria host a large growing population on its watersheds (Lopez-Carr et al. 2013) and has been subjected to diverse and strong anthropogenic pressures (e.g., habitat degradation, introductions and invasion of invasive Nile perch, Nile tilapia and water hyacinth, and pollution/eutrophication) (Verschuren et al. 2002, Juma et al. 2014, Nassali et al. 2020, Olokotum et al. 2020). Consequently, large changes have been reported in physical characteristics of Lake Victoria (Awange 2020), water quality (Mwamburi et al. 2020) and biological communities (e.g., Witte et al. 2013).

After the introduction of Nile perch and Nile tilapia that resulted in the crash/decline in indigenous fish diversity (Witte et al. 2013, Taabu-Munyaho et al. 2016), there were changes in the biological communities of the lake. One of the most important changes in biological communities related both to the functioning of the lake and to sanitary impact is the increase of cyanobacteria biomass and its dominance in phytoplanktonic communities in different parts of the lake. Such dominance of cyanobacteria, including potentially toxic ones, appeared as mainly related to the level of eutrophication of the sampled areas (Ochumba and Kibaara 1989, Abonde et al. 2015, Olokotum et al. 2020, Olokotum et al. 2021).

However, factors controlling the dominance of cyanobacteria may differ in their nature and/or intensity from one area to another especially in large lakes such as Lake Victoria. For instance, it is well known that the level of eutrophication differs greatly between the central area of the lake and its embayments (bays and gulfs), but also among them (Olokotum et al. 2020). Moreover, changes in hydrodynamic connections between the open lake and the embayments may modify their trophic status (Simiyu et al. 2022) as well as mixing regime of the water column (Dermendjian et al. 2021) may change the composition of phytoplanktonic communities. Results from field studies do not always make it possible to disentangle and identify the magnitude of the different factors controlling the cyanobacterial biomass and composition. This could be due to: i) low sampling frequency that might not record fast planktonic responses to environmental changes; ii) the hydrodynamic events that mix different water masses or transport them away from the sampling stations which imply that the observations may refer to planktonic communities originating from different locations; and iii) some potential controlling factors may not be measured in the field, or when measured do not refer to conditions before sampling events related to the changes in planktonic communities. Therefore, one way to access a better understanding of the effect of some bottom-up and top-down factors on microbial and planktonic communities is to perform *in-situ* experiments using mesocosms.

Mesocosms have been used in several ways for many years to address the effects of local and/or global environmental changes including nutrients involved in local eutrophication process (e.g., Schindler 2012), grazing pressure (e.g., He et al. 2018) as well as climate related variables (e.g., Stewart et al. 2013). However, such mesocosm approaches have also received criticisms, for instance one of the main criticisms is that biological communities in “control” (no treatment) mesocosms may often diverge compared to *in-situ* conditions (e.g., Dzialowski et al. 2014). This divergence is often related to the size of mesocosms and duration of experimentations. Consequently, large facilities with different kinds of controlled

experimentations and connected through transnational networks and analytical facilities are now available in the Western world (e.g., Gall et al. 2017, Jeppesen et al. 2021, Urrutia-Cordero et al. 2021). In Africa, *in-situ* mesocosm experimentations have been rarely used to identify the bottom-up and top-down factors controlling the phytoplankton biomass and composition in African lakes (e.g., Ahoutou et al. 2022) and, to our knowledge, never been used in Lake Victoria. This has been mainly because such experiments were mainly based on expensive devices that limit their use in African countries.

In this study, we used similar simple and low-cost mesocosm system that were successfully deployed in Aghien Lagoon in Ivory Coast (Ahoutou et al. 2022) to test short-term effect of inorganic nutrients (bottom-up effect) and of potentially planktivorous fishes (top-down effect) on phytoplankton and zooplankton communities. We therefore performed these mesocosm experiments in two embayments of the Lake Victoria, with known contrasted eutrophication status and conditions (Olokotum et al. 2021), expecting to get different responses of planktonic communities under the same controlled treatments. Thus, in this study, controlled laboratory (indoor microcosms) and outdoor *in-situ* mesocosm experiments were carried out in Napoleon Gulf and Murchison Bay to investigate the vulnerability and responses of phytoplankton and zooplankton community to different nutrient concentrations and fish addition. A special attention to cyanobacteria community response through their magnitude of toxin production after nutrient addition and its potential transfer into fish tissues such as intestines, liver, and the muscle of the Nile tilapia (*Oreochromis niloticus* L.). Nile tilapia was selected because it dominates tilapiine fisheries in the Lake Victoria (Yongo et al. 2021) and of its omnivorous capacity including cyanobacteria (Moriarty 1973; Salazar Torres et al., 2016).

Materials and methods

Pre-experimentation period

The pre-experimentation and the conditions for the experimentation were conducted between 23rd and 29th April 2019 where the condition of macro and trace nutrients for controlling of phytoplankton biomass tested. During this experimentation, four stock solutions were used (Z8I = 15 μ L stock solution containing N, Z8II = 28 μ L stock solution containing P, Z8 III = 28 μ L stock solution containing Fe+EDTA and Z8 trace solution containing a combination of: W, Mo, K, Zn, Cd, Co, Cu, Nk, Cr, Al, V, Bo, Mn), and control with Z8 solution only. Each enrichment was performed in 50 mL flasks filled with 20 ml of water from Lake Victoria (NG, MB, and Open Lake). Phytoplankton from NG, MB and open lake were

concentrated with 20µm phytoplankton net while the zooplankton were filtered out from the water using 60µm net. On the day of sampling of water from the lake, N and P concentrations were measured. The pre-experimentation was performed for four days during which optical density (OD) were measured daily while chlorophyll a were measured on Day zero (start of experiment) and day four (end of experiment).

From the pre-experimentation, the overall optical density (OD) was highest in the treatments with N+P; N+P+Iron+EDTA and N+P+Trace+Iron+EDTA in all the water samples from MB, NG, and Open Lake (Figure 5.1). Thus, based on existing literature on secondary limitation of phytoplankton growth (Guildford et al., 2003) and our preliminary results we decided to conduct mesocosm experimentations to include the effect of Fe and EDTA (micronutrients).

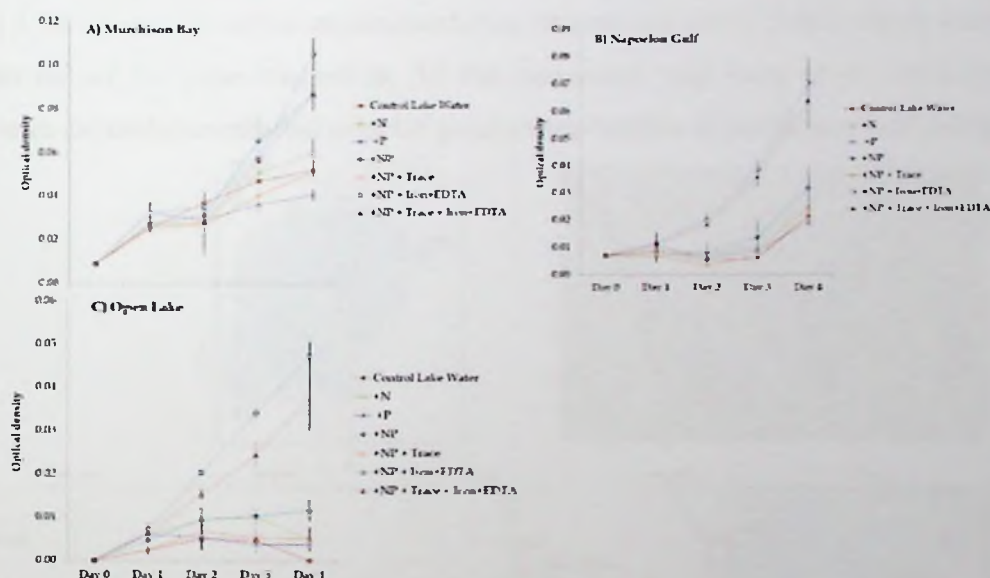


Figure 1:

Figure 5. 1: The optical density measured during the microcosm experimentation during four days with Murchison Bay, Napoleon Gulf and Open Lake water during the microcosm experimentation in April 2019.

Study sites, mesocosm conditions and experimental set up

The in-situ mesocosm experimentation were undertaken in two embayments of northern Lake Victoria, Murchison Bay, and Napoleon Gulf. These embayments have been extensively described in Olokotum et al. (2021). Two sets of 15 transparent plastic mesocosms (diameter 180cm, depth 150cm) on a metallic buoyant frame were set in these embayments of northern Lake Victoria (Figure 5.2).



Figure 5. 3: The experimental design with two independent sets of 15 mesocosm bags in Murchison Bay and Napoleon Gulf randomly inoculated with treatment factors. B) The 7-day experimentation period considered nutrient inoculation on Day-0, addition of fish on Day 3 and concluding on Day 7.

Treatment sampling designs and measured variables

The physical-chemical conditions (temperature, electrical conductivity, turbidity) were measured daily using YSI Sonde EXO2 multiprobe meter (Sonde 16B102461). Before each sampling, the mesocosm were gently mixed. For chlorophyll-a analysis about 700 mL of water samples were collected every morning (9:30 - 10:30 am) for the seven days of the experiment while for nutrients, the same volume was collected on Day 0, Day 3 and Day 7. For phytoplankton and cyanotoxins analyses about 1.5L were taken on Day 0 (start), Day 3 (mid-way) and Day 7 (end of experimentations). On the other hand, on Day 0 and Day 7, vertical zooplankton hauls were taken from at least 0.5m using a 5L Schindler trap (0.25m mouth opening and 60µm nitex mesh) (**Figure 5.3**). On Day 3, fish were added and continually observed for any dead individuals and when this occurred the dead fish were noted and removed from the mesocosm bags. At the end of the experimentation, the fish were collected with ice anesthesia, weighed, measured, and later dissected. From the fish, the liver, intestine, and muscle were removed, and these tissues were frozen in liquid nitrogen (-96°C) and later stored at -20°C awaiting further cyanotoxin extraction and analysis.

Sample processing and analysis

Chlorophyll a (Chl-a) concentration. 100-400 mL of water were filtered (Whatman GF/C filters), followed by hot 90% ethanol extraction of the filtered biomass according to ISO-10260 (1992) and spectrophotometric analysis (Jenway 6505 UV/vis).

Nutrient concentrations: Soluble reactive phosphorus (SRP) was determined using the ammonium molybdate method (Wetzel and Likens 2000b), nitrate (NO₃-N) by the sodium salicylate method (Müller and Wiedemann 1955), ammonium (NH₄-N) using the indophenol blue method (Krom 1980), and soluble reactive silica (SRSi) using the yellow molybdate-silicic acid method (Wetzel and Likens 2000b). The unfiltered water samples were used for total phosphorus and nitrogen analysis using the persulphate digestion oxidation methods described in Wetzel and Likens (2000b).

Phytoplankton communities. Taxonomic identification, taxa abundance, and biovolume estimation were performed as described by Catherine et al. (2017). The phytoplankton samples were fixed using Lugol's iodine solution and 2-5 mL of sample were sedimented for 6-12 hours (Utermöhl 1958). To ensure even distribution of cells, the samples were diluted and vortexed for one minute, whereas the sedimented volume was guided by the Chl-a concentrations as described in Olokotum et al. (2021) for subsequent cell counting and enumeration.

Zooplankton communities. The samples were preserved in 5% sugar-formalin solution to mitigate the ballooning of cladocerans for easy identification. Each sample was washed and diluted to a suitable concentration to remove excess preservative as described in Kiggundu et al. (2012), from which sub-samples of 2 and 5 ml were taken, transferred to a counting chamber and observed under an inverted microscope at x40 and x100 magnification. The taxonomic identification and enumeration were done using zooplankton taxonomic guides (Boxshall and Braide 1991, Korinek 1999).

Identification and quantification of microcystins in phytoplankton and fish

Extraction of intracellular MC from filters. About 100 mL from MB mesocosm and 400 mL from NG mesocosms were filtered (Whatman GF/C filters), and biomass were extracted using 75% aqueous methanol (Fastner et al., 1998; Cerasino et al., 2017), following an adapted double-extraction protocol as described by Olokotum et al. (2022). Briefly, biomass was conditioned with 4 mL of 75% aqueous methanol in sterile 15 mL glass vials. The first extraction followed sonication on ice using an ultrasound probe (Sonics Vibra cell) (100% amplitude, 130 W, 20 kHz) for 3 minutes at 30-second interval pulses to aid extraction and supernatant collected. The second extraction used with 2 mL of 75% aqueous methanol and iced ultrasonic bath for 15 minutes. The extracts were pooled, centrifuged at 13,000 rpm for 10 minutes and part of the supernatant stored in 2 mL amber glass vials at -20°C, prior to liquid chromatography-mass spectrometry (LC-MS) analysis.

Extraction of MC from fish tissue: Approximately 0.12g of fresh muscle and intestine, and 0.03g of liver tissues were weighed (Mettler Toledo) and used for extraction using an adapted protocol according to Colas et al. (2020). Briefly, the muscle and intestine were conditioned with 1000 µL and liver with 500 µL of 75% ice-cold methanol before homogenization. The tissues were homogenized using the Omi general laboratory homogenizer (GLH 850) with a 7mm sharp tooth generator probe at 25000 rpm for 90 seconds (3 times in intervals of 30 seconds). The tissue homogenates were later sonicated for 1 minute (2 pulses of 30 seconds) using an ultrasound agitation probe (Sonics Vibra Cell) (60% 130 W, 20kHz). Later, the tissue mixtures were centrifuged at 13400 rpm for 15 minutes at 4°C. The supernatant was collected and stored in 2 mL amber glass vials at -20°C, prior to LC-MS analysis.

LC-MS/MS characterization and quantification of MC. From the extracts, 30 µL were transferred into Verex glass vials from which 5 µL was injected into Ultra-High-Performance Liquid Chromatography (UHPLC) chromatographic chain (Elute UHPLC-Bruker) coupled

upstream to a High-Resolution Mass Spectrophotometer system (Compact QTOF-Bruker) for identification and quantification of cyanotoxins. The extracts were separated on a grafted-C18 stationary phase column (Acclaim RSLC polar advance 2, 2.2 μm , Thermo Fisher® 2.1 $\times$ 100 mm) along a 15-min linear gradient (5-90%; 1 $\mu\text{L}\cdot\text{min}^{-1}$ flow rate) of acidified acetonitrile 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, then quantified according to the area-under-the-peak signal determined for analytical standards of seven MC variants (MC-LR, MC-LA, MC-LF, MC-LW, MC-LY, MC-RR and MC-YR)(Novakit®) using the TASQ software) (Bruker®). Except from MC-RR, MC-LR and MC-YR, other MC structural variants were quantified according to MC-LR equivalence calculated from the regression curves of the MC-LR analytical standard.

Statistical analysis

Indices of biological diversity in phytoplankton and zooplankton were calculated using taxa richness, Pielou evenness, Shannon and Simpsons diversity indices from the '*vegan*' package (Oksanen et al. 2007). The differences in the Chla, phycocyanin, biological indices, phytoplankton biovolume, zooplankton densities, and MC concentrations between the NG and MB mesocosms were compared using Mann-Whitney test, while amongst the treatments using non-parametric one-way ANOVA. The effect of treatment duration was tested using three-way ANOVA. During the water experimentation, the effect of the fish addition was calculated based on the differences between the with and without fish treatments. All statistical tests and graphical outputs were produced in R software.

Results

Initial conditions

At D0, the water temperature in the mesocosms in both embayments were within the same range ($26.40 \pm 0.01^\circ\text{C}$ in Murchison Bay and $26.92 \pm 0.04^\circ\text{C}$ in Napoleon Gulf respectively). The main differences between the two sites were observed in the electrical conductivity, turbidity, Chl-a biomass, and the nutrient concentrations (**Table 5.1**). At the beginning of the experiments, the water of the two bays differed in their nutrient concentrations regarding TN was $2586.39 \pm 331.50 \mu\text{g/L}$ in MB and $543.21 \pm 125.39 \mu\text{g/L}$ in NG, $\text{NH}_4\text{-N}$ was $125.38 \pm 164.46 \mu\text{g/L}$ in MB and $14.71 \pm 5.55 \mu\text{g/L}$ in NG. However, $\text{NO}_3\text{-N}$ was lower in MB $65.63 \pm 31.07 \mu\text{g/L}$ than in NG $253.72 \pm 161.97 \mu\text{g/L}$ (mean values $\pm$ SD) and planktonic biomasses were higher in MB $296.22 \pm 59.47 \mu\text{g. Chla L}^{-1}$ than in NG where it was $25.02 \pm 2.08 \mu\text{g. Chla L}^{-1}$.

The initial phytoplankton communities in NG and MB were dominated by cyanobacteria. However, this dominance was quite absolute in MB waters while other taxonomic groups were observed in NG (Bacillariophyceae, Chlorophyceae). The initial taxa richness was slightly higher in MB (34) than in NG (27). The initial dominant cyanobacteria taxa in the two bays were different: *Dolichospermum circinale* or *Planktolyngbya circumcreta* in NG versus *Microcystis aeruginosa* in MB (**Table 5.2**).

Zooplankton community in NG and MB mesocosms comprised of three taxonomic groups namely, cladocerans, copepods and rotifers. Rotifera had the highest zooplankton richness (21), followed by Copepoda (11) and Cladocera had only 6 species. Generally, the species richness was higher at the NG station compared to the MB station. Nonetheless, the initial zooplankton community was dominated by young forms such as Nauplii and copepodites. For instance, in NG, there was no significant dominant zooplankton species while in MB mesocosms, the community was dominated by Nauplius larvae ($113,589.3 \text{ Ind.m}^{-2}$), and cyclopoid copepodites ($46,772.1 \text{ Ind.m}^{-2}$) (**Table 5.3**).

Table 5. 1: The Mean±SD of the physical chemical conditions and nutrient concentrations in the lake water on Day 0 at the two experimental stations of Murchison Bay and Napoleon Gulf, northern Lake Victoria.

Station	Temperature (°C)	Conductivity (µS/cm)	Turbidity (NTU)	NO ₂ -N (µg/L)	NO ₃ -N (µg/L)	NH ₄ -N (µg/L)	TN (µg/L)	SRP (µg/L)	TP (µg/L)	Chla (µg/L)
Murchison Bay	26.40±0.01	199.68±25.56	89.48±16.96	2.45±0.17	65.63±31.07	125.38±164.46	2586.39±331.50	11.17±0.00	80.54±10.50	296.22±59.47
Napoleon Gulf	26.92±0.04	124.28±28.09	4.44±3.03	1.25±0.17	253.72±161.97	14.71±5.55	543.21±125.39	7.80±0.00	40.43±23.87	25.02±2.08

*NO₂-N= Nitrite; NO₃-N= Nitrate; NH₄-N= Ammonium; SRP=soluble reactive phosphorus; TN = total nitrogen; TP = total phosphorus; and Chla = chlorophyll-a

Table 5. 2: Mean biovolume and composition of the dominant phytoplankton species in lake water on D0 at the two experimental stations of Murchison Bay and Napoleon Gulf, northern Lake Victoria

Site	Group	Taxa	Biovolume (mm ³ /L)
Murchison Bay	Bacillariophyceae	<i>Nitzschia_fonticola</i>	0.166±0.109
		<i>Nitzschia_acicularis_var.acicularis</i>	0.100±0.022
		<i>Nitzschia_bacta</i>	0.026±0.000
		<i>Synedra_cunningtonii</i>	0.026±0.000
	Chlorophyceae	<i>Scenedesmus_acuminutus</i>	0.012±0.000
		<i>Scenedesmus_perfolatus</i>	0.012±0.000
		<i>Actinastrum_gracillium</i>	0.010±0.000
		<i>Chlorella_vulgaris</i>	0.002±0.003
		<i>Chlorella_sp</i>	0.002±0.000
		<i>Microcystis_aeruginosa</i>	5.900±0.294
		<i>Microcystis_flos-aquae</i>	3.290±1.075
	Cyanobacteria	<i>Microcystis_wessenbergii</i>	1.515±0.714
		<i>Microcystis_viridis</i>	1.010±0.000
		<i>Planktolyngbya_circumcreta</i>	0.382±0.299
		<i>Dolichospermum_circinale</i>	0.367±0.184
		<i>Chroococcus_minutus</i>	0.232±0.025
		<i>Planktolyngbya_limnetica</i>	0.109±0.062
Napoleon Gulf	Bacillariophyceae	<i>Synedra_cunningtonii</i>	0.131±0.000
		<i>Epithemia_argus</i>	0.078±0.054
		<i>Nitzschia_acicularis_var.acicularis</i>	0.064±0.021
	Chlorophyceae	<i>Pediastrum_simplex</i>	2.270±0.000
		<i>Oocystis_gigas</i>	1.460±0.000
		<i>Phacus_longicauda</i>	0.445±0.000
		<i>Scenedesmus_acuminutus</i>	0.110±0.000
		<i>Scenedesmus_apiculata</i>	0.049±0.000
		<i>Ankistrodesmus_falactus</i>	0.007±0.004
	Cyanobacteria	<i>Dolichospermum_circinale</i>	1.710±0.208
		<i>Planktolyngbya_circumcreta</i>	1.289±0.516
		<i>Spirulina_platensis</i>	0.406±0.440
		<i>Planktolyngbya_limnetica</i>	0.126±0.082
		<i>Planktolyngbya_tallingii</i>	0.054±0.000
		<i>Pseudanabaena_limnetica</i>	0.051±0.000
		<i>Chroococcus_minutus</i>	0.045±0.028

Table 5. 3 The mean zooplankton abundance and composition of the dominant zooplankton groups and taxa in lake water on D0 at the two experimental stations of Murchison Bay and Napoleon Gulf, northern Lake Victoria

Bay	Group	Taxa	Density (Ind/m ²)
Murchison Bay	Copepoda	Nauplius-larvae	113589.3±0.000
		Cyclopoid-copepodite	46772.1±0.000
		Calanoid-copepodites	3340.9±0.000
		<i>Thermocyclops emini</i>	1113.6±0.000
	Rotifera	<i>Synchaeta sp.</i>	28954.1±0.000
		<i>Lecane bulla</i>	27840.5±0.000
		<i>Keratella tropica</i>	1113.6±0.000
Napoleon Gulf	Copepoda	Nauplius-larvae	10956.8±7795.7
		Cyclopoid-copepodite	4720.5±3047.9
		<i>Tropocyclops tenellus</i>	1342.5±525.0
		Calanoid-copepodites	649.6±551.3
		<i>Tropocyclops confinnis</i>	97.5±45.9
		<i>Harpacticoida</i>	65.0±0.0
		<i>Thermodiaptomus galeoides</i>	65.0±0.000
		<i>Lecane bulla</i>	3681.1±1120.2
	Rotifera	<i>Brachionus calyciflorus</i>	877.0±780.9
		<i>Polyarthra vulgaris</i>	389.8±0.000
		<i>Brachionus angularis</i>	259.9±183.8
		<i>Keratella tropica</i>	259.9±183.8
		<i>Brachionus hidentatus</i>	194.9±0.000
		<i>Cephalodella sp.</i>	129.9±0.000
		<i>Trichocerca cylindrica</i>	129.9±0.000
		<i>Brachionus falcatus</i>	65.0±0.000
		<i>Keratella cochlearis</i>	65.0±0.000
		<i>Moina micrura</i>	129.9±0.000
	Cladocera		

Responses of planktonic communities to nutrient additions

Physical and chemical variables: In both NG and MB mesocosms, there were an increase in conductivity between D0 and D3 in the N+P treatment, followed by a slight decrease until D7. On the other hand, water temperature decreased during the experimentation in period while no changes were observed in turbidity in all treatments (**Supp Figure 5.1**).

For the nutrients, there are observable trends in soluble nutrients added (e.g., TP, NO₂-N and NH₄-N decreased between D3 and D7; **Supp Figure 5.2**) with a large decrease both in SRP and NO₃ in NG and in MB mesocosms. Given the initial differences between the MB and NG mesocosms, the observed rate of nutrient decrease appeared quite faster in MB than in NG mesocosms (**Figure 5.4**). However, considering the rate of decrease per unit of phytoplankton biomass (Chl_a), the rate of nutrient consumption per biomass was higher in NG than MB mesocosms (**Table 5.4**).

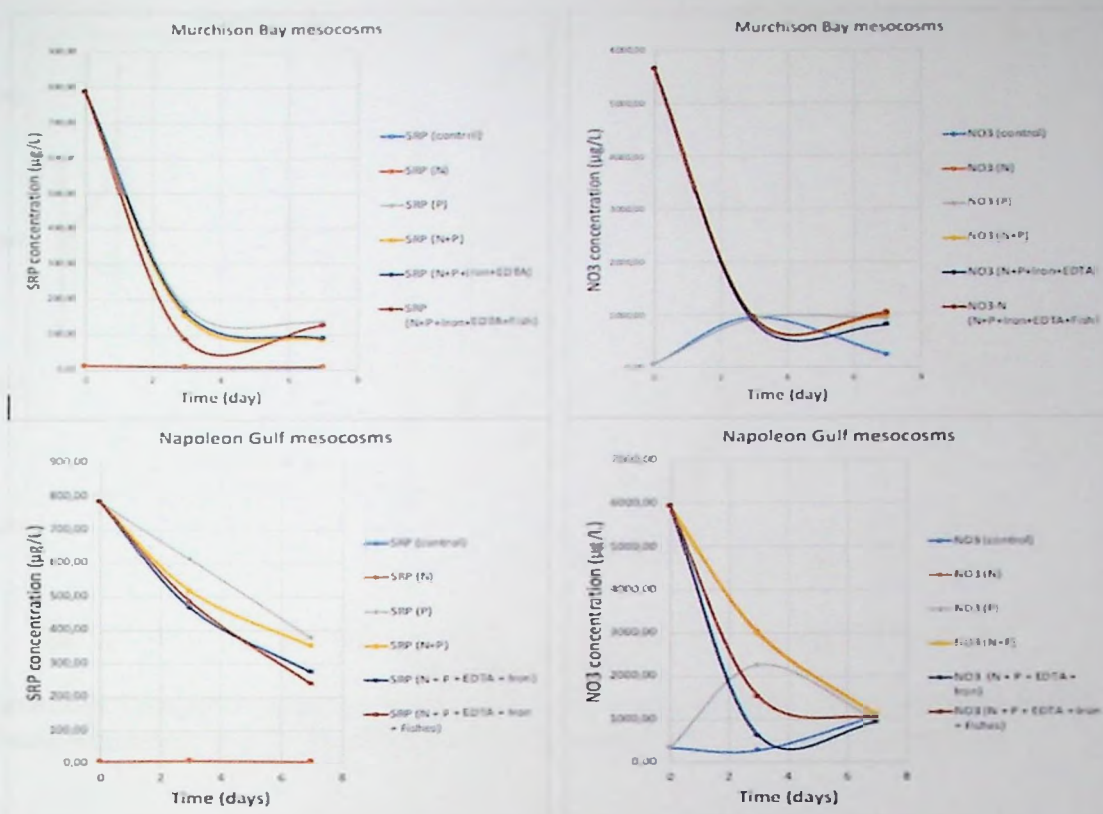


Figure 5. 4: The dynamics of SRP and NO₃ concentrations in the different treatments of the mesocosms operated in Murchison Bay and Napoleon Gulf, northern Lake Victoria.

Phytoplankton biomass: In NG mesocosms, from D0 to D5, the chlorophyll-a concentration showed a fast and large increase following the addition of N+P, while adding N or P alone do not imply a significant increase in chlorophyll-a concentrations (Kruskal-Wallis $\chi^2 = 111.01$, $p < 0.0001$). After D5, there was a decrease in chlorophyll-a concentration (Figure 5.5).

In MB mesocosms, the addition of nutrients does not cause an increase in chlorophyll-a concentrations (Kruskal-Wallis, $\chi^2 = 7.637$, $p = 0.3657$). Instead, there was an overall decreasing trend in chlorophyll-a concentrations from the beginning to the end of the experiment whatever the treatment and even in the control. However, the observed large variations in chlorophyll-a concentrations do not allow to detect significant statistical differences during the experiment (Figure 5.5).

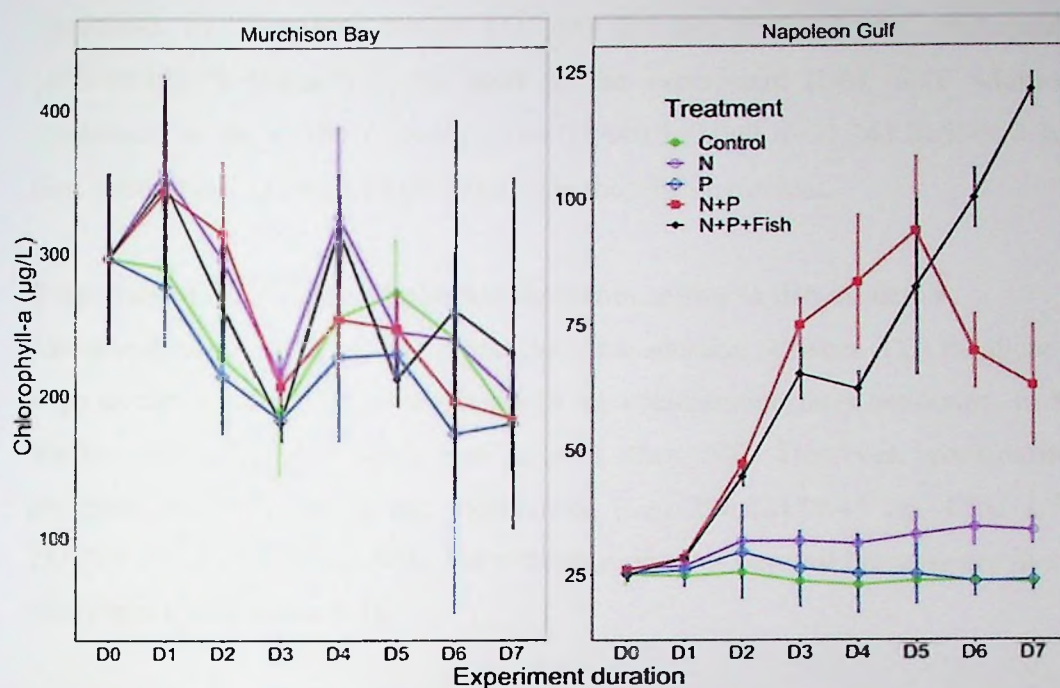


Figure 5.5: Temporal variation in the chlorophyll-a concentration (Mean±SD) under the different treatments at Murchison Bay and Napoleon Gulf, northern Lake Victoria.

Planktonic community structure and composition

Phytoplankton: In NG and in MB, the initial phytoplankton community was composed by cyanobacteria, chlorophyceae, bacillariophyceae and other groups such as dinophyceae, and cryptophyceae. The addition of nutrients does not modify the dominant status of cyanobacteria species. In MB, *M. aeruginosa* remain the dominant taxa, while in NG it was *D. circinale* and/or *P. circumcreta*. However, following N+P addition the taxonomic richness in MB showed a large decrease (from 35 taxa in the control mesocosm at D0, to 27 at D3 and 15 at D7), while it was the reverse for NG (from 27 taxa in the control mesocosm to 42 at D3 and then 34 at D7). At MB station, the addition of N resulted in an increase taxonomic richness from 20 at D3 to 24 at D7 while after P addition, the richness decreased from 29 at D3 to 22 at D7. In NG mesocosms, N addition increased phytoplankton richness from 37 at D3 to 42 at D7 while P addition saw the phytoplankton richness remain relatively the same at 36 taxa at D3 and 35 at D7.

Zooplankton: Generally, following N+P addition, there was a large change in the dominant taxonomic group: from copepods at the beginning of the experiment (D0) to rotifers at the end (D7). In NG mesocosms, the community shifted from Nauplius larvae ($10956.8 \pm 7795.7 \text{ Ind.m}^{-2}$) to the dominance of rotifers mainly *Brachionus calyciflorus* in the N+P treatment (from 877.0 ± 780.9 to $1,693,321.9 \text{ Ind.m}^{-2}$). Although at MB the zooplankton community was

dominated by Nauplius larvae ($113,589.3 \pm 0.000 \text{ Ind.m}^{-2}$) and cyclopoid copepodites ($4720.5 \pm 3047.9 \text{ Ind.m}^{-2}$) at the start of the experiment (D0), N+P addition shifted the community to the rotifer *L. bulla* (from $27,840.5 \pm 0.000$ to $31,243.2 \pm 5966.3 \text{ Ind.m}^{-2}$) and *L. luna* (from 0 to $111,362.1 \pm 0.00 \text{ Ind.m}^{-2}$) in the +fish treatment.

Responses of nutrients and planktonic communities to fish addition

Nutrient dynamics and consumption: After the addition of fishes (D3) there was a fast and large increase in the nutrient as shown in the consumption rates estimated. In MB, there is a higher nutrient consumption rate in MB than NG. However, considering the initial phytoplankton biomass in the mesocosms (*i.e.*, $296.22 \pm 59.47 \text{ } \mu\text{g. Chla L}^{-1}$ in MB and $25.02 \pm 2.08 \text{ } \mu\text{g. Chla L}^{-1}$ in NG), the consumption rates per unit biomass are much higher than NG (**Figure 5.4; Table 5.4**).

Phytoplankton: After the addition of fishes (D3) there was a fast and large increase in chlorophyll-a concentration in NG mesocosms (from 65.1 ± 5.1 on D3 to $121.2 \pm 3.4 \text{ } \mu\text{g. L}^{-1}$ on D7), while in MB mesocosm this treatment did not modify in a large way the observed chlorophyll-a values (*i.e.*, from 180.6 ± 12.9 on D3 to $229.1 \pm 122.9 \text{ } \mu\text{g. L}^{-1}$ on D7; **Figure 5.5**). Concerning the different phytoplankton groups in NG mesocosms, after the addition of fishes the biovolume of cyanobacteria decreased from 17.7 to $9.1 \text{ mm}^3/\text{L}$ (48.6% decrease) and bacillariophyceae from 1.51 to $0.48 \text{ mm}^3/\text{L}$ (68.2% decrease). In the MB mesocosms, fish addition was followed by a decrease of cyanobacteria, from 64.7 to $19.9 \text{ mm}^3/\text{L}$ (69.2%), and Bacillariophyceae, from 0.26 to $0.13 \text{ mm}^3/\text{L}$ (**Figure 5.6; Supp Figure 5.3A&B**).

Zooplankton: Following fish addition, no significant changes in the zooplankton community but rather in the densities were observed. We observed a large decrease in the density of Copepoda and replacement with an increase in the rotifers (**Figure 5.7**). For instance, in NG mesocosms, there was a 79.9% decrease in total zooplankton density ($7,742,215.1 - 1,549,036.6 \text{ Ind.m}^{-2}$) mainly due to the 77% decline in *B. calyciflorus* from $1,922,851.5 \pm 55,502.9 \text{ Ind.m}^{-2}$ to $440,761.7 \pm 373,395.1 \text{ Ind.m}^{-2}$. In MB there was a 9.04% decline in the total zooplankton densities ($164,258.9$ to $149,410.8 \text{ Ind.m}^{-2}$) depicted in the decline in the density of Nauplii's ($113,589.3$ in control to $2,784.1$) and cyclopoid copepodites ($46,772.1$ in control - $13,920.3 \text{ Ind.m}^{-2}$; **Figure 5.7; Supp Figure 5.4A&B**).

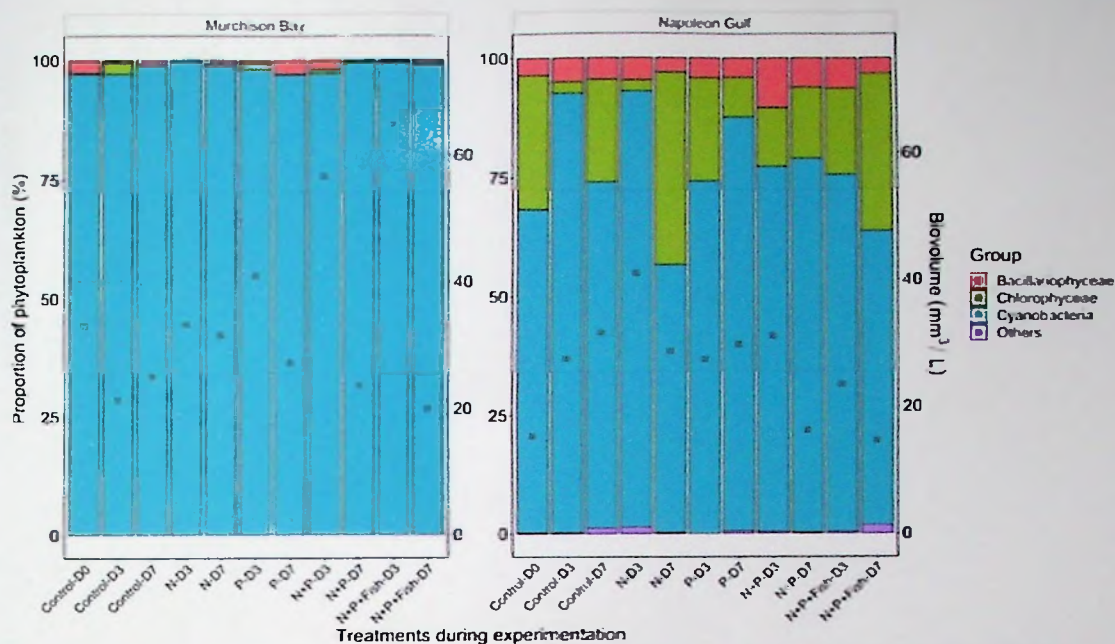


Figure 5. 6: The proportion of phytoplankton groups (%) and temporal variation in the total phytoplankton biovolume under the different treatments at Murchison Bay and Napoleon Gulf, northern Lake Victoria.

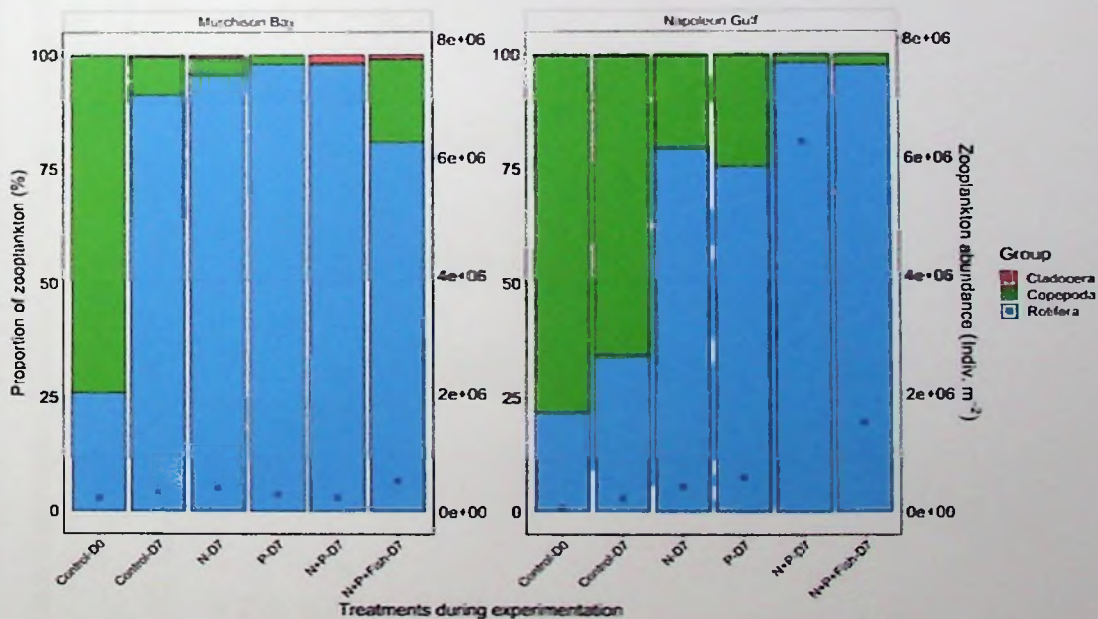


Figure 5. 7: The proportion of zooplankton groups (%) and temporal variation in the total zooplankton abundance under the different treatments at Murchison Bay and Napoleon Gulf, northern Lake Victoria.

Table 5. 4 : The estimated rate of nutrient decrease in the different mesocosms operated in Murchison Bay and Napoleon Gulf, northern Lake Victoria. Note: The different initial phytoplankton biomass in the mesocosms (*i.e.*, 296 $\mu\text{g Chla/L}$ in MB and 25 $\mu\text{g/L}$ in NG).

Treatment/Rate of decrease	Murchison Bay				Napoleon Gulf			
	SRP ($\mu\text{g/L/D}$)	SRP/biomass ($\mu\text{g/L/D}/\mu\text{g Chla}$)	NO ₃ ($\mu\text{g/L/D}$)	NO ₃ /biomass ($\mu\text{g/L/D}/\mu\text{g Chla}$)	SRP ($\mu\text{g/L/D}$)	SRP / biomass ($\mu\text{g/L/D}/\mu\text{g Chla}$)	NO ₃ ($\mu\text{g/L/D}$)	NO ₃ / biomass ($\mu\text{g/L/D}/\mu\text{g Chla}$)
N	-	-	1572.53	5.31	-	-	970.85	388,338,962
P	202.66	0.68	-	-	56.87	227,481,481	-	-
N+P	210.94	0.71	1562	5.27	88.31	353,259,259	983,355,222	393,342,089
N+P+Fe+EDTA	207.28	0.7	1586.7	5.36	105.02	420,074,074	1765,09	706,037,523
N+P+Fe+EDTA+Fish	233.67	0.78	1565.67	5.28	99.06	396,222,222	1462,82	585,128,622
Mean $\pm$ SD	213.64 $\pm$ 13.78	0.72 $\pm$ 0.05	1571.73 $\pm$ 9.44	5.31 $\pm$ 0.03	87.31 $\pm$ 21.44	349 $\pm$ 0.86	1295.53 $\pm$ 335.91	51.82 $\pm$ 15.52
CV	6.45	6.45	0.6	0.6	24.56	24.56	25.93	29.94

Dynamics of cyanotoxins in water and fishes

Cyanotoxins in water: All the samples in both MB and NG mesocosms were negative for Anatoxins (ATX), Saxitoxins (STX), Cylindrospermopsins (CYN) but positive for microcystins (MCs): MCs were found in 100% of the samples in MB mesocosm treatments (n=54) and in 72.5% of the samples from NG mesocosms (n=37). The main MC congeners in the water constituted of MC-RR, MC-YR, MC-LR, with all congeners being detected in MB mesocosms while MC-RR the most frequently occurring congeners in NG mesocosms (**Figure 5.8A**). Generally, the total MC concentration were significantly higher among the treatments in MB mesocosms than those from NG mesocosms (Wilcoxon rank $W = 2646$, $p < 0.0001$; **Figure 5.8B**). In MB, the mean total MC concentration in water ranged between 4.09 ± 1.08 $\mu\text{g/L}$ in control to 8.26 ± 3.78 $\mu\text{g/L}$ in the N+P treatment. Furthermore, significant differences in the mean MC concentrations were observed amongst the treatments (Kruskal-Wallis $\chi^2 = 20.943$, $p = 0.003856$) depicted by the general increase in the nutrient (N, N+P) and fish treatments. However, no significant difference was found due to duration of the experiment (day) ($F=0.279$, $p=0.840$) and no interaction effect of treatment and day (time) was observed ($F=0.184$, $p=0.992$).

Cyanotoxins in fish tissue: No MCs were detected in fish tissue (muscle), and liver in all the fishes from NG station. At the MB station only 4 fishes (7% of the fishes) tested positive for MCs in the muscle ($0.004 - 0.3$ $\mu\text{g MC-LR g}^{-1}$ of fresh weight (FW)). At both stations, MCs were detected in the intestine of fish from both the control (mesocosms and lake), and the +fish treatment. The MCs concentration in the intestine of fish from MB mesocosms ranged from 0.03 to 27.5 $\mu\text{g MC-LR g}^{-1}$ of fresh weight (FW) while those from NG mesocosms ranged from 0.02 to 0.4 $\mu\text{g MC-LR g}^{-1}$ FW. These concentrations of MC in the intestines of fish from the MB treatments were significantly higher than those from NG mesocosms (Wilcoxon rank $W = 324$, $p=0.002$; **Figure 5.8C**).

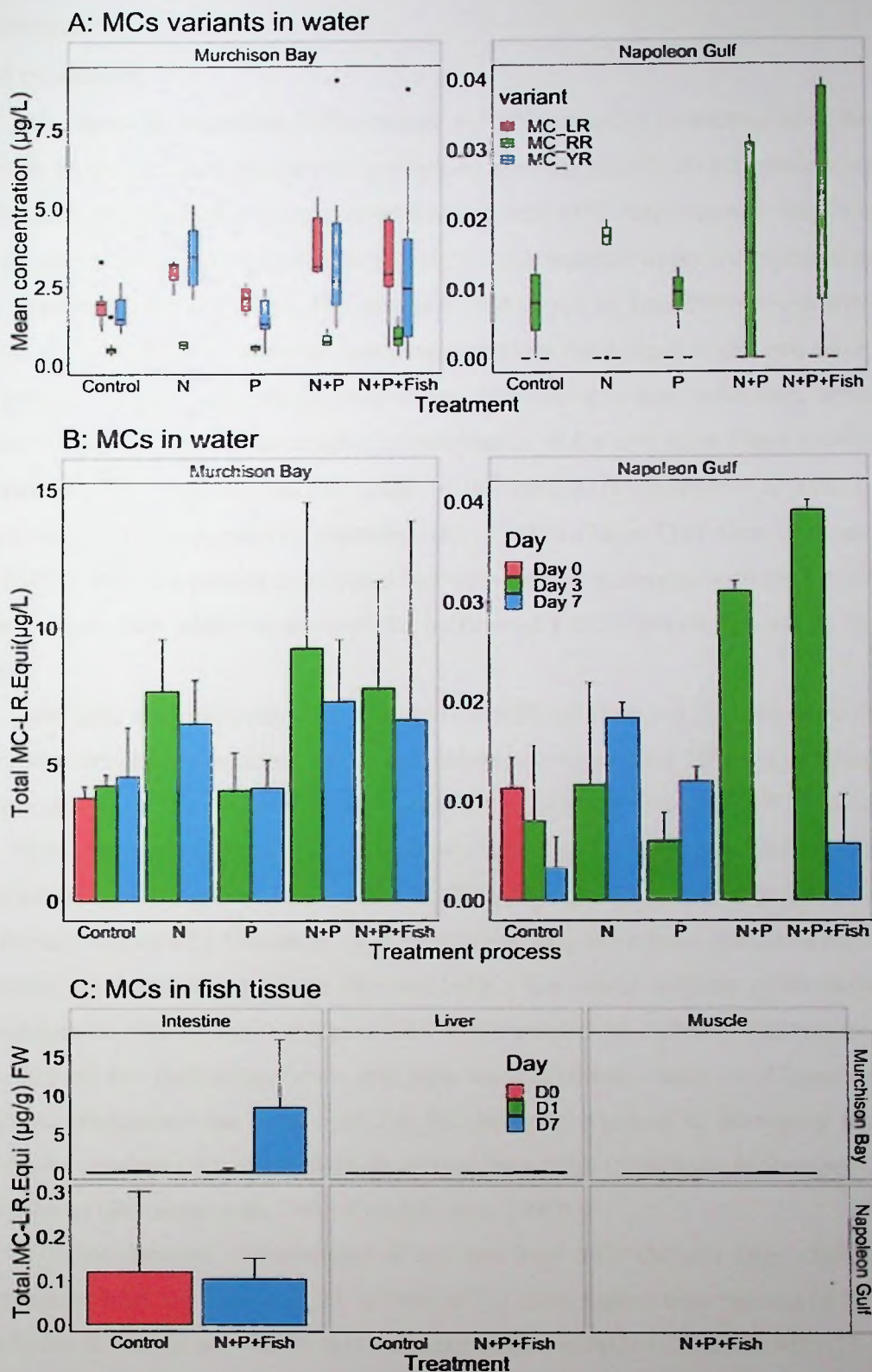


Figure 5. 8: The concentration of the MC variants detected in the different treatments (A); temporal variation in the concentration (mean $\pm$ SD) of MCs in water (B) and different fish tissues (C) under the different treatments at Murchison Bay and Napoleon Gulf, northern Lake Victoria.

Discussion

Initial conditions

As expected, regarding both nutrient and chlorophyll-a concentrations, the two bays where the mesocosm experiment were performed, showed quite different eutrophication status. The values of these variables measured at the beginning of the experiments were in accordance with the recent results of Olokotum et al. (2021) who reported hyper-eutrophic status for MB and a mesotrophic status for NG. The most probable reason for this difference in eutrophication are first the difference between the wastewater volume discharged in the two bays which are quite higher in MB than in NG given the large differences in their catchment areas (256 and 188 km², respectively) and the number of inhabitants of the two main cities located near MB (Kampala, 5 million people) and NG (Jinja, 90,000 people) (Olokotum et al. 2021). Secondly, the hydrodynamic conditions and characteristics of the two bays. Therefore, following Mbonde et al. (2015), MB is regarded as a closed bay with limited exchange with the open lake while NG as an open bay which location at the outflow of Lake Victoria allowed a higher water renewal.

Not only the chlorophyll-a concentration differed between the two bays but also the composition of phytoplanktonic and zooplanktonic communities. While cyanobacteria were the dominant group in the two bays, the dominant taxa were different. In MB the dominant taxa were *Microcystis aeruginosa* and in NG two other taxa dominated the phytoplanktonic communities: *Dolichospermum circinale* or *Planktolyngbya circumcreta*. These differences were already reported by Olokotum et al. (2021) and they have been related to the differences in nutrient concentrations between the two bays. The lower nutrient concentration in NG selected species able to cope with potential N limitation with different strategies: heterocyst differentiation for *Dolichospermum* and high surface/volume ratio for *Planktolyngbya*. *M. aeruginosa* dominance has been linked to its ability to regulate its buoyancy through their intracellular aerotopes which allow them to have the access to the light in the upper part of the water column (Bormans et al. 1999; Brookes et al. 1999).

The zooplankton communities of the two bays also showed large differences. MB zooplanktonic total abundance (2.23×10^5 Ind.m⁻²) is quite higher than the one of NG (2.25×10^4 Ind.m⁻²), but it was the reverse for taxa richness: higher in NG (11) than in MB (7). In MB and NG dominant species were from the Copepoda group which agrees with other authors who reported the dominance of cyclopoid copepods (Kiggundu et al., 2012; Waya et al., 2014). This

is attributed to the versatile feeding habits and ability to avoid predation in turbid and eutrophic conditions (Zhang et al. 2023).

Dynamics of planktonic communities in the control mesocosms

From the control mesocosm under such short-term experimental durations (7 days), it is expected that the enclosure of planktonic communities in mesocosms do not produce large changes in these communities. Therefore, as observed in the chlorophyll-a concentration in NG control mesocosms, there was no significant changes during the 7 days of the experiment. In the same way, the dominant taxa that were *Dolichospermum circinale* or *Planktolyngbya circumcreta* remained the same from the beginning to the end of experiments. Similarly, the zooplankton community remained dominated by young forms of Nauplius and copepodites. This can be explained by the fact that zooplankton community and abundances are controlled by phytoplankton community and abundance/biovolume.

In MB control mesocosms, chlorophyll-a concentrations were unstable compared to that in NG. An unexpected dynamic in the control mesocosm occurred, like the other ones observed in other mesocosms with different treatments. Our short-term experimentation in MB showed a decreasing trend in chlorophyll-a concentrations in all mesocosms with strong simultaneous covariation in all mesocosms. *Microcystis* sp., mainly *M. aeruginosa*, remained the dominant species from the beginning to the end of the experimentation. Such trends can be explained by an unbalance and mismatch between bottom-up and top-down factors.

The decreasing trend in both the control and treatment mesocosm (*i.e.*, being similar) may be seen as a common “*mesocosm effect*” which do not allow the high initial phytoplanktonic biomass ($296.22 \pm 59.47 \mu\text{g. Chla. L}^{-1}$) to be maintained during the experimentation. Due to such high biomass, the phytoplanktonic community is known to experience a low light availability as it reported for most bays of Lake Victoria (Loiselle et al., 2008) and particularly Murchison Bay (Haande et al., 2011). Additionally, the plastic walls of the mesocosm bags may also have reduced the quantity of light available inside the mesocosms. However, in NG, the chlorophyll-a concentrations recorded in the same mesocosms do not show a decreasing trend. Therefore, most likely bottom-up limiting factor for the observed decreasing trend in chlorophyll-a concentration in both the control and all the MB mesocosms appeared to be self-light limitation (see next section). On the other hand, top-down factors such as grazing by zooplankton, may potentially contribute to these decreasing trends. However, during the experiment, the abundance of micro- and mesozooplanktonic species known to be able to graze the dominant taxa (*Microcystis* sp.) (Davis and Gobler, 2011) did

not increase. As reported by Kâ et al. (2012) *M. aeruginosa*, especially in its colonial form, are not easily edible by zooplankton such as cladocerans and rotifers.

Responses of phytoplankton and zooplankton communities to nutrient enrichment

The planktonic communities of the two embayments exhibited different responses to the nutrient addition. As reported before, nutrient addition in MB mesocosms did not induce a different dynamic of chlorophyll-a concentration than the one observed in the control mesocosms, nor a change in the dominant phytoplanktonic taxa (*M. aeruginosa*). This result demonstrates and confirms that the phytoplanktonic community in MB was not limited by the inorganic nitrogen or phosphorus concentrations, but rather by light availability through self-shading process.

The high abundances of initial dominant zooplankton taxa (*i.e.*, Copepoda Nauplius-larvae and copepodites), were dramatically reduced after 7 days of experiment. Such a quick decrease could be explained by a short maturation time of the individuals of this stage. However, no adult stages of copepods were detected at the end of the experiment. In fact, development time from nauplii to adult stage in tropical/subtropical lakes compiled by Melao and Rocha (2004) are quite higher than the duration of our experiments. It may also be attributed to relatively large size of *M. aeruginosa* colonies (dominant phytoplankton) limited or impaired their grazing by the zooplankton (Lüring, 2021).

In addition, one of the constraints that mesocosms generate on the water column is that they suppress the vertical and horizontal hydrodynamical exchanges. Such exchanges may have significant effects on both phyto- and zooplankton community dynamics. Especially, in hyper-eutrophic ecosystems with very high amount of chlorophyll-a, vertical transport and turbulences are the most efficient way to allow phytoplanktonic cells to adapt to low light conditions that enter in the surface layer of the water column. In the absence of mixing, it may be that a large part of *M. aeruginosa* cells cannot access to enough light, impairing their growth and causing their sedimentation, which imply their decrease in the water column of the MB mesocosms. Moreover, the lack of turbulences may reduce the encounter rate of planktonic organisms, which may reduce the zooplankton grazing efficiency (Alcaraz et al. 2007).

In NG mesocosms, only the simultaneous addition of N and P resulted into a large increase in chlorophyll-a concentration indicating a co-limitation of phytoplankton by these two nutrients. Such co-limitation appears quite common in freshwater, marine and terrestrial systems as reported by Harpole et al. (2011). Co-limitation was also reported for large tropical lakes such as Lake Tanganyika (Järvinen et al. 1999; North et al. 2008), Lake Kiwu (Sarmiento

et al. 2009), Lake Malawi (North et al. 2008). In Lake Victoria, North et al. (2008) reported an N+P colimitation only for offshore areas with relatively low chlorophyll-a concentrations (10 µg/L) which are in the same range at the beginning of the experiment in NG.

The initial dominant cyanobacteria taxa (*D. circinale* and/or *P. circumcreta*) were also dominant at the end of the nutrient addition treatment. Thus, these taxa appeared to be able to quickly respond to nutrient addition. Reynolds et al. (2002) reported that *Dolichospermum* is diazotrophic, tolerant to low nitrogen conditions but sensitive to mixing and low light, using gas vesicles to regulate its buoyancy to optimize nutrient concentrations and light conditions for growth. This cyanobacterium can produce akinetes, that allow its survival when adverse environmental conditions occurred (e.g., Li et al. 2016). It is considered as a potentially toxigenic cyanobacterium (Olokotum et al. 2022) while the filamentous *P. circumcreta* that co-dominated the phytoplanktonic communities both in the initial conditions and after nutrient enrichment is considered as non-toxigenic taxa. After the growth phase, a significant decrease in phytoplankton biomass occurred, which can be attributed to either a decrease in available nutrients or a response of the zooplankton community able to graze the dominant phytoplanktonic species, or a combination of these two factors.

While Copepods stages, especially copepodites, showed a limited increase, it was rotifers (*Synchaeta* sp. and *Brachionus calyciflorus*) that exhibited the largest increase in abundances from the beginning to the end of the experiment. It seems that the growth of phytoplankton induced two different responses of the zooplankton community following their size-class. The limited increase in largest zooplanktonic taxa may be related to their lower growth rate than the one of rotifers (Allan, 1976). The ability of *Brachionus* species to feed on a wide range of filamentous cyanobacteria, including *Planktolynghya* and *Dolichospermum* species, as well as colonies of *Microcystis* and *Dolichospermum* (formerly *Anabaena*) has been extensively documented (see in the review of Gilbert (2022)). However, not so much information on the grazing ability of *Synchaeta* species on large cyanobacteria is available, much as it seemed to feed on large phytoplanktonic cells during our experiment. Thus, the decrease in phytoplanktonic biomass after the Day 5, may be caused by the rotifers that proliferated in the N+P mesocosms.

Responses of phyto and zooplankton communities to fish addition

The addition of fish was performed three days after the beginning of the experiment in the N+P treatments. Therefore, we expected an increase in the phytoplankton biomass to be able to observe the potential controlling effect of fishes on cyanobacteria dynamics. As

previously reported in MB mesocosms and the N+P treatments, no increase in phytoplankton biomass was observed. Similarly, the addition of fish (Nile tilapia, *Oreochromis niloticus*) did not cause changes in chlorophyll-a concentrations when compared to the other treatments or to the control. Even if the N+P treatment do not imply an increase in the phytoplanktonic biomass, one can expect that, compared to other mesocosm treatments, adding planktivorous fishes may contribute to a decrease in the large Chla biomass. An explanation may be that no significant grazing of fishes occurred on the dominant phytoplanktonic taxa (*M. aeruginosa*). This could be attributed to no significant grazing of fish occurred on the dominant phytoplanktonic taxa (*M. aeruginosa*). This is supported by studies by Beveridge et al. (1993) who showed that *O. niloticus* grazing on toxic *M. aeruginosa* strain was significantly depressed compared to grazing rate on non-toxic strains.

In NG mesocosms, the addition of N+P caused a large increase of phytoplankton biomass while addition of fishes did not cause the expected decrease in phytoplankton biomass through grazing but rather the opposite (*i.e.*, a supplementary increase in chlorophyll-a concentration). The effects of tilapias on phytoplankton biomass have been largely documented with two main opposite results: i) an effective control of the phytoplankton biomass including cyanobacteria (e.g., Moriarty and Moriarty 1973; Lu et al., 2006; Torres et al., 2016; Vasconcelos et al., 2018, Mayer 2020); ii) and/or an increase in phytoplankton biomass through nutrient release and/or zooplankton grazing by fishes (e.g., Attayde and Hansson 1999; Dantas et al. 2019; Zhang et al. 2022).

Ahoutou et al. (2022) using a similar experimental design in Aghien lagoon showed that the addition of Nile tilapia to the N+P mesocosms resulted in a drastic reduction in the phytoplankton biomass. In Mwanza Gulf (Lake Victoria), Witte et al. (2012) estimated that losses of phytoplankton by fish grazing only represented near 10 % of the daily net phytoplankton production and thus that fishes were unable to control phytoplanktonic blooms in this gulf. One possible explanation of the differences observed on the effects of fish addition in MB and NG mesocosms, may be dependent of the relative abundances of fish and cyanobacteria. We already underlined that a large abundance of *M. aeruginosa*, such the one observed in MB, including toxic clones, may depress the grazing rate of *M. aeruginosa* by *O. niloticus*. Furthermore, large biovolume of *M. aeruginosa* might clog the fish gill (Jewel et al., 2003) consequently inducing death due to suffocation associated with low oxygen concentration.

Dynamics of cyanotoxins in water and fishes

The results of cyanotoxins analyses on water samples from control mesocosms showed a very large difference between the initial concentrations of MCs of the two bays, with significantly higher concentrations in MB than in NG, which can be related to the difference in abundances of potentially toxin-producer taxa in the two bays. (Olokotum et al., 2021). Addition of nutrients in MB did not significantly modify the MCs concentration during the experiment. Observable increases occurred in NG for the N+P and N+P+Fish treatments, but the maximum MC concentration reached in NG mesocosms were far below the ones observed in MB mesocosms. These results agree with both the standing stock of cyanobacteria and their dynamics in the mesocosms of the two bays.

Intestines of fishes incubated during four days in the N+P+Fish mesocosms contained MCs concentrations which also can be related to the concentration of potentially toxin-producer cyanobacteria. However, no MCs have been detected in the muscle and the liver of the fishes, except 7% of the MB fish that had MCs in their muscle. Based on these findings, it was possible to reject the hypothesis of a “toxigenic” effect of MCs on fish and their grazing behavior, at least during this short-term experiment. However, it may be that increasing the exposure time in the mesocosm may produce adverse toxigenic effects on fish. Although the sanitary consequences of tilapia grazing on cyanobacteria was not the focus of this study, this question must be considered in future management of cyanobacterial blooms in the Lake Victoria.

Lessons from this study for management of cyanobacterial blooms in Lake Victoria

Our results highlight several interconnected consequences of man-induced actions against cyanobacterial blooms. First, the differences both in the ecological status and the responses of planktonic communities in the two bays support the idea of site-specific assessments and decisions that is currently supported by international organizations. It is certainly advisable to limit sewage inputs into the lake, but even if implemented, it will take a long time to observe their effects. This is not to imply that it is not vital to start reducing these inputs; if we do not take meaningful action against this primary cause of eutrophication, all other measures will be ineffective. MB status is a good example of the consequences of cumulative sewage inputs, with a permanent bloom of potentially toxigenic cyanobacteria which is no longer sensitive to nutrient addition since they are readily available in large amounts. Such embayments become permanent source of potentially toxic cyanobacteria that can colonize other areas. On the other hand, NG serves as an example of an ecological status

where a decrease in nutrient inputs may first allow the maintenance of its mesotrophic status and then possibly an improvement. We have also shown that in this gulf increasing Nile tilapia stocks may have the opposite effect of the expected one, i.e., increasing instead of decreasing phytoplankton biomass. Thus, applying systematic strategy based on increasing top-down control, by introducing or eliminating fishes such as tilapias at the lake scale may not be the most effective way to reduce the eutrophication and the associated cyanobacterial blooms. Decreasing fish stocks also may not be an acceptable solution from a social and economic point of view. At the opposite increasing fish stocks may be attractive for fishermen and riverine populations. Growing aquaculture in the bays in its present form (i.e. monoculture) may be at risk for several reasons: (i) large density of fishes produce a large amount of inorganic nutrients and organic matter, (ii) high nutrient concentrations may favor cyanobacterial blooms, (iii) high amount of organic matter sinking on the sediment will increase the dissolved oxygen consumption with the creation of anoxic areas, (iv) reared fishes may accumulate toxins produced by toxic cyanobacteria.

Therefore, Nature Based Solutions (NBS) need to be identified and proposed to help to improve both the ecological and sanitary status of bays of the Lake Victoria. They must be tailored to the current situations unique in each embayment with a strong participative strategy. Above all, none of the adapted solutions will have anticipated and favorable benefits unless significant efforts are made to reduce nutrient inputs into the lake. Such approaches include multitrophic aquaculture process to replace the today monoculture paradigm (Outa 2021) or the sustainable use/restoration of wetlands around the lakes (Maithya et al. 2022) or new ecosystem-based fisheries strategy (Kolding et al. 2008).

Conclusions

From the data above, it is evident that additional nutrient loading into (mesotrophic) bays, will result into changes in the richness and diversity, and increase in the biovolume in phytoplankton dominated by toxigenic cyanobacteria, increase in the small sized zooplankton communities especially rotifers. At a site level, increasing nutrient loading in hypertrophic systems will increase the cyanobacteria since there is low richness and diversity. The species of these trophic communities were dependent on *Brachionus* and *Lecane* spp. However, the introduction of the Nile tilapia that tolerated high biovolume of cyanobacteria, influenced the dynamics and shifts the structure of these planktonic communities. The added fish were exposed to high concentrations of MC, to levels might exceed the TDI for human consumption.

Acknowledgement

We are grateful to the BSc and Msc students, partners, and collaborators from Makerere University, and colleagues from National Water and Sewerage Corporation (NWSC), Directorate of Water Resources Management (DWRM) and National Fisheries Resources Research Institute (NaFIRRI) who provided a helping hand during the mesocosms experimentations. Financial support for this study was provided by through the WaSAf project that funded MO, PhD training. There was enough support from colleagues at the lab during toxin extraction and analysis at MNHN, Paris, France.

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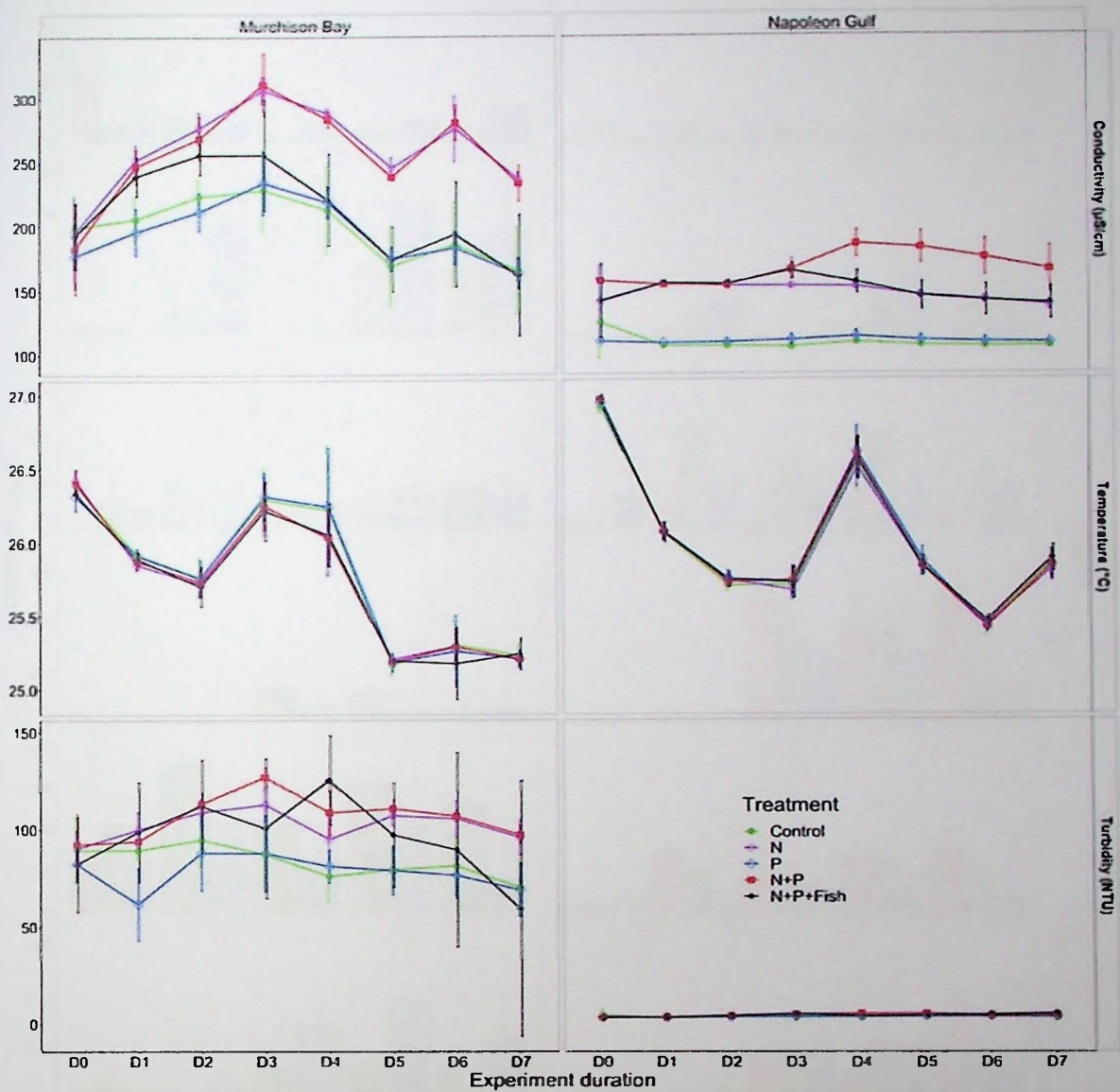
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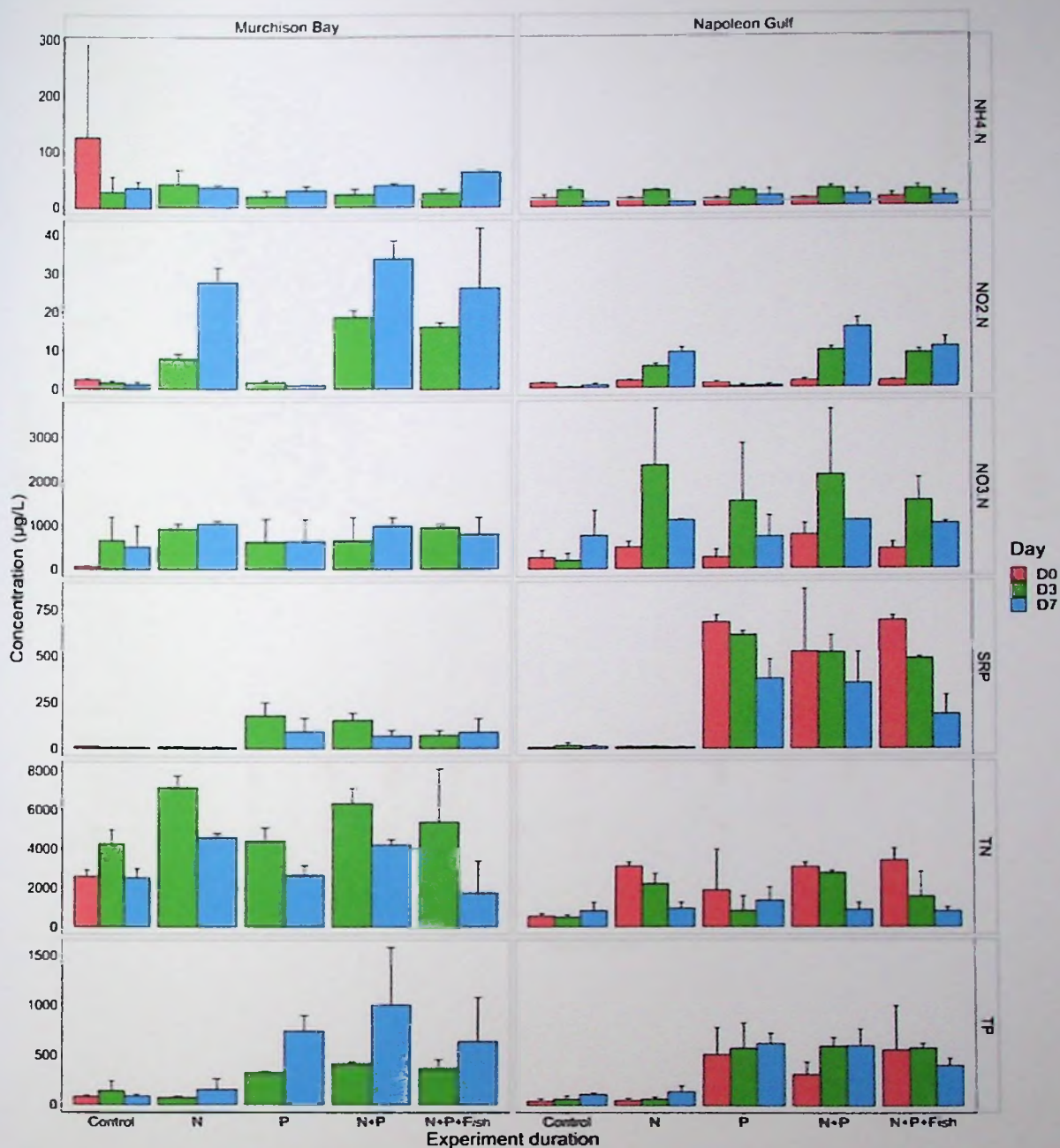
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Supplementary materials

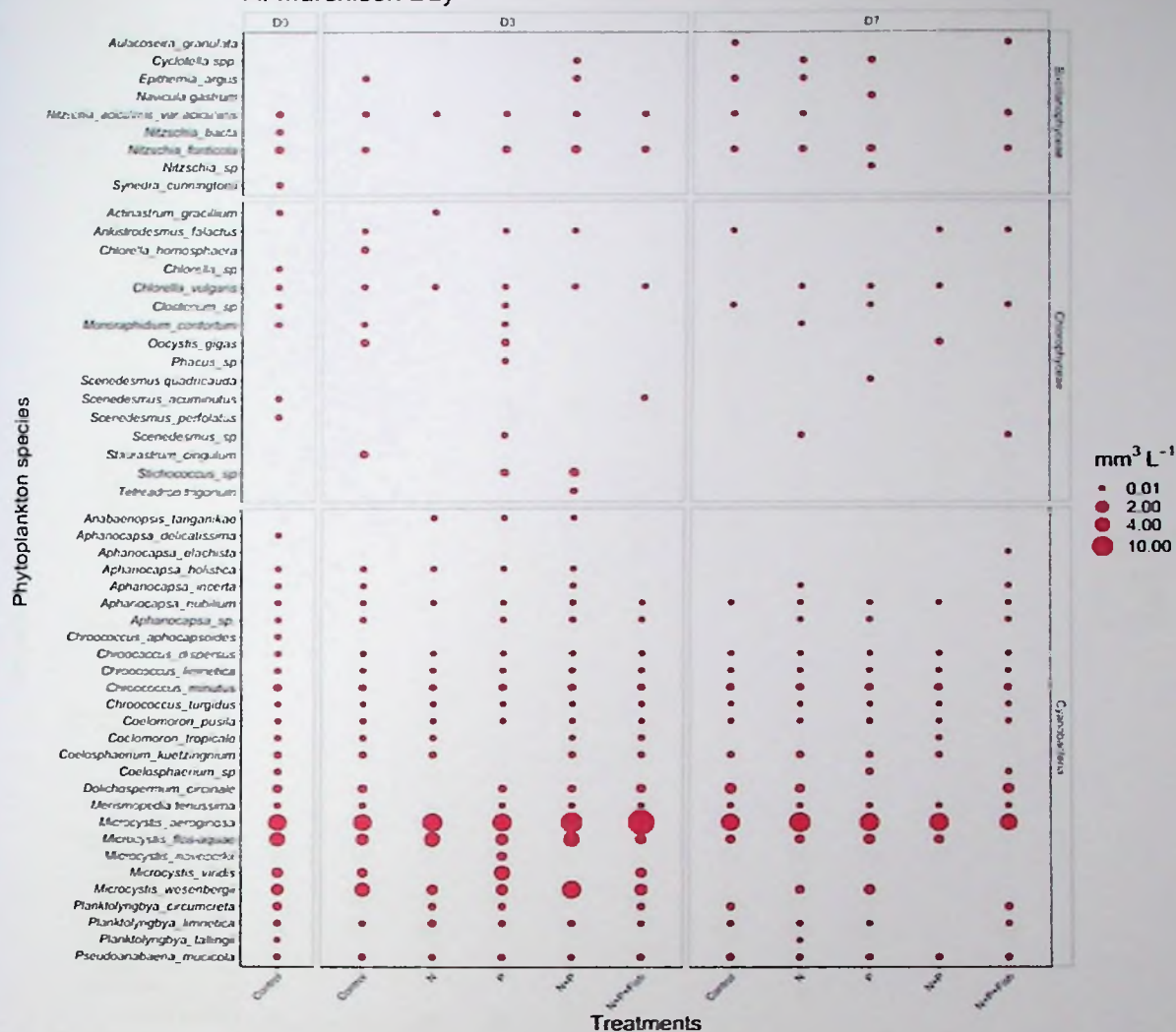


Supp Figure 5. 1: Temporal variation in the physical chemical conditions of the mesocosms (mean $\pm$ SD) under the different experimental treatments at Murchison Bay and Napoleon Gulf stations, Lake Victoria.



Supp Figure 5. 2: Temporal dynamics in the nutrient concentrations (mean±SD) under the different experimental treatments at Murchison Bay and Napoleon Gulf stations, Lake Victoria.

A: Murchison Bay

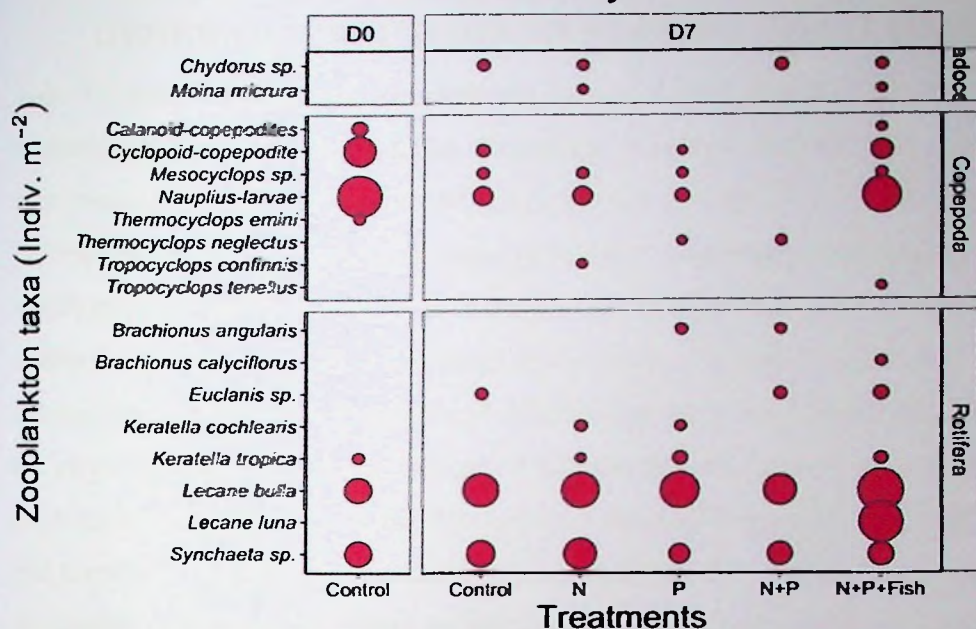


B: Napoleon Gulf

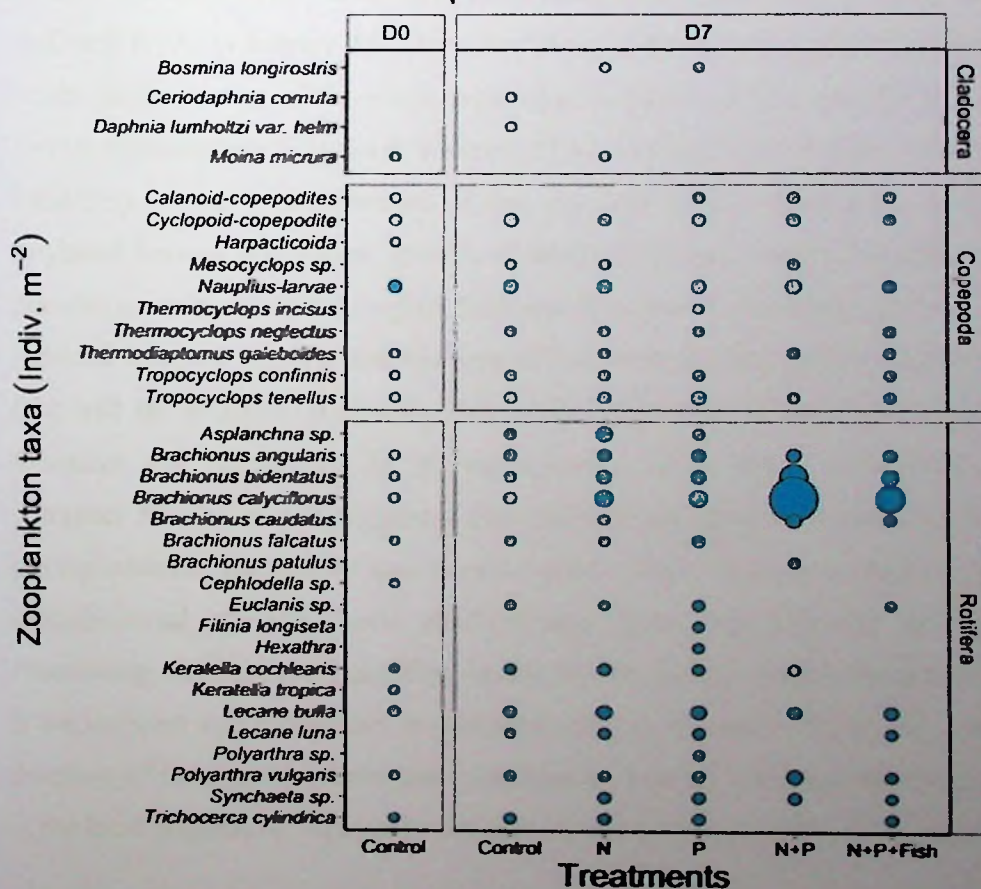


Supp Figure 5. 3: Temporal dynamics in the phytoplankton taxa composition, richness and biovolume under the different treatments at Murchison Bay and Napoleon Gulf stations, Lake Victoria.

A: Murchison Bay



B: Napoleon Gulf



Supp Figure 5. 4: Temporal dynamics in zooplankton taxa composition, richness, and abundance under the different treatments at Murchison Bay and Napoleon Gulf stations, Lake Victoria.

CHAPTER SIX

GENERAL DISCUSSION, SYNTHESIS AND PERSPECTIVES

Lake Victoria is faced with recurrent occurrence of cyanobacterial blooms particularly in the numerous bays and gulfs (B&Gs). Therefore, based on the literature review (**Chapter 2**), numerous studies have been carried out in the last two decades, to understand the causes and consequences of cyanobacterial blooms in the lake. These studies emphasize on; i) the potential health threats associated with cyanotoxins, ii) the need for long term monitoring, iii) assess the sanitary risks and efforts to reduce the exposure to cyanotoxins (see **Chapter 2**). As summarized in **Figure 6.1**, this thesis affirms that the proliferations of cyanobacteria disrupt the physico-chemical and the biological functioning of the lake, particularly where only 25 phytoplankton species out of the 135 species identified influence the phytoplankton dynamics and turnover. Indeed, the two embayments (MB and NG) phytoplankton communities were dominated by potentially toxic cyanobacteria (*Microcystis aeruginosa* and *M. flos-aquae*; chapter four and five) which were associated with detection of two families of cyanotoxins (MC and HTX) (**Chapter 4**). These toxigenic cyanobacteria and cyanotoxins have an impact on the different uses of the water, including the water supply, recreation, and fish production and/or consumption (Chorus & Welker, 2021). This was also premised on the spatial-temporal variability in the concentrations of the cyanotoxin MC. Therefore, in the context of the predicted human population growth of about 2.5% per annum (Bremner et al., 2013) and possible increase in nutrient inputs (Gikuma-Njuru et al., 2005; Njagi et al., 2022), there is the potential increase in pollution, changes in the water quality, and biological functioning of the lake will be at stake. It is why this study explored this potential phytoplankton growth, dynamics and functioning in the embayments of the lake, using experimental approach (**Chapter 5**). The results suggested that nutrient and light availability are the main drivers of phytoplanktonic biomasses and communities' structure, with a shift to potential toxigenic cyanobacterial species. Such modifications have consequences on aquatic ecosystem functioning, structure, and potential health threats. In this chapter the following are discussed: i) the increase and projection in eutrophication in NG and MB, ii) the consequences on the diversity of the cyanobacteria and cyanotoxins, and iii) the potential routes of contamination of the local population especially through treated water and fish consumption.

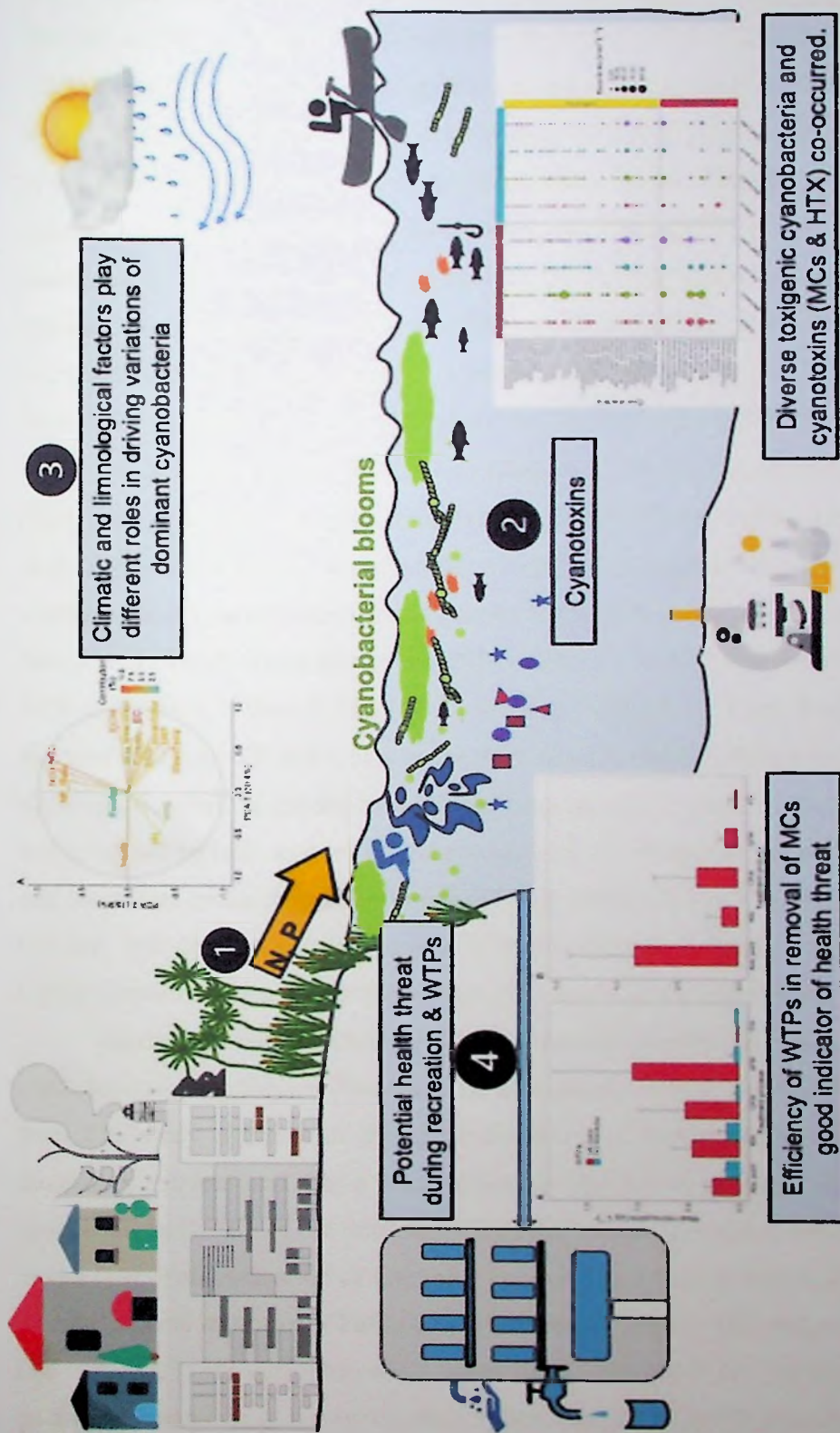


Figure 6. 1: Graphical summary of the results obtained during this study where a gradient is observed in the nutrient loading (1), that resulted into similar gradient in the cyanotoxins (2), and potential treats arising during recreation and drinking water treatment (3).

6.1 Projection in eutrophication in the embayments

Since the 1990s, the population of the Jinja region has increased by 1.5 times, from 289,476 in 1991 to 387,573 in 2002 to 471,242 in 2014 (including 288,500 in Jinja city) (UBOS, 2017b). On the other hand, this has more than doubled in the Kampala Metropolitan Area (KMA) from 755,000 people in 1990 to 2,117,000 in 2011 and to 2,843,000 in 2017, both in the urban and rural parts of the embayments MB and NG (UBOS, 2017a). These have made Lake Victoria more vulnerable to the eutrophication with different sets of metrics used to guide management. The primary set of indicators for eutrophication assessment have been silica, nitrogen, phosphorus, and Chl-a concentrations. In the lake, the phosphorus concentrations increased from 1.1 to 2.9 $\mu\text{moles L}^{-1}$ between the 1960s and 1990s and that eutrophication manifested noticeably towards the end of the 1980s (Hecky 1993; Hecky et al., 2010). In the B&Gs Chl-a L^{-1} are often $>30 \mu\text{g}$ (*i.e.*, Chapter 2, Table 2 as published in Olokotum et al., 2020), with cyanobacteria often comprising more than 80% of the total phytoplankton biomass. In the main lake, Chl-a L^{-1} are generally $<25 \mu\text{g}$, and changes from a dominance of diatoms to a dominance of cyanobacteria are frequently found (Kling et al., 2001; Verschuren et al., 2002; Hecky et al., 2010). From this study (2018 – 2019), Chl-a L^{-1} were much higher in MB (28.1 - 1058 $\mu\text{g Chl-a L}^{-1}$) than in NG (11.8 - 50.36 $\mu\text{g Chl-a L}^{-1}$). Even if the basic eutrophication indicators such as TP and Chl-a in the lake have increased, future assessments of the lake's eutrophication will be guided by additional measures that quantify community changes. These metrics could include trait-based indicators, such as changes in the phytoplankton community and functional group dynamics, have evidently reflected eutrophication in the system. This is because, trait-based indicators reveal different adaptation strategies and productivity of the highly adapted species within the system and vary with intensity of the eutrophication.

Increase in nutrient concentrations, enhances growth of existing plankton community, and consequently produces changes in the community structure (O'Sullivan, 1995). The results from this study agree with this general theory of eutrophication based on the observed increasing nutrient and Chl-a concentrations, and the irreversible dominance exhibited by cyanobacteria ($>70\%$ of the total phytoplankton biomass) (Hasler, 1969). Based on the OECD model (1982) classification of eutrophic state of water bodies which uses both biological and physiochemical indicators (**Table 6.1**). For instance, when Chl-a ranges of 25 - 75 $\mu\text{g L}^{-1}$ or TP (35-100 $\mu\text{g L}^{-1}$) are eutrophic while when Chl-a $>75 \mu\text{g L}^{-1}$, TP $>100 \mu\text{g L}^{-1}$ and secchi depth $\leq 1.5 \text{ m}$, the water body is hypertrophic. Then, based on OECD classification (**Table 6.1**), NG ranges from mesotrophic - eutrophic while MB being eutrophic – hypertrophic systems.

Therefore, since the late 1970s, Lake Victoria has exhibited eutrophic conditions based on the Chl-a concentration and secchi depth values. These conditions saw the dominance of *Nitzschia* spp. diatoms due to bottom- up effect (nutrient dynamics) (Stager et al., 2009), and later in the 1980s, these dominant diatoms were replaced by bloom forming cyanobacteria (Verschuren et al. 2002). These changes were explained by seasonal depletion of the dissolved silica and light limitation (Loiselle et al., 2006), given that some cyanobacterial species can be advantageous (control vertical position in the water column) than diatoms during nutrient limitation.

Table 6. 1: The general classification of the trophic status of the rivers and lakes according to the OECD (1982)

Trophic status	Total Phosphorus (µg/L)	Chlorophyll-a (µg/L)	Secchi depth (m)	Trophic Level Index (TLI)
Ultraoligotrophic	<4	<1	>12	-
Oligotrophic	<10	1-2.5	12 - 6.0	≤30
Mesotrophic	10-35	2.5-8	6 - 3.0	30-60
Eutrophic	35-100	5 - 25.0	3-1.50	60-70
Hypertrophic	>100	>25	<1.5	>90

Earlier studies on eutrophication and nutrient limitation in Lake Victoria showed that phytoplankton growth and dynamics were limited by either P in Nyanza Gulf (*e.g.*, Kenya Njuru 2009) or by N in MB (*e.g.*, Haande et al. 2011). Generally, the shallow shoreline areas are deficient in P while the open lake is deficient in N (Gikuma-Njuru and Hecky 2005). Similarly, experimental approaches performed in the mid 1990s in NG and Bugaya Channel, reported N limitation (Lehman and Branstrator, 1994). Additionally, reports of secondary limitation either by light or iron (Fe) was suggested by Guildford et al. (2003). Nonetheless, the concept of nutrient limitation in Lake Victoria can be subject to debate. For instance, this study primarily describes N limitation in NG and secondary light limitation (shelf shadowing) in MB. Thus, the findings from NG mesocosm suggests that an increase in phytoplankton biomass could be foreseen with increase in N concentrations in the future. On the other hand, in MB that exhibited a slight N+P co-limitations, agrees with Guildford et al. (2003) who indicated that when light increases, the second limiting factor is N.

Eutrophication can be irreversible if accelerated by human activities in the catchment and thus making it appear permanent unless there are significant changes nutrient loading (Walter et al., 1988). This irreversibility has been over time, assessed by the response of the water body to phosphorus reduction (Carpenter et al., 1999). Both studied embayments of Lake Victoria indicates increasing eutrophication. Furthermore, light limitation arising from

dominance, increased biomass and self-shading by cyanobacteria is expected. This scenario is already existent in MB where there is increased Chl-a biomass and high biovolume of toxigenic cyanobacteria (this study). This therefore calls for further assessment and efforts to controls increased eutrophication in the lake. Since the late 1960s, the control of eutrophication has been targeting nutrient particularly P sources (Schindler, 2006; Lewis et al., 2011). To control the increase of eutrophication different approaches have been used. Water managers have tried several expensive, impracticable for larger ecosystems, and occasionally unsuccessful measures, such diverting excess nutrients (Edmondson, 1970), alteration of N:P ratios (Downing et al., 2001) and physical mixing (Huisman et al., 2004) among others. Although, external loading of nutrients might be controlled, internal loading from the sediments (*e.g.*, Søndergaard et al. 2003) and the growing cage aquaculture in the lake (>105 installations with over 12,000 cages) (Musinguzi et al., 2019; Orino et al., 2021) may increase eutrophication and prevent improvement in water quality in the lake.

Other efforts to control eutrophication include ecological measures such as use of macrophytes, establishment of wetland buffers, and use of microorganisms have been recommended to rehabilitate and restore eutrophic systems (Le et al., 2010). In general, microbial aggregates or single species of microorganisms can be used to control eutrophication. (Sun et al. 2018). Nonetheless, this depends on the intended mechanisms, such as quick reduction in algal cell density, prevention of harmful algal growth, and/or lysis of dangerous algae, which call for biofilms, periphytons, and algae-bacteria and -fungus flocculation (Sun et al., 2018). This study using mesocosms experimentation also demonstrated slow grazing potential of Nile tilapia on cyanobacteria. Therefore, the use of Nile tilapia is not a solution for control of eutrophication and cyanobacterial management since did not significantly reduce by fish. The possible consequences associated with extensive aquaculture would also be the discharge of particulate and dissolved nutrients through uneaten feeds. This has already been observed in some of the closed embayments of the Lake in Tanzania (Kashindye et al., 2015) with a need for caution during site suitability studies and concentrations of cages might compromise the water quality. Consequently, other approaches such as bivalve filtration or a combination of phytoplanktivorous fish and bivalves could be attempted given that the use of macrophytes and denitrifying bacterial have shown to be effective within 20 days in China (Li et al., 2011).

6.2 Eutrophication and consequences on the dynamics of dominant cyanobacteria and cyanotoxins

Due to eutrophication and a lesser extent to climatic changes, the causes, and consequences of cyanobacterial dominance over the years in numerous freshwater bodies including Lake Victoria have been independently examined (Chapter 2). The start of the 20th century, there was an increase in the proliferation of water hyacinth (Opande et al., 2004) and increase in frequency and intensity of cyanobacterial blooms in Lake Victoria (Haande et al., 2011). Despite the recorded dominance by cyanobacterial in the lake, the different embayments have responded differently due to existing nutrient limitation. In NG, where N limitation was observed, the cyanobacterial population was observed was dominated by N-fixing cyanobacterium *Dolichospermum* while in MB where secondary limitation (light and Fe) was probable based on the microcosm experimentation, the cyanobacterial population was dominated by *Microcystis*. Furthermore, Reynolds et al. (2002) elaborated that the buoyant and colony forming cells such as *Microcystis* can migrate vertically, thus efficiently on the bottom of the water column) within the water column. Meanwhile, shade-adapted genus as well as ability to synthesize phycoerythrin under certain light conditions (Reynolds, 2002; Kehoe, 2010) such as *Planktolyngbya* form thin filaments to compensate for sinking away from the nutrient-rich water column (Komárek, 2003) and suggests the coexistence with *Dolichospermum* in NG. Future studies could delve into the pigment synthesis of either phycoerythrocyanin or phycoerythrin for adaptations in the face of the predicted eutrophication in Lake Victoria.

The dominance of cyanobacteria: *Dolichospermum* and *Microcystis* have consequences on the potential cyanotoxins concentration particularly MC (Chorus & Welker, 2021). These genera are among the most prominent MC producing cyanobacteria genera in the world (Carmichael, 1994; Chorus & Bartram, 1999). Following the recommendations of the WHO on the provisional guidelines for MC-LR, numerous countries in the north have adopted the 1.0 µg. equi. MC-LR L⁻¹ in lifetime and 12 µg. equi. MC-LR L⁻¹ short term, 24.0 µg. equi. MC-LR L⁻¹ for recreational water, and 0.04 µg equi. MC-LR/kg body fresh weight/day as the Tolerable Daily Intake for chronic exposure fish consumption (WHO, 2020; Chorus & Welker, 2021, Table 6.2) during cyanobacterial studies however, the extent of adoption in the global south is questionable. This is mainly because most countries in the south lack data or harmonized monitoring systems for sanitary risk assessment of toxigenic cyanobacteria and cyanotoxins.

Table 6. 2: Alert levels for cyanobacterial biomass indicators of possible occurrence of cyanotoxins concentrations reaching the guideline values that should trigger management responses. Adapted from Chorus & Welker (2021)

Alert Level	MC/Biovolume (µg/mm ³)	MC/Chl-a ≤1:1(µg/µg) cyanobacteria dominant	(with Basis for estimates of toxin/ biomass
Alert level I in drinking water	0.3 mm ³ /L	1 µg/L	MC lifetime value for drinking water: 1 µg/L
Alert level II in drinking water	4 mm ³ /L	12 µg/L	MC short-term value for drinking water: 12 µg/L
Alert level II in recreation water	8 mm ³ /L	24 µg/L	MC value for recreational water: 24 µg/L

As recommended in the alert level frameworks, monitoring of source waters can start with simple site inspections for any visible blooms and possibly assessing transparency using a Secchi disc (WHO, 2020). However, not all MC producers form surface blooms, as a result, microscopic investigation for the presence of cyanobacteria that can potentially produce MC is crucial if the presence of cyanobacteria is suspected. Monitoring can be enhanced as blooms grow to include quantitative measurements of cyanobacterial biomass, such as cyanobacterial biovolumes or chlorophyll a, that could point to possible toxin concentrations, or direct analyses of MC concentrations. This is because concentrations associated with blooms can vary substantially. In this study for example, changes in cyanobacterial community composition from the toxigenic *Dolichospermum* dominance to non-toxigenic *Planktolyngbya* dominance did not affect MC concentrations in NG-. In addition, focus on cyanotoxins research should attempt to identify and classify other classes such as CYN, Furthermore, one cannot assume that cyanotoxins are produced because cyanobacteria predominate in the lake as a whole; one must instead concentrate on the toxigenic cyanobacterium *Microcystis*.

6.3 Potential threats through water and fish consumption

Humans may be exposed to cyanotoxins orally, inhaled and, or through the skin (dermal routes). The effects of this reported in humans include gastrointestinal illness, hepatic toxicity, and pneumonia. Furthermore, where blooms were treated with copper sulfate, high concentrations of copper may explain the highly reported cases of diarrhoea, vomiting, stomach cramps and nausea (WHO, 2020). The ingestion may happen through drinking water (treated and untreated water), or accidentally drinking water during recreation and fish consumption (Chorus & Welker, 2021). This study identified and quantified the possible cyanotoxins produced by the bloom forming cyanobacteria, and main the exposure areas such as WTPs, inshore areas and recreational areas and fish. It is important to note that until 2020 (McHua et

al., 2020), there was no available data on other cyanotoxins from Lake Victoria (ATX, STX, or CYN) that could be potentially present in the lake.

Potential threats through exposition to raw water: The embayments are increasingly vulnerable to eutrophication with dominance of cyanobacteria, that have disrupted of water uses such as drinking water supply (cost, quality, and quantity), disruptions of recreation, and exposure to high concentrations of the MC. This study presents the first report of HTX and the first report on the concentrations of MC in the environment (lake water) and during water treatment for two contrasting embayments of Lake Victoria. The WHO's threshold levels of MC for safe water usage were repeatedly exceeded during this study in MB rather than in NG. Therefore, it is likely that human population living in the area surrounding the MB are significantly exposed to MC. Direct Lake users, livestock, and wildlife, and for recreationist during the bloom periods are likely to be at a higher risk of exposure (see **chapter 4** and **Table 6.2**). Additionally, eating fish during these bloom periods might greatly increase the amount of MC that humans are exposed to. Based on the findings, research scientists, water managers, and recreationist (citizen science approach) are required to undertake urgent and long-term monitoring of cyanobacteria and cyanotoxins in the embayments of the lake. Therefore, the consumption of contaminated raw water that is infested by toxigenic cyanobacteria can give rise to some outbreaks of illness e.g., gastroenteritis, abdominal pain, flu-like symptoms, irritation, and rashes, to kidney and liver damage (Funari & Testai, 2008; US EPA, 2015).

Potential threats through exposition to treated water: In East Africa there is no available data on the MC concertation in treated drinking water although there is numerous data on the concentrations of MC in the lake (Olokotum et al., 2020). It reduces the ambiguity of using the WHO guideline for drinking water especially when conclusions from literature are being made based on untreated lake water (environmental samples). This lack of data could be attributed to limited interest, and possibly the assumption that toxins are removed by WTPs. It should be noted that WTPs all-over the world draw water from water bodies where cyanobacteria dominate, and the embayments of Lake Victoria are no exception. In addition to the obvious threats associated to cyanotoxins which are directly linked to bloom forming cyanobacteria, there is likelihood that organic carbon (raw water) will be produced during water treatment (Sanchez et al., 2013). It is also imperative to know that large amounts of total organic matter affect disinfection processes, generates large amounts of sludge, and support the development of pathogenic bacteria (Kitis, 2004). Consequently, managers of WTP must consider several

characteristics of cyanobacteria during water production: the ability to float, and produce cyanotoxins, unpleasant odours and tastes (“off-flavours”) and soluble Extracellular Polymeric Substances (EPS) (Chorus & Bartram, 1999; Chorus & Welker, 2021). Most of these molecules are intracellular, that are released mainly at the death of the cells (Sakai et al., 2009). In addition, the spatial distribution of cyanobacteria during their blooms is very heterogeneous, both at the horizontal and vertical (water column) scales. Moreover, this spatial distribution can change very quickly under the influence of wind and/or currents. Therefore, areas of focus for WTPs during blooms events are location and technology for the water intake (water abstraction points), pre-oxidation treatment (e.g., pre-chlorination) and the efficiency of filtration step (e.g., slow vs. rapid filtration system), and the final removal of dissolved cyanotoxins.

Conventional water treatment methods (flocculation, coagulation, filtration, and chlorination) can typically remove low quantities of MC and intact cyanobacterial cells (*Microcystis* sp. and *Dolichospermum* sp.) from source waters provided that the cells are not damaged (Chorus & Bartram, 1999). However, to effectively remove cyanobacterial cells and MC from the treated water, modifications to the WTP and treatment methods are needed. Indeed, rapid sand filtration can be considered effective in removing intracellular MC through the removal of cyanobacterial cells. Data from this study showed that intracellular MC were detected in larger proportions in the raw water samples from MB-Gaba III than NG-Walukuba but were not detected anymore in final water. Nonetheless, the treatment process of rapid sand filtration may be more susceptible to cell lysis (Chorus & Bartram, 1999; Chorus & Welker, 2021). Therefore, conventional WTPs might face a challenge arising from dissolved MC. In this study, an increase of dissolved MC during rapid sand filtration was observed probably due to filter clogging that require frequent backwashing. Possibly a higher cyanobacteria biomass in the abstracted raw water causes a higher dissolved MC concentration. This is in agreement with studies in Finland where Tarczyska et al. (2001) found that on average $0.8 \mu\text{g.L}^{-1}$ of MC were detected in treated water. This further suggests that conventional methods are ineffective in removing dissolved MC from treated water. Furthermore, in case of recycling filter wash water (used during back-washing), extra caution must be considered to ensure no significant amounts of toxigenic cyanobacteria and/or dissolved cyanotoxins are present before reuse (back-washing). Otherwise, special, and additional treatment must be applied before continuing to recycle or use the same water for safety reasons. Since an increase in *Microcystis* in raw water cannot be excluded in the future, regular monitoring of dissolved MC during the water treatment process is recommended.

For effective removal of cyanotoxins, several approaches have been installed including nanofiltration and reverse osmosis that can remove between 90% to complete MC and ATX in treated water (Newcombe et al., 2017; Munoz et al., 2019; Munoz et al., 2021). Nonetheless, these approaches are expensive, thus developing countries such as Uganda could adopt approved approach of activated carbon removal particularly charcoal rather than hard coal (Cook & Newcombe, 2002; Campinas & Rosa, 2010). There are few studies that have tested the efficiency of coal *vs.* charcoal in the south (e.g., Westrick et al., 2010). This has been outside the scope of this study but could be an area of further research and experimental approaches for MC removal in Uganda WTPs. Other approaches that can be recommended are oxidation using either ozone, permanganate, or chlorine (Newcombe et al., 2017). However, use of chlorine results into formation of by-products that could be potentially dangerous to human health. These by-products include monochloro-microcystins, monochloro-dihydroxy-microcystins, and tri-halo-methanes (Zong et al., 2015). From this study, there is pre-chlorination at MB-Gaba and therefore the chlorine by-products might not be ruled out. Consequently, it is a potential area of further research to assess the undesired odorous and tasteless/funny taste by-productions of chlorination in treated water such as trihalomethanes.

Potential threats through fish consumption: The detection of MC in the intestine and some muscle of fish (**chapter 5**) represents a potential source of MC exposure to consumers. Even though the biomass of toxigenic cyanobacteria had significantly increased, studies for the measurement of cyanotoxins in fish and fish tissue had stopped in the early 2000s. These MC can occur in fish as free or cell bound forms, but the challenge over the years have been mainly the use of ELISA. Semyalo et al., (2010) reported $\leq 5 \mu\text{g MC-LR kg}^{-1}$ in Nile tilapia muscle from the fisherman's catch similarly, Poste et al. (2011) reported up to $\leq 57 \mu\text{g MC-LR kg}^{-1}$ from Nile tilapia muscle. This study under controlled conditions, supports and confirmed that there is transfer of MC and a possible biomagnification of MC in the fish muscle ($\leq 0.3 \mu\text{g MC-LR g}^{-1}$ of fresh weight (FW)) way below the data reported from the lake (Semyalo et al., 2010; Poste et al., 2011). Although only 7% of the fish had MC in the muscle, from a standpoint of chronic exposure to MC, the presence of MC in water throughout the year especially in MB indicates possible persistent exposure to humans. For risk assessment, an adult consuming 200-300 g of fish is $0.04 \mu\text{g MC-LR kg}^{-1} \text{ day}^{-1}$ (equivalent to $8 \text{ ng g muscle}^{-1}$) fish muscle might have exceeded the total daily intake (TDI) for MC-LR for chronic exposure (Chorus & Bartram, 1999). Therefore, locals should desist from daily consumption of Nile tilapia caught from severely affected embayments of Lake Victoria. Of course, the studies from the lake

survey are scanty but locals can still use a precautionary approach towards consumption of whole fish or poorly gutted Nile tilapia. Some research focus on the concentrations of MC in fish tissue of different size classes (bioaccumulation of MC during ontogenic shift) and along the trophic group (for biomagnification as well as bio-dilution e.g., in studies by Kozłowsky-Suzuki et al., 2012). For example, Mohamed et al. (2003) reported up to $102 \mu\text{g kg}^{-1}$ MC-LR in farmed tilapia in Egypt, Magalhães et al. (2003) reported maximum of $103.3 \mu\text{g kg}^{-1}$ MC-LR in Sepetiba bay, Brazil, while Xie et al. (2005) reported as low as $1.81 \mu\text{g MC-LR g}^{-1}$ dry weight in freshwater fish from eutrophic Lake Chaohu, China. These studies reaffirm the widespread concern about MC accumulation in fish. Study by Flores et al. (2018) reported that omnivorous fishes generally had the highest tissue MC concentrations as opposed to the general hypothesis that higher MC concentrations are from carnivorous to omnivorous to planktivorous fishes. Further studies could be undertaken to assess biomagnification and bio-dilution of MC in fish of Lake Victoria because there may be a difference in MC content in the tissues of fish based upon species.

Potential threats through multiple exposure routes: Although MC concentration in the lake provides a good overview of the potential risks associated with toxigenic cyanobacteria and MC. It should be noted that there are reports of MC in fish in the lake (Semyalo et al., 2010; Poste et al., 2011; Simiyu et al., 2018), therefore such phenomena in addition to drinking water constitutes several exposure routes that must be considered in the evaluation of the sanitary risks linked to MC exposition. This is because chronic exposure to MC has been associated with increase in prevalence in liver cancer in China (Ueno et al., 1996) and Serbia (Svirčev et al., 2009). Therefore, humans can be exposed to chronic MC exposure even if they may not be directly exposed to acute quantities of MC. From this perspective, it is important to monitor fish in waterbodies containing toxic cyanobacteria and assess the potential threats of human exposure.

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CHAPTER SEVEN

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The conclusions arising from this study are:

The physical-chemical characteristics of NG and MB were influenced by nutrients (e.g., TN, TP, NO₃-N, NH₄-N) from the point and non-point sources consequently, NG can be classified as mesotrophic – eutrophic while MB is eutrophic – hypertrophic.

Similar phytoplankton richness and diversity exists except that the ecosystem functioning of the two embayments is dependent on 25 species dominated by different cyanobacteria species: *Microcystis* spp. in MB and *Dolichospermum circinale* and *Planktolyngbya circumcreta* in NG. The biomass of these dominant cyanobacteria were influenced by that both climatic variables (e.g., wind, solar radiation) and levels of inorganic dissolved nutrients (e.g., SRP, NO₃-N, and NH₄-N). In addition, the micro conditions influencing the MC concentration were biomass of *Microcystis*, influenced through solar radiation, mean wind speed (N-S direction), and turbidity.

There was detection of two classes of cyanotoxins (MC and HTX) in lake water and only MC in treated water and fish. This detection of two families of toxins adds to the sanitary threats from toxigenic cyanobacteria in Lake Victoria. The seasonal and spatial diversity, and dynamics of MC in lake water used WTPs were established for the first time. Despite the removal of MC in treated water by the WTPs (MB-Gaba), 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 daily consumption of untreated water by locals and people engaging in recreation requires an early warning system especially during cyanobacterial bloom events. From the decision support tree (DST), *Microcystis* biomass or cells abundance estimation can be used as early warning method.

The experimental approaches revealed that continuous eutrophication will enhance phytoplankton biomass and specifically increased the growth of *Microcystis* spp. However, the introduction of the Nile tilapia that tolerated high biovolume of cyanobacteria, influenced the dynamics and shifts in the structure of these phytoplankton and zooplankton communities. Consequently, the fish exposed to MC, bioaccumulated the MC, that might exceed the TDI (0.04 µg equiv. MC-LR kg⁻¹ day⁻¹) for an adult consuming 200-300g of fish.

7.2 Recommendations

The overall recommendations arising from this study are:

Due to the demographic growth, there will be need to reduce nutrient loading proper wastewater management and disposal within the catchment, and wetland restoration, although this stretches beyond the scope of this study.

Sensitization of people during bloom events and biomass are recommended to prevent health threats. Additionally, warning signs, closure, should be undertaken during bloom events especially at recreational sites. The local population should desist from using lake water (raw water) for domestic purpose (cooking, drinking) but rather use treated water, or filter excess cyanobacterial cells and reduce recreational activities during bloom events.

For the WTPs, integration of *Microcystis* biovolume estimation is highly recommended in MB-Gaba to ensure water safety. Similar approach could be used as an early warning tool for recreational services providers. These can benefit from the developed checklist from this study for easy reporting and comparison of the dominant cyanobacteria and MC concentration. ELISA result should be verified with LC-MS to confirm the variants of MC if detected.

As the case for many developing countries, pre-oxidation by WTPs should be avoided due to the likely cell lysis and release of cyanotoxins into the water, and the formation of disinfection by-products. In the future, high technology, new approaches and designs such as powdered activated carbon adsorption or ultrafiltration should be considered in the removal of toxigenic cyanobacteria and MC. The WTPs should be done with clear knowledge of water quality inflows, and thermal stratification of the water column, and avoid installing intake points in closed bays, where toxigenic cyanobacteria can preferentially develop and/or accumulate.

The presence of Homoanatoxin-a (HTX) an analogue of Anatoxin-a (ATX) at the inshore areas of MB requires further dedicated evaluation. Furthermore, future assessments of the lake's eutrophication should be guided by additional measures that quantify community change by incorporating trait-based indicators, such as changes in the phytoplankton community, functional group dynamics and molecular approaches.

Based on the high biomass of toxigenic cyanobacteria and occurrence of cyanotoxins, there is need for legal frameworks (policies, rules, and regulations) for toxigenic cyanobacteria and cyanotoxins. A dedicated cyanotoxin research working group (water, fish, and recreation experts) should be formed to implement long term monitoring programs of cyanobacteria and cyanotoxins in Lake Victoria.

Curriculum vitae



Mark Olokotum was born on the 7th of October 1987 in Apac district, Northern Uganda. He went through his studies in primary and secondary school in Rwenzori High School, Kasese and St Leos College, Kyegobe – Fort portal. Later he joined Makerere University in 2007 for a 3-year Bachelor of Science in Fisheries and Aquaculture. After the bachelor's degree he was taken up as a Research Assistant with NaFIRRI in 2011 after the successful internship programme. Between 2011 and 2014, Mark studied his first Master of Science in Zoology of Makerere University. The following year, Mark secured a fellowship for a Master of Sciences in Environmental Science (Limnology and Wetland Ecosystem Management) a joint programme offered by the University of Natural Resources and Life Sciences (BOKU), Austria, UNESCO Institute of Water Education of UNESCO-IHE, The Netherlands and Egerton University (Kenya). Mark got an award for a 3-year PhD fellowship from the FFEM under the WaSAf programme tenable at Makerere University. During the PhD life cycle, Mark returned to Uganda from France and was taken up by NARO and appointed a Research Officer – Fish Biologist/Stock Assessment to work with NaFIRRI based in Jinja (Uganda). Mark's 11 years' experience in fish, water quality and wetlands management saw him rise to the position of the Ag, Programme Leader of the Capture Fisheries and Biodiversity Conservation Research Programme of NaFIRRI. At workplace, Mark is keen to understanding the interactions between fish stock dynamics, ecosystem functioning, data quality and management actions. He has led several projects aimed at rescuing freshwater biodiversity data and making it freely accessible by the public. Some of the tools created to date include the online freshwater biodiversity data portal for Uganda (<https://freshwaterbiodiversity.go.ug/>), where he serves as the scientific data manager and editor before publication.