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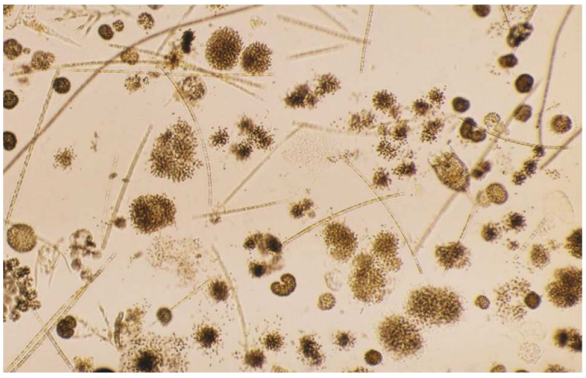
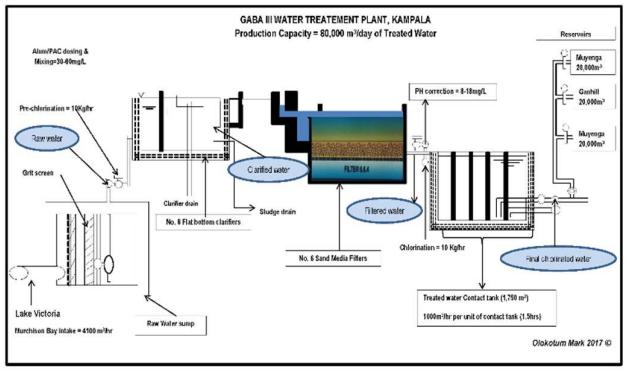
The occurrence and elimination of cyanobacterial toxins in potable water in Uganda

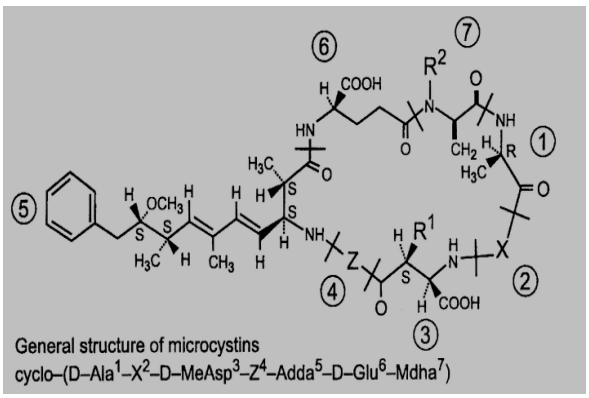
A Case of Gaba III and Walukuba Waterworks

Olokotum Mark

MSc Thesis 17192







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The occurrence and elimination of cyanobacterial toxins in potable water in Uganda

A Case of Gaba III and Walukuba Waterworks

Thesis submitted for the award of the title
"Master of Science"
by

Olokotum Mark

Supervised by

***Prof. Rainer Kurmayer – Research Institute for Limnology of the University of Innsbruck, Austria**

Dr William Okello - National Fisheries Resources Research Institute (NaFIRRI), Jinja

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The cover photos clockwise a micrograph of cyanobacteria viewed under a microscope at 100x magnification, Gaba III waterworks flow diagram, an aerial view of Gaba waterworks with persistent algal bloom and the general structure of microcystins (MCs)

I Olokotum Mark declare that this study is my original work and has not been submitted before for the award of any degree at any university. The findings and their interpretation in this study do neither represent the views of BOKU, Egerton University nor UNESCO-IHE Institute for Water Education.

Dedication

To my beloved family and friends. You have always been part of this wonderful journey in life and I am glad we have always achieved the goals.

Acknowledgements

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List of Abbreviations

a.s.l.:	above sea level
ACN:	Acetonitrile
ADDA:	3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4, 6-dienoic acid
Alum:	Aluminium sulphate [$Al_2(SO4)_3$]
ELISA:	Enzyme Linked Immunosorbent Assay
HABs:	Harmful algal blooms
HPLC:	High-Pressure Liquid Chromatography
MCs:	Microcystins
MS:	Mass Spectrometry
NaFIRRI:	National Fisheries Resources Research Institute
NWSC:	National Water and Sewerage Corporation
PAC:	Poly-Aluminium Chloride
PWSs:	Public Water Systems
SPE:	Solid Phase Extraction
TFA:	Trifluoroacetic acid
v/v:	volume/volume
w/v:	weight/volume
WHO:	World Health Organization
WTP:	Water Treatment Plant

Abstract

Surface bloom-forming cyanobacteria (blue-green algae) such as Microcystis sp. produce hepatotoxic microcystins (MCs) which impair water quality. However, less is known about the occurrence and potential breakthrough of MCs in the course of drinking water treatment in Uganda. Phytoplankton composition and MC concentrations were monitored from November 2016 to January 2017 in Murchison Bay and Napoleon Gulf (Lake Victoria) which serve for Gaba and Walukuba waterworks raw water abstraction. Both waterworks use flocculation, gravity sand filtration and chlorination as major treatment steps. During the treatment process the ratio of chlorophyll a/dry weight decreased significantly from 1% in raw water to 0.1% in the final water implying that algae were significantly removed. Among the cyanobacteria Microcystis, Planktolyngbya, and Aphanocapsa were found dominating in the abstracted raw water at Gaba III (average±SD, 8.2±1.8 mm3/l) and Walukuba waterworks (2.7±1 mm3/l). Microcystis sp. formed >70% or >50% of cyanobacteria biovolume in Gaba and Walukuba raw water, respectively. Overall cyanobacteria biovolume had a linear relationship with cellular-bound total MC (MC-LR equivalents) concentrations (R2 = 0.27, n=20). On average cellular bound MC concentrations were 0.9±0.3 µg/l and 0.1±0.1 µg/l of MC-LR equivalents in abstracted raw water in Gaba and in Walukuba respectively. Analysis with LC-MS revealed six MC structural variants dominated by MC-YR and [MeAsp3]-MC-RY. ELISA detected total dissolved MCs in the raw water and occasionally also in the final treated water from both waterworks. Using both techniques the estimated concentrations were much below the WHO drinking water guideline value (<1 µg/l of MC-LR). The occasional presence of low amounts of dissolved MCs in final water requires early warning tools for MC concentrations possibly exceeding the WHO guideline value during seasonal development with highest cyanobacteria (Microcystis) biovolume.

Keywords; Cyanobacteria; Microcystins; Health risk; Drinking water; water treatment

CHAPTER ONE

1.0 Introduction

1.1 Cyanobacteria and cyanotoxins

Cyanobacteria, also known as blue-green algae, are microscopic unicellular or filamentous bacteria that carry out photosynthesis and are considered the first plants on earth (Altermann et *al.,* 2006). They occur suspended in the water column or attached on sediments (Chorus, 2012). Naturally, cyanobacteria are found as free-living cells or in colonies in surface waters including lakes and rivers (Hoek *et al.*, 1995; Catherine *et al.*, 2013). Despite the importance in photosynthesis and nitrogen fixation in aquatic ecosystems (Hoek *et al.*, 1995), cyanobacteria have caused a number of water quality issues including toxin production. Like any other organism, under favourable conditions of growth resources, cyanobacteria multiply rapidly thereby causing "blooms". The blooms can proliferate due to increase in nutrient discharge from catchments and around major towns and cities e.g. in Jinja and Kampala, Uganda. Population upsurge is also associated with the unethical discharge of domestic and industrial wastes and consequently increased nutrient loads that enhance proliferation of harmful cyanobacterial blooms.

Harmful algal blooms (HABs) in eutrophic aquatic systems such as Lake Victoria have environmental, social, and economic impacts. The effects of HABs range from acute morbidity and mortality of biota to economic impacts across the world (Anderson *et al.*, 2000; Hoagland *et al.*, 2002; Nyenje *et al.*, 2010). Due to public health concerns, raw water containing cyanobacteria requires an appropriate or additional water treatment process to eliminate the toxins in potable water for domestic consumption. The effectiveness of the process to remove cyanobacteria cells or to inactivate the cyanotoxins are still unknown for Public Water Systems (PWSs) whose raw water sources are reportedly dominated by cyanobacteria.

Cyanotoxins are classified into three categories based on the chemical composition, i.e., cyclic peptides (microcystins and nodularins), alkaloids (anatoxin-a, anatoxin-a (S), saxitoxins, aplysiatoxin, cylindrospermopsin, lyngbiatoxin-a) and lipopolysaccharides (Kaebernick & Neilan, 2001). Cyanotoxins are either cell-bound or dissolved in water. The toxins are typically inside the cyanobacterial cells and can be released in high concentrations during cell lysis (Sivonen & Jones, 1999). Microcystins (MCs) are cyclic peptides with seven amino acids. Several variants of MCs are formed because of the different α-amino acid found at position 2 and 4 for

example in MC-LR (L= leucine and R = arginine). The characteristic moiety of the microcystin structure is the unique C_{20} amino acids 3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4, 6-dienoic acid (ADDA) linked to D-glutamate (Carmichael, 2001). Therefore variants are chemically similar compounds differing in variable amino acids which also influences determines the toxicity of the MC (Figure 1)

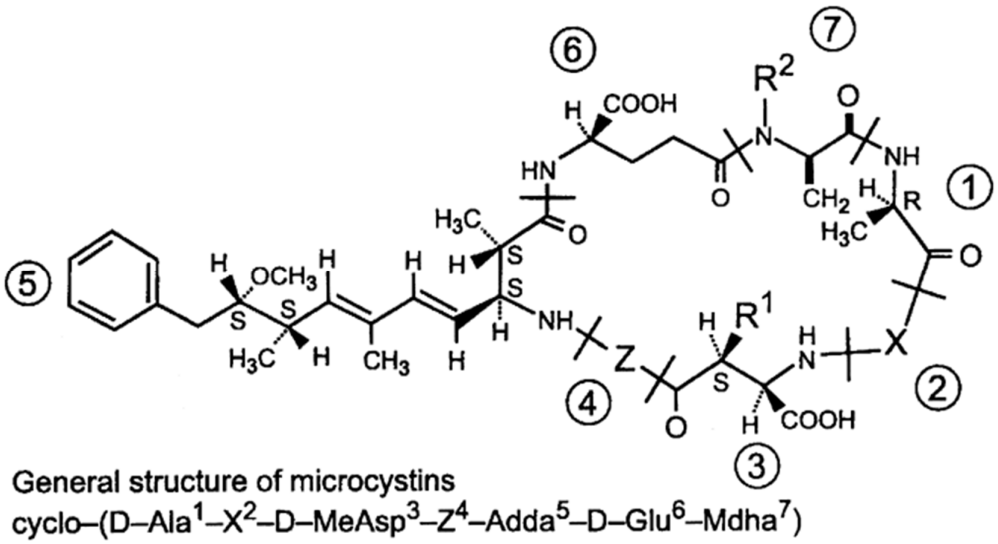

Figure 1: The general structure of microcystins (MC-LR, MC-RR, and MC-YR) and its common variants. X and Z are variable L- amino acids. (In MC-LR, X=L-leucine [L] and Z=L-arginine[R]); R1 and R2 are H (demethyl microcystins) or CH_3. Source: (Carmichael, 2001).

There are over 200 known active microcystin variants with the structural variant MC-LR considered as the most toxic (Spoof & Catherine, 2016). The toxic microcystins are reportedly produced by the planktonic genera *Microcystis, Anabaena, Anabaenopsis* and *Planktothrix* (Sivonen & Jones, 1999). However, by 2015, *Microcystis* was the major bloom causing genus in tropical African countries which produced an array of microcystins (MC–RR, (Asp^3)-MC–RR, MC–YR, (Asp^3)-MC–YR, MC–LR, MC–RY, (Asp^3)-MC–RY) (Okello *et al.*, 2010a; Okello *et al.*, 2010b; Haande *et al.*, 2011; Sitoki *et al.*, 2012; Ho & Michalak, 2015). Toxic secondary metabolites produced by different cyanobacteria species/strains are harmful to several aquatic and terrestrial organisms, including humans (Chorus & Bartram, 1999). With the diversity of cyanobacteria in Lake Victoria, the situation becomes complex because different species produce different toxins and it is not easily predictable which species will bloom and produce toxins. Until recently, cyanotoxins have become an emerging

drinking water contaminant requiring special attention due to the health concerns. Therefore, in 1996, the World Health Organization (WHO) established 1.0 µg/l as a provisional guideline on allowable concentrations of the structural microcystin variant MC-LR in drinking water. Furthermore, recreational WHO guidelines were developed for chlorophyll-a and cyanobacterial abundance but mainly Cyanobacteria *Microcystis* sp. capable of producing MC-LR (Table 1) (Chorus & Bartram, 1999). This calls for measures by existing PWSs to ensure that treatment technologies are able to reduce, eliminate or degrade the cyanotoxins and to protect human health at public recreational facilities.

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

Cyanobacteria (cells/ml)	Microcystin-LR (µg/l)	Chlorophyll-a (µg/l)	Probability of acute health effects
<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

While MC-LR is one of the most abundant MC in the world, it is being replaced by other structural variants such as MC-RR and MC-RY in Uganda's surface waters (Okello *et al.*, 2010a; Okello *et al.*, 2010b). The variant MC-RR is known to be substantially less toxic to humans and vertebrates compared to MC-LR (Zanchett & Oliveira-Filho, 2013) but so far, the levels of the most abundant structural variants MC-RR and MC-RY in drinking water have not been assessed.

1.2 Problem statement

Over the past decades, there has been an increasing nutrient enrichment and greening in northern Lake Victoria (Verschuren *et al.*, 2002). Consequently, there has been dominance and persistence of cyanobacteria associated with microcystin production in Murchison Bay and Napoleon Gulf in Lake Victoria (Haande *et al.*, 2011; Okello & Kurmayer, 2011) (**Figure 2**). These reports of MCs is a threat to PWSs quality for the cities and towns that heavily depend on the lake for domestic water. Amidst the increasing algal blooms in these bays, the National Water and Sewerage Corporation (NWSC) has to supply potable water to thousands of inhabitants. In addition, NWSC heavily relies on conventional treatment technologies that may not effectively remove MCs. In principle, 90% of microcystins are both cell-bound that could enter the

treatment plants after cell lysis while the dissolved microcystins would definitely enter the treatment plants (Chorus & Bartram, 1999). This is a major threat to drinking water quality, as dissolved microcystins cannot be removed by conventional treatment technologies. Furthermore, the cyanobacterial occurrence and MCs in potable water have not been documented since it is an emerging drinking water quality concern. Although 1.0 µg/l is the WHO guideline for allowable concentration of MC-LR in drinking water, Uganda lacks an appropriate system for monitoring the presence of cyanobacteria, analysis and detection of cyanotoxins in drinking water. Therefore, this information is needed to support policy formulations, management of Lake Victoria (water abstraction points) and treating drinking water for safe human consumption.

Figure 2: An aerial photo of Gaba waterworks in Murchison Bay, Kampala with persistent algal blooms. Source: Google Maps January 2016.

1.3 Research question

What is the capacity of conventional water treatment processes in eliminating cyanobacteria toxins in drinking water in Uganda?

1.4 Research hypotheses

Ho_1: There is a significant difference in the cyanobacterial species and MC concentrations in Murchison Bay and Napoleon Gulf and the respective water treatment plants of Gaba and Walukuba.

Ho_2: There is a significant reduction in both cyanobacteria and MCs during the water treatment.

1.5 Objectives

1.5.1 Overall objective

This study assessed the occurrence of cyanobacteria species and MCs in two bays in northern Lake Victoria used as drinking water abstraction points and the potential elimination of toxins from potable drinking water.

1.5.2 Specific objectives

1. Determine the physical-chemical parameters in Murchison bay and Napoleon Gulf and during drinking water treatment process,
2. Determine the cyanobacterial occurrence in two bays of northern Lake Victoria used as abstraction points for drinking water;
3. Determine and quantify the major cyanotoxins (microcystins) found in raw water and along the drinking water treatment processes.

1.6 Justification

MCs are the widest spread cyanotoxins and an emerging contaminant of drinking water that require immediate attention. It is necessary to clarify which cyanobacterial taxa are responsible for MC production and to identify the most severely affected area. Although NWSC is committed to providing water of good quality, they might be challenged with MCs, which are cell-bound or dissolved in water released naturally or during the treatment process.

Persistent cyanobacterial blooms have been reported in both Napoleon Gulf and Murchison bay, northern Lake Victoria. Both Gaba III and Walukuba waterworks operated by NWSC, abstract raw water from the two bays but are relying on the flocculation and rapid gravity sand filtration system. These water treatment processes are susceptible to toxic cyanobacterial blooms. Therefore, there is a need to control the potential release of cyanotoxins in drinking water in the event of contamination of raw water with cyanobacteria.

Without sufficient data, no legislative measures can be adopted to implement the recommended WHO guidelines. A better understanding of the cyanobacteria and cyanotoxin proliferation and its elimination during water treatment is required to advise management. MCs concentrations also vary in time and space as climatic conditions changes and anthropogenic activities increase therefore it will be necessary to develop monitoring and management strategies. This study aims to determine the cyanobacteria, MCs in raw water and potable water to support drinking water quality standards.

CHAPTER TWO

2.0 Literature review

2.1 Occurrence of cyanobacterial toxins in Uganda

Uganda is endowed with rivers, lakes, and wetlands that cover about 18% of its 241,000 km^2 total surface area. Despite Uganda being well-endowed with freshwater resources, some of these water bodies are either naturally eutrophic e.g. Lake George (Viner & Smith, 1973), or are becoming increasingly eutrophic due to human activities such as Lake Victoria (Mugidde, 1993; Kling *et al.*, 2001; Mugidde, 2001; Verschuren *et al.*, 2002). All these authors documented eutrophication processes particularly in Lake Victoria leading to increased biomass of phytoplankton dominated by Cyanobacteria.

Cyanobacteria have existed for over 3.5 billion years and have played a major role in oxygenation of the air (Altermann *et al.,* 2006). In addition, they provide a wide range of contribution to human wellbeing that is both useful and harmful. Some people in Chad and Mexico are known to have consumed *Spirulina* for centuries (Carmichael, 1994) while the nitrogen-fixing species contribute globally to soil and water fertility (Chorus & Bartram, 1999). On the other hand, abundant growth of cyanobacteria in reservoirs and surface water reduces water quality with adverse implications for water supplies, biodiversity, and aquatic productivity. Human health might be at risk if exposed to the harmful cyanotoxins. The major exposure routes for these toxins are both oral and dermal which include consumption of drinking water, certain food supplements and recreation on lakes and rivers. The development of MC-producing cyanobacteria is a common experience in eutrophic water bodies all over the world including Lake Victoria.

In Lake Victoria, there are reports on the increasing importance and dominance of cyanobacteria over green algae, diatoms, and dinoflagellates (Ochumba & Kibaara, 1989; Lung'ayia *et al.*, 2000; Haande *et al.*, 2011; Okello & Kurmayer, 2011; Sitoki *et al.*, 2012). This has been linked to the eutrophication of Lake Victoria and consequently the production of cyanotoxins. In all the Ugandan lakes that showed the dominance of cyanobacteria such as *Microcystis* species (*M. aeruginosa*, *M. botrys*, *M. flos-aquae* and *M. wesenbergii*), occurred with varying concentrations of MCs (Okello & Kurmayer, 2011).

For Lake Victoria, the first report of the occurrence of cyanobacteria and MC production was in Nyanza Gulf (Krienitz *et al.*, 2002). Over the years a number of cyanobacterial studies on Lake Victoria have reported MC occurrence, i.e. in Mwanza

Gulf (Sekadende *et al.*, 2005), Nyanza Gulf (Sitoki *et al.*, 2012), and in Murchison Bay and Napoleon Gulf, northern lake Victoria (Okello *et al.*, 2010a; Okello *et al.*, 2010b; Semyalo *et al.*, 2010; Haande *et al.*, 2011). These studies have indicated varying concentrations of MCs for example in Nyanza Gulf from < 1 µg/l (Krienitz *et al.*, 2002) up to 81 µg/l (Sitoki *et al.*, 2012), 1.8 µg/l in Murchison Bay and < 1 µg/l in Napoleon Gulf (Okello *et al.*, 2010a). Semyalo *et al.* (2010) reported both MCs in water (0.2 - 0.7 µg/l) and in the gut, liver and muscle tissue of Nile tilapia (*Oreochromis niloticus*) from Murchison Bay. Most of these studies focused on the community structure and dominance of cyanobacterial species, genotypic differences and MC production.

Although these studies have clarified cyanobacterial species responsible for the MC production, the levels in drinking water have not been addressed. None of the studies reported above determined MC concentrations in drinking water. This study, therefore, will determine the cyanobacteria, MC composition in raw water and treated drinking water and thus help to ascertain the quality of drinking water provided by NWSC to its customers.

2.2 Drinking water production in Gaba and Walukuba waterworks

In Uganda, drinking water treatment plants and PWSs are operated and managed by the NWSC. In Kampala, NWSC operates Gaba I, II and III waterworks. Gaba Water Treatment Plant (WTP) I was constructed in 1928 with a capacity of 72,000 m^3/day but operates at approx. 50,000 m^3/day. Seven decades later, Gaba WTP II was constructed in 1992 with a design capacity of 80,000 m^3/day is operating at 67,000 m^3/day. In order to meet the increasing demand of about 200,000 m^3/day of water within Kampala Metropolitan Area, Gaba WTP III was constructed with a capacity of 80,000 m^3/day and operating at about 70,000 m^3/day (Kalibbala *et al.*, 2006).

All the WTP in Gaba abstract raw water from inner Murchison bay, Lake Victoria which is already threatened by eutrophication and persistent cyanobacterial blooms. Gaba I and II have intake pipes about 30 m offshore and at 5 m deep into the water, while Gaba III is 1.5 km offshore and 8 m deep to abstract about 400 m^3 of raw water every hour. The intake pipes are screened with 3 inches mesh and smaller to prevent abstraction of course material such as sticks, plastics, e.t.c. The raw water undergoes the conventional drinking water treatment process of chemical coagulation and flocculation/sedimentation and clarification; mechanical rapid sand filtration; and

chemical chlorination before distribution (**Figure 3**). However, Gaba III WTP does pre-treatment with 10kg of gaseous chlorine per hour to aid the subsequent coagulating and settlement of algae.

Coagulation is done for the agglomeration of suspended material into larger particles that can easily be removed. This is achieved with the use of Poly-Aluminium Chloride (PAC) and Alum [$Al_2(SO_4)_3$] at approximately 30-70 mg/l. During this process, the pH is monitored and controlled at pH 6-8. After coagulation, water flows into the flocculation chamber for about 10-30 minutes which helps agglomeration of smaller particles. The water then flows through four flat bottom clarification lamella settling basins used for sedimentation in all the WTPs. After the solids have settled at the bottom, they are removed mechanically by sludge collection device to the sludge drying beds (Nayebare *et al.*, 2014). This is done to avoid blocking the filtration system. The retention time within the clarifier basins is 2 hours and an overflow rate of 2.4 m/hr or 58.4 m/day. Due to the increased consumption and water demand, and the fear of hydraulic loading, the clarifier is sometimes bypassed at peak demands/hours (Kalibbala *et al.*, 2006).

The water from the clarifier basins flows through the rapid gravity sand filtration system. This is a sand filter bed of about 1.25 m depth and surface area of approximately 376 m^2 where the water undergoes rapid sand filtration. Depending on the quality of the raw water the filters run for about 12 – 18 hours. The filter beds are cleaned and maintained regularly by backwashing and scrapping of the clogged upper layers of sand. The backwash lasts about 30 - 40 minutes and sand layers are replaced as and when deemed necessary. Disinfection is done primarily by chlorination whereby the water is treated so that the residual chlorine is 0.5 mg/l during the distribution while pH is maintained between 6.7 -7.2 using calcium carbonate (Ca_2CO_3) (Kalibbala *et al.*, 2006; Nayebare *et al.*, 2014).

In Jinja, the NWSC abstracts water from Napoleon Gulf, northern Lake Victoria to supply the Walukuba WTP. Constructed in 1950 and renovated in 2006, the plant has a design capacity of about 50,000 m^3/day but is currently operating at 60% of the capacity producing approximately 30,100 m^3/day. Walukuba waterworks abstracts about 880 m^3 of raw water per hour but there is no pre-treatment with chlorine as in Gaba III WTP. The raw water then undergoes conventional treatment processes mainly by flocculation using 8-18 mg/l of Alum or PAC depending on availability. The water then goes through both the vertical and horizontal sedimentation chambers for agglomeration of algal particles. The water filtrated through the sand filters, and

disinfected using liquid chlorine. The treated water is allowed to settle in the contact tank for about 1 hour prior to distribution and storage in the Rubaga (24,000 m^3) and Walukuba (4,000 m^3) reservoirs (**Figure 4**).

Walukuba waterworks is a much smaller WTP than Gaba III. Both operating at a capacity to supply drinking water to inhabitants of Jinja and the greater Kampala. Water samples were collected along the production chain: Raw water, Clarified water (flocculation treatment), Filtered water (Sand filtration) and Final Water (after chlorination).

Gaba III and Walukuba WTPs were selected because they abstract raw water from Murchison Bay and Napoleon Gulf which display contrasting ecological status in terms of eutrophication (Okello *et al.*, 2010b), probably because of different population densities.

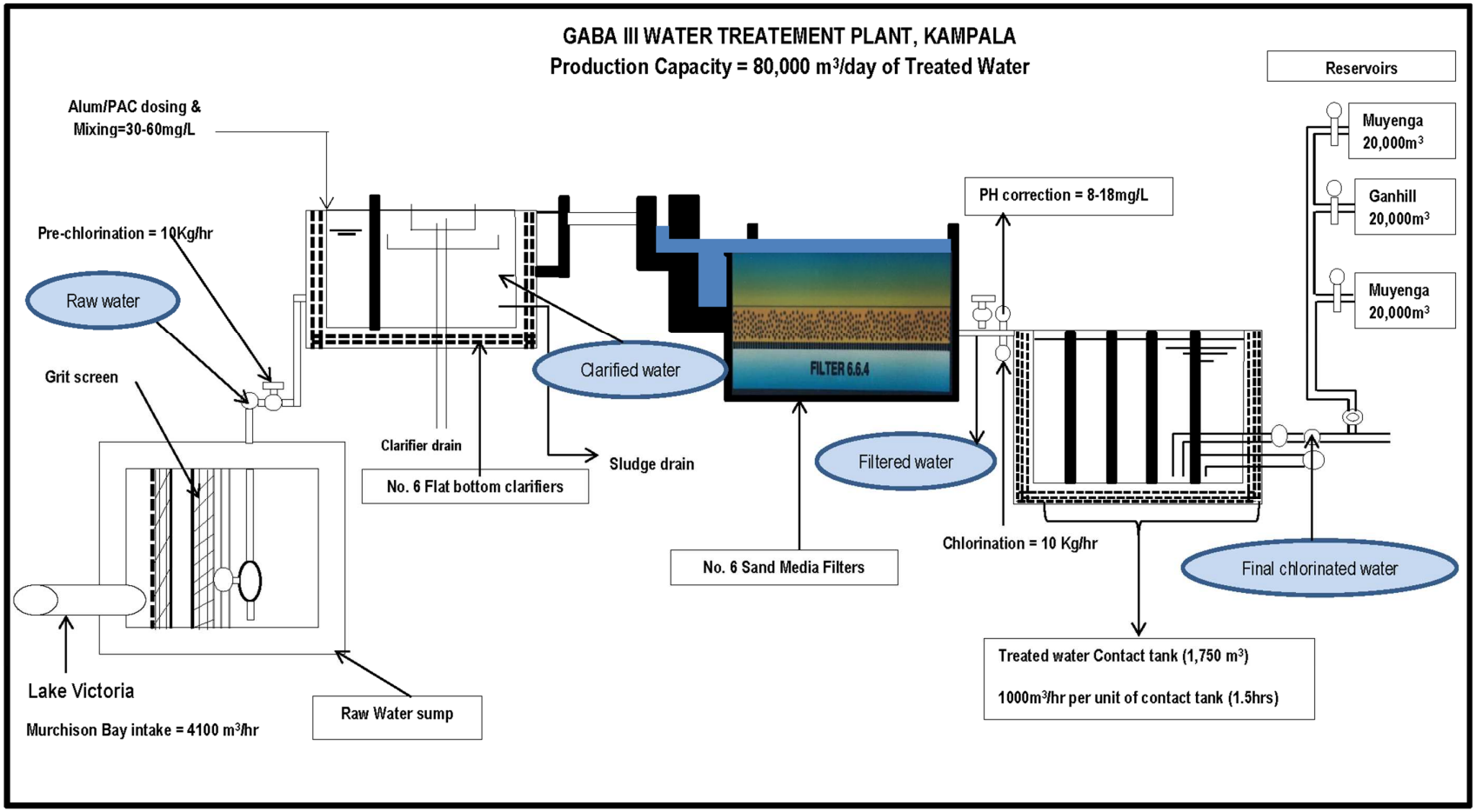

Figure 3: Typical process flow diagram at Gaba III water treatment plant. Inset are the locations for the sampling of raw water, clarified water, filtered water and final water before distribution.

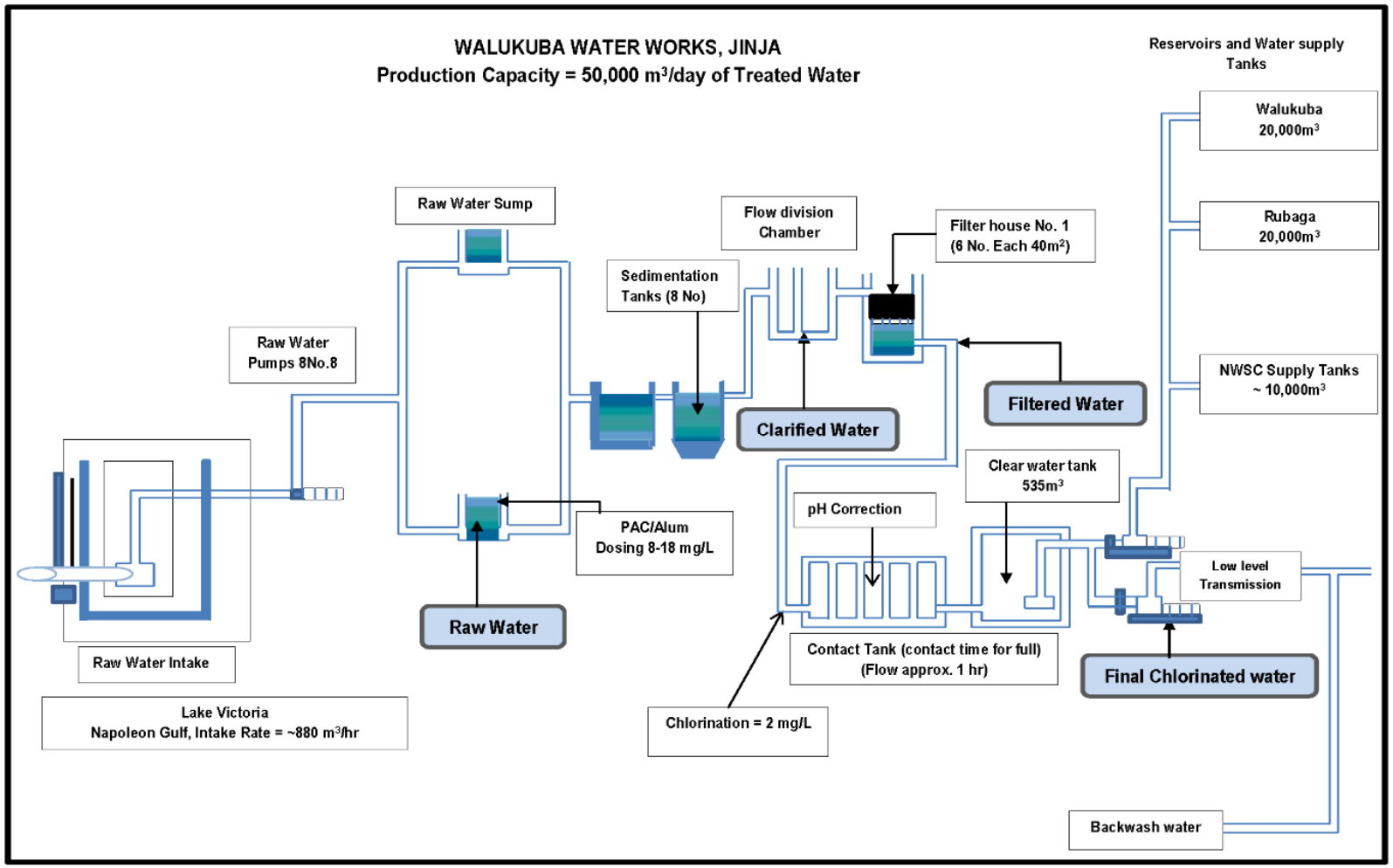

Figure 4: Typical process flow diagram at Walukuba water treatment plant. Inset are the locations for the sampling of raw water, clarified water, filtered water and final water before distribution.

2.3 Removal of cyanotoxins in drinking water

Over the years, there has been a growing interest and knowledge in the occurrence of toxin-producing cyanobacteria. This is has been mainly because of the close proximity of society and nature to drinking water sources (Haande *et al.*, 2007). Some of the surface waters are dominated by bloom forming cyanobacteria. The toxicity of a bloom is difficult to establish because not all cyanobacterial blooms cause toxin production if the bloom consists of strains not actively producing toxic metabolites (Okello & Kurmayer, 2011; Sitoki *et al.*, 2012). Until recently it has also been established that 50-70% of cyanobacterial blooms are toxic especially if there is the presence of *Microcystis* species (WHO, 2003). The seasonality of *Microcystis* sp. occurrence makes it difficult for drinking water production from surface waters, such as those in Uganda, using rapid sand filtration treatment methods.

The best way to eliminate the potential risk of toxins in drinking water is to reduce eutrophication to control the cyanobacterial blooms. If the raw water contains cyanobacteria, there is a need for an efficient water treatment process. Conventional treatment methods including flocculation and rapid sand filtration are relatively efficient in removing cyanobacterial cells (Chow *et al.*, 1999; Ho *et al.*, 2012; Roegner *et al.*, 2014) and thus could reduce the concentrations of microcystins. Following raw water abstraction, coagulation and flocculation could be an efficient way to reduce the cyanobacterial cells from water but the soluble cyanotoxins might not the eliminated. This process could also cause cell lysis, which leads to the release of secondary metabolites or toxins prior to oxidation. Some studies, however, reported few cases of cell lysis (Rositano & Nicholson, 1994; Westrick, 2003). Filtration, which is done to remove any contaminants in drinking water are effective in removing cyanobacterial cells (Gijsbertsen-Abrahamse *et al.*, 2006). Disinfection with chlorination is efficient due to its persistence in the distribution lines but also leads to transformation of cyanotoxins (Merel *et al.*, 2009). The by-products from chlorination include monochloro-microcystin, monochloro-dihydroxy-microcystin, dichloro-dihydroxy-microcystin and trichloro-hydroxy-microcystin (Merel *et al.*, 2009; Merel *et al.*, 2010).

Additional treatment stages such as oxidation with activated carbon have been reportedly efficient to reduce MC levels down to 0.05 - 0.2 µg/l in the treated water (Donati *et al.*, 1994; Lambert *et al.*, 1996; Roegner *et al.*, 2014). Furthermore, the dosage, duration, and concentration of the oxidant determine the effectiveness in removal of MCs; for instance with 15 mg/l hypochlorite at a pH <8, or 0.5 mg/l chlorine

after 30 minutes contact time the MCs are destroyed (Merel *et al.*, 2009). To effectively remove toxins from potable water, a multi-barrier approach has to be administered (Westrick *et al.*, 2010) and special attention must be taken to deal with high algal biomass to avoid recontamination. Therefore, the final step in controlling cyanotoxins will depend on the technology of water treatment and the physical-chemical and biological properties of the raw water.

The NWSC uses the flocculation and rapid sand filtration methods for potable water treatment. The most critical information is to understand the specific cyanotoxins and its seasonal dynamics since different toxins are eliminated differently by the different treatment technologies (Westrick, 2003). For instance, chlorination and ozonation are efficient in removal and inactivation of MCs but with at least a dosage of 3 mg/l to obtain a residual of about 0.5 mg/l during 30 minutes incubation time (Chorus & Bartram, 1999).

To potentially eliminate cyanotoxins in potable water, it is imperative to know the actual toxin concentrations in the raw water. This knowledge is necessary to determine the extent the treatment methods are eliminating the toxins. Several studies in the US have assessed the concentrations of MCs during water treatment. In Alberta, raw water MC concentration ranged from 0.15 to 4.3 µg/l, and after treatments, the MCs were reduced to 0.09 to 0.64 µg/l (Hrudey *et al.*, 1994). In Victoria Beach on Lake Winnipeg, MC-LR concentrations were reduced from 300 µg/l to 0.2 µg/l (Gurney & Jones, 1997). It is also challenging to deal with dissolved MCs because they are reportedly variable in concentrations in drinking water. In Australia MC-LR persisted for about 21 days (Jones & Orr, 1994) while in some studies from the United Kingdom and Canada MCs resisted chemical treatment (copper sulfate or lime), biological degradation and persisted for about 4 days (Kenefick *et al.*, 1993; Tsuji *et al.*, 1994; Cousins *et al.*, 1996).

In summary, it is concluded that flocculation and rapid sand filtration treatment methods can only partially eliminate the cyanotoxins (microcystins). The efficiency of the removal of MCs is influenced by other environmental abiotic factors such as temperature and biotic factors such cell densities of cyanobacteria. These factors need to be considered to generate a safe water production process.

CHAPTER THREE

3.0 Materials and methods

3.1 Description of study areas

The study was undertaken in two bays; Murchison Bay and Napoleon Gulf, northern Lake Victoria, and at Gaba III and Walukuba waterworks (**Figure 5**). Murchison Bay is located in the north-eastern part of Lake Victoria (00°16.911'N and 032°38.398'E) near Kampala and covers an area of about 60 km^2 at an altitude of 1,135 m a.s.l. (Semyalo *et al.*, 2010). The bay is the main water source for over 1,000,000 inhabitants and raw water processed by Gaba waterworks in Kampala. Murchison Bay is also a recipient of treated wastewater and runoff from the city through Nakivubo channel and wetland. Napoleon Gulf, located also in the north-eastern part of Lake Victoria (00°40.281'N and 033°29.806'E). It covers an area of about 200 km^2 at an elevation of 1,133 m a.s.l. Napoleon Gulf is a source of water to over 500,000 from Jinja and the surrounding region. When compared to Kampala, Jinja has fewer inhabitants but pollution, runoff and cage fish farming increasingly threaten the gulf.

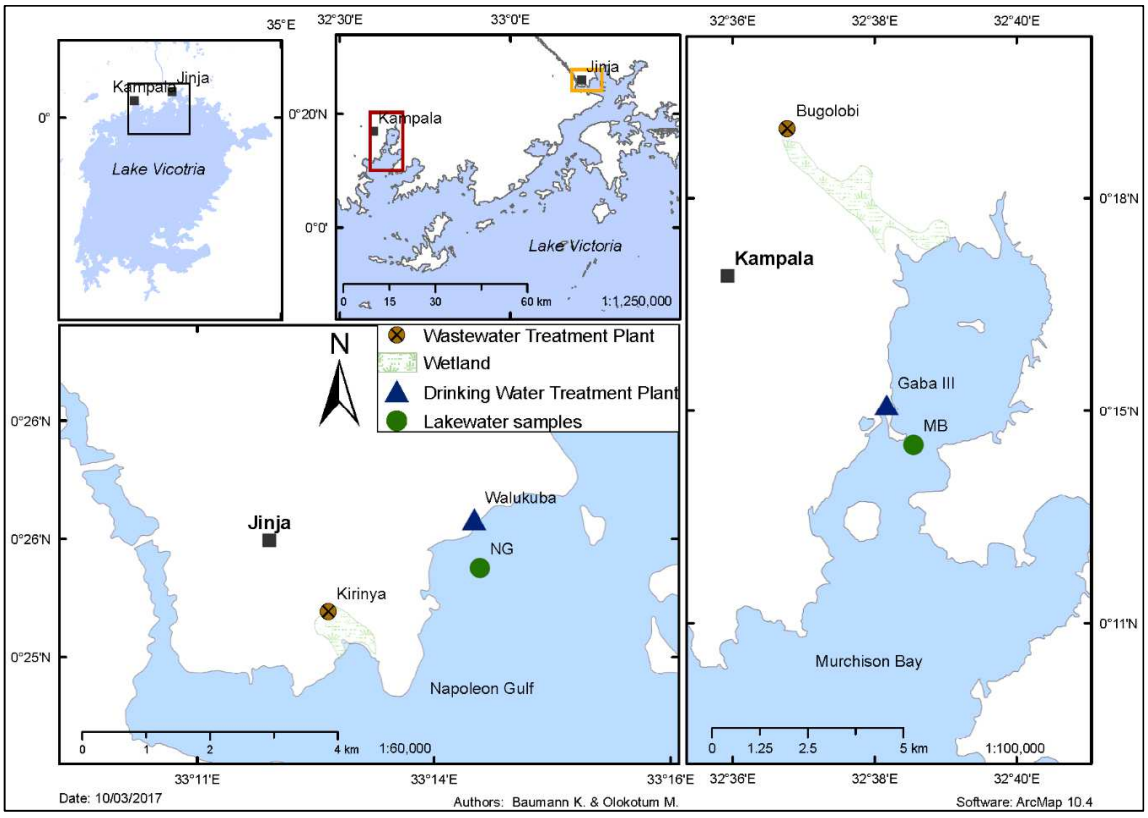

Figure 5: Maps of Napoleon Gulf and Murchison Bay with the locations of raw water sources and drinking water treatment plants. Inset are maps of northern Lake Victoria.

3.2 Study design and sample preparation

Weekly sampling in Murchison Bay and Napoleon Gulf were done from November 2016 to January 2017 between 8 and 11 am and 1-2 hours later in the respective water treatment plants. The samples were processed and analysed at the National Fisheries Resources Research Institute (NaFIRRI) - Jinja in Uganda and the Research Institute for Limnology of the University of Innsbruck, Mondsee in Austria.

3.3 Data and sample collection

3.3.1 Data from Napoleon gulf and Murchison Bay

The total depth of the sampling stations (water abstraction points) in Napoleon Gulf and Murchison Bay were determined using portable Eco sounder (Hondex PS-7). Water transparency was measured using a black and white Secchi disk (Ø = 20 cm). Water transparency was determined by taking the average depth at the disappearance and re-appearance of the Secchi disk in the water column. At every 1m, an integrated water sample was collected using a 2-litre horizontal van Dorn sampler as described by Wetzel & Likens (2000). The water was collected in a 20-litre bucket from which a composite sample for phytoplankton composition, chlorophyll-a, algal biomass and microcystins were collected. A vertical phytoplankton net (30-µm mesh size) was used to concentrate phytoplankton (**Figure 6**).

Water temperature, pH, electrical conductivity and dissolved oxygen concentration were measured for every 1m depth using a HACH Multiprobe meter (HQ40d). Turbidity was measured using the YSI Sonde EXO2 Multiprobe meter (Sonde 16B102461).

Figure 6: An integrated water sample in a 2-litre Van Dorn sampler from a known depth (Left) and concentrated water sample in a 30 µm plankton net (Right).

3.3.2 Data from Walukuba and Gaba III waterworks

At the respective water treatment plants, two litres of water samples (raw water, clarified water, sand filtered water and final chlorinated water) were obtained from designated sampling taps/points along the treatment process (**Figure 3 and 4**). The water samples were collected for determining phytoplankton composition, chlorophyll-a, algal biomass and microcystins during the water treatment process. At each of the sampling points, water temperature, pH, electrical conductivity and dissolved oxygen concentration were measured using a HACH Multiprobe meter (HQ40d). Turbidity was measured using the YSI Sonde EXO2 Multiprobe meter (Sonde 16B102461).

About 20 ml of the water samples from the lake and the water treatment plants were fixed using Lugol's solution (Utermöhl, 1958), stored away from light for phytoplankton identification and biovolume estimation as described in Wetzel & Likens (2000). All the water samples were transported in a cooler box to NaFIRRI – Jinja for further processing and subsequent analysis at the Research Institute for Limnology, Mondsee, Austria.

3.4 Laboratory sample preparation and analysis

3.4.1 Phytoplankton identification and biovolume estimates

About 2 ml of the fixed lake water and raw water samples from the treatment plants were sedimented in a sedimentation chamber for 4 - 5 hours (Utermöhl, 1958) awaiting identification under an inverted microscope (Nikon Eclipse TS-100) at 400x magnification. The phytoplankton species were identified using taxonomic guides and keys based on their morphological characteristics as described in Talling (1987) and Cronberg & Annadotter (2006).

Determination of phytoplankton biovolume

Phytoplankton species counts were done at 400x magnification. For each sample, 2-3 transects in the sedimentation chamber were counted and the average number recorded. In order to determine the algal biovolume from cell numbers and cell size measurements, both the eyepiece and digital Nikon software were calibrated using a micrometre scale (1 unit in the scale = 2.45 µm while 1 unit on the eyepiece = 24.5 µm at 400x). *Planktolyngbya* and *Nitzschia* were counted as filaments and their total length and width measured. Other species such as *Anabaena*, *Cryptomonas* and *Chroococcus* and others were counted as single cells. Both *Microcystis* and *Aphanocapsa* were counted as colonies. The number of cells in a colony was estimated from the size of the colony. *Merismopedia* were counted as packets of eight cells each. Empty or dead specimens especially frustules of diatoms were not considered during the counting. At least 400 cells of the most abundant species were counted to reach a 90% confidence interval and 10% error as described in Wetzel & Likens (2000). Cell dimensions (length and width) were determined for bio-volume calculations. Twenty (20) randomly selected specimens from the dominant species were measured and their volumes calculated by assuming a geometric shape. i.e. for *Microcystis*, it is a "sphere" the formula $(\pi/6)d^3$ was used where "d" denotes cell diameter (Hillebrand *et al.*, 1999; Wetzel & Likens, 2000). The biovolume was then calculated from;

Biovolume (mm^3/l) = Cell volume (μm^3/cell) x Cell density (cells/ml).

3.4.2 Phytoplankton biomass (dry weight) and chlorophyll-a

The total phytoplankton biomass was estimated using the dry weight from cells harvested on the filters. The filters (Whatman GF/C filters (Ø 47 mm)) were pre-weighed and stored in a desiccator. Depending on the turbidity (from lake and treatment plants) about 200 – 1000 ml of the water samples were filtered using the pre-weighed filters and dried in the oven at 50°C for 48 hours. After the 48 hours, the filters were weighed and recorded to the closest 10^{-3}g using a digital weighing scale (Mettler Toledo, AB204-S) to obtain the dry weight of algae. The dried filters were then folded and transferred into Eppendorf tubes for cell bound microcystin extraction and determination. The Eppendorf tubes were closed, covered in zip-lock bags and frozen at -20°C in NaFIRRI, Uganda before extraction and analysis in Mondsee, Austria.

Determination of Chlorophyll-a

Chlorophyll-a analysis was based on the hot ethanol extraction spectrophotometric method according to the international standard method (ISO-10260, 1992). Between Depending on the turbidity from lake and treatment plants, 250 - 1000 ml of water samples were filtered through the Whatman GF/C filters (Ø 47 mm) using a low-vacuum hand pump. Each filter was folded, placed in Eppendorf tube and dried in the oven at 50°C for 24 - 48 hours and stored frozen. The frozen chlorophyll-a filter paper was transferred from the Eppendorf tube to 15 ml Falcon tube into which 10 ml of 90% (v/v) hot ethanol were added to tube and incubated in a water bath for 2 minutes at 78°C. Thereafter, the tubes were transferred to a sonicator for 15 minutes at 78°C in order to accelerate the lysis of pigments. The extract was then placed in the dark for 1 hour at room temperature. After cooling, the filter was squeezed with a forceps to facilitate extraction and the extract filtered again using the 0.2 µm mesh size membrane filter (Millex, GS). Absorbance measurement was done at 665 nm and corrected for turbidity by subtracting the absorbance measured at 750 nm using a spectrophotometer (Jenway 6505 UV/Vis). To the measured extract in the cuvette, 100 µl of 0.25 M-hydrochloric acid was added, shaken and absorbance measured again at 665 nm and 750 nm. Chlorophyll-a concentration in µg/l was then calculated according to the equation:

$$Chl\text{-}a\ (\mu g/l) = [(E665-E750)-(Ea665-Ea750)]*((2.43*12.19*Ve)/(Vs*d))$$

Where:

E665: absorbance at 665 nm wavelength before acidification

E750: absorbance at 750 nm wavelength before acidification

Ea665: absorbance at 665 nm wavelength after acidification

Ea750: absorbance at 750 nm wavelength after acidification

Ve: volume of extract (ml)

Vs: filtered sample volume (l)

d: cuvette path length (cm)

3.4.3 Determination of microcystin concentration

Both the dissolved and cell bound microcystins were determined from the lake water and water treatment plants. The filtrate was used for the determination of dissolved microcystins while the biomass collected for dry weight estimation was used for cell bound microcystin extraction.

3.4.3.1 Determination of dissolved (extracellular) MCs by ELISA

The principle of Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA is an immunological (antigen-antibody reaction) detection technique. It has been used because of its sensitivity, selectivity and ease of operation. For the analysis of MCs, the indirect competitive ELISA was used (Abraxis-manufacturer's information). In principle, in the indirect competitive ELISA, a MC specific antibody is attached to a microtiter plate. The MCs present in a sample and a microcystin different conjugate compete for binding sites of the anti-microcystin antibodies. Once the plate is washed, the conjugate will cleave a substrate leading to a colour. The developed colour intensity is inversely proportional to the MCs concentrations. i.e. the lighter the colour the higher the MC levels and the darker colour indicates lower MC levels (Carmichael & An, 1999).

About 1.0 ml of the filtrates and additional 1.0 ml final water treated (quenched with 10 mg Ascorbic acid in 100 ml of filtrate) were frozen at -20°C and used for the determination of dissolved microcystins. ELISA results from both the quenched and unquenched final water were then compared. The quenching was done since the concentration of chlorine in the final water was > 0.1 mg/l according to the EPA Ohio ELISA guidelines (2015). The quenching should avoid false negative detection.

The ELISA procedure

The frozen 1.0 ml filtrates were allowed to thaw at room temperature and applied directly to commercial Abraxis Microcystins/Nodularins (ADDA) ELISA Kit (Product No. 520011 and Lot No. 16H0667) for the analysis of the dissolved microcystins. The samples were added and treated according to the manufacturer's protocol. To the 96 well microtiter plate, 50 µl of the standard solutions (1-5), control and filtrates were added to the wells in duplicate. Thereafter, 50 µl of the antibody solution were added to the individual wells, covered with parafilm and mixed by moving the microtiter plate in a circular motion for 30 seconds. The microtiter plate was then incubated at room temperature in the dark for 90 minutes. After incubation, the parafilm was removed and the contents emptied into the sink. Afterwards, the wells were washed three times using 250 µl (each time) of the wash buffer solution. The remaining buffer solution in the wells was completely removed by patting the plate dry on a stack of tissue paper. After washing, 100 µl of enzyme conjugate solution were added to the individual wells, covered with parafilm and the contents were mixed again by moving the plate in a circular motion for 30 seconds. The microtiter plate was then further incubated at room temperature in the dark for 30 minutes. After the second incubation, the contents were then emptied into the sink and washed again three times using 250 µl (each time) of the wash buffer solution. The remaining wash buffer was further removed by patting the microtiter plate on a stack of tissue paper. Thereafter, 100 µl of substrate solution were added to the individual wells and the plate incubated at room temperature in the dark for 25 minutes. After substrate addition, a blue colour whose intensity is inversely proportional to the concentration of microcystins is generated. Finally, 50 µl of the stop solution were added to the individual wells in the same sequence. The absorbance at 450 nm was determined using an ELISA plate reader (LEDETECT 96, SN: 1357) in duplicate within 15 minutes (**Figure 7)**.

Standard calibration curves were constructed using the microcystin standard (150, 400, 750, 1000, 2000 and 5000 ng/l) from which the concentration of the microcystins in the samples were calculated (**Figure 8**). In this study the concentrations of MCs were calculated from $y = -0.8547x + 1.6774$ (November 17th 2016), $y = -0.5265x + 1.9415$ (November 24th 2016), $y = -1.1464x + 2.156$ (January 3rd 2017) and $y = -0.3092x + 1.474$ (January 12th 2017). Where y = Microcystin concentration and x = the absorbance at 450 nm for the respective dates when the measurements were done.

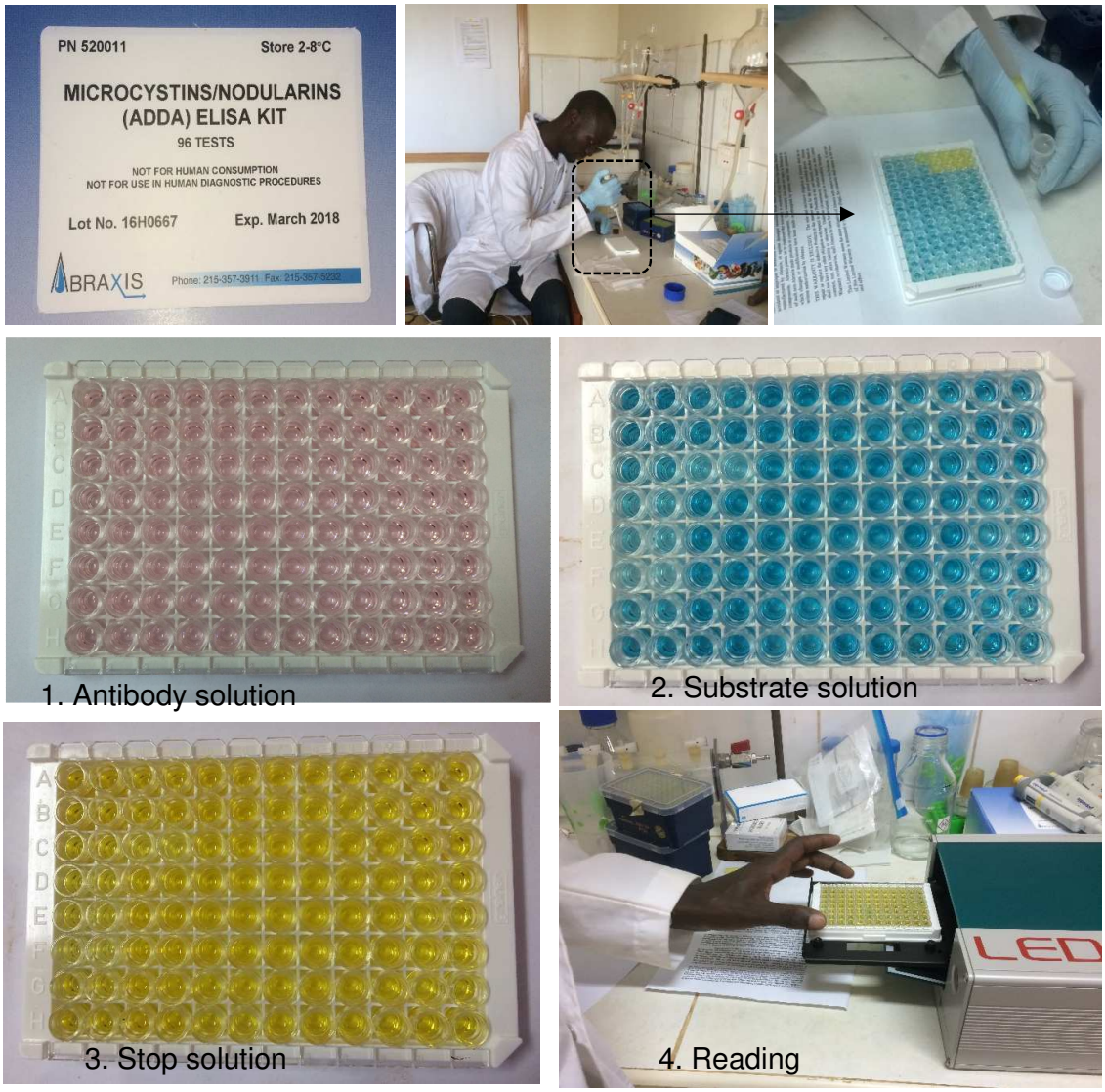

Figure 7: The sequence of preparation of the microtiter plate for determination of dissolved microcystins using the microcystin ELISA (ADDA) kit.

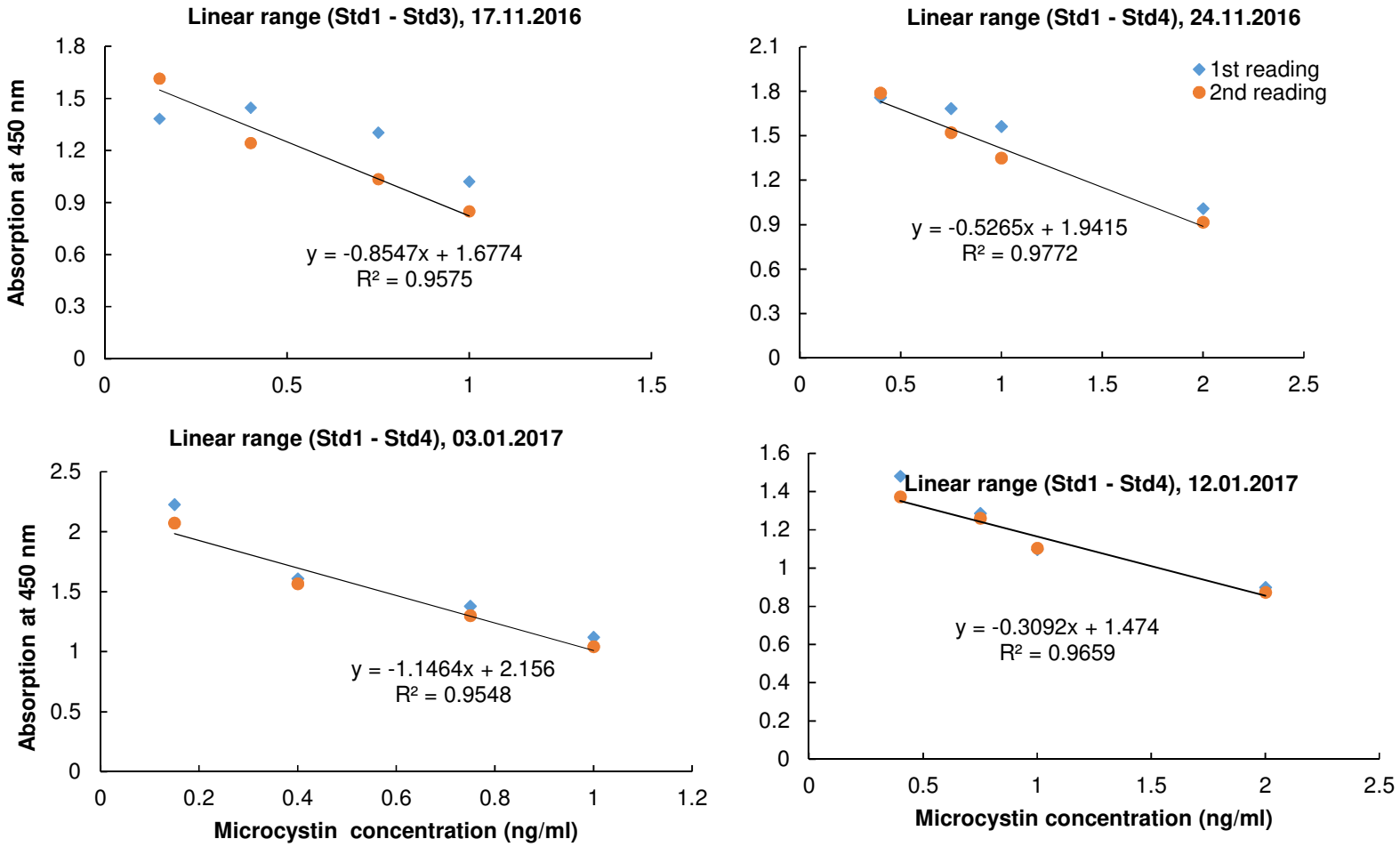

Figure 8: The standard calibration curves with selected linear ranges used to calculate total dissolved (extracellular) microcystin concentrations in the water samples. According to manufacturer's instructions calibration curves were determined for each individual tests separately which is indicated by the date.

3.4.3.2 Concentration of dissolved microcystins using Solid Phase Extraction (SPE)

Additional samples for dissolved microcystins were concentrated using Solid Phase Extraction (SPE) (C_{18} SPE columns) according to Dean (1998). The C_{18} SPE columns (Sep-Pak® C18, Waters Corporation, US) were conditioned using 1.0 ml of 80% (v/v) methanol followed by 1.0 ml of distilled water. Thereafter, between 500-700 ml of the filtrates were concentrated through the conditioned C_{18} SPE columns by suction using the hand vacuum pump. The flow through rate through the C_{18} SPE columns was maintained at 2-4 drops/second to allow maximum contact time with the sorbent. Each of the C_{18} SPE columns was dried at 50°C for 48 hours thereafter stored frozen at -20°C. The frozen C_{18} SPE columns were transported to the Research Institute for Limnology, Mondsee in Austria. In Mondsee, each C_{18} SPE columns were brought to room temperature before elution with 1.0 ml of 80% (v/v) methanol. The elute was injected into the High-Pressure Liquid Chromatography coupled to a Diode Array Detector (HPLC-DAD) and Mass Spectrometry (MS) for analysis.

SPE external controls

For purpose of quality assurance, SPE was performed and standardised in September - October 2016 at the Research Institute for Limnology, Mondsee. During September - October 2016, 100 ml of Mondsee water, tap water and Millipore water were spiked with analytical MCs standards (MC-RR, MC-YR and MC-LR) and thereafter concentrated in the C_{18} SPE columns in duplicate. Half of the columns were eluted immediately (wet) while the other half were dried at 50°C for 48 hours. Using 1.0 ml of 80% (v/v) methanol for eluation, 100 µl of the eluate were injected into the HPLC-MS for analysis. This was done to ensure that the C_{18} SPE columns retrieve microcystins quantitatively (**Figure 9**). Between November 2016 and January 2017, SPE samples (filtrates) from the lake and water treatment plants were also spiked with analytical MCs standards (MC-RR, MC-YR and MC-LR) as external controls.

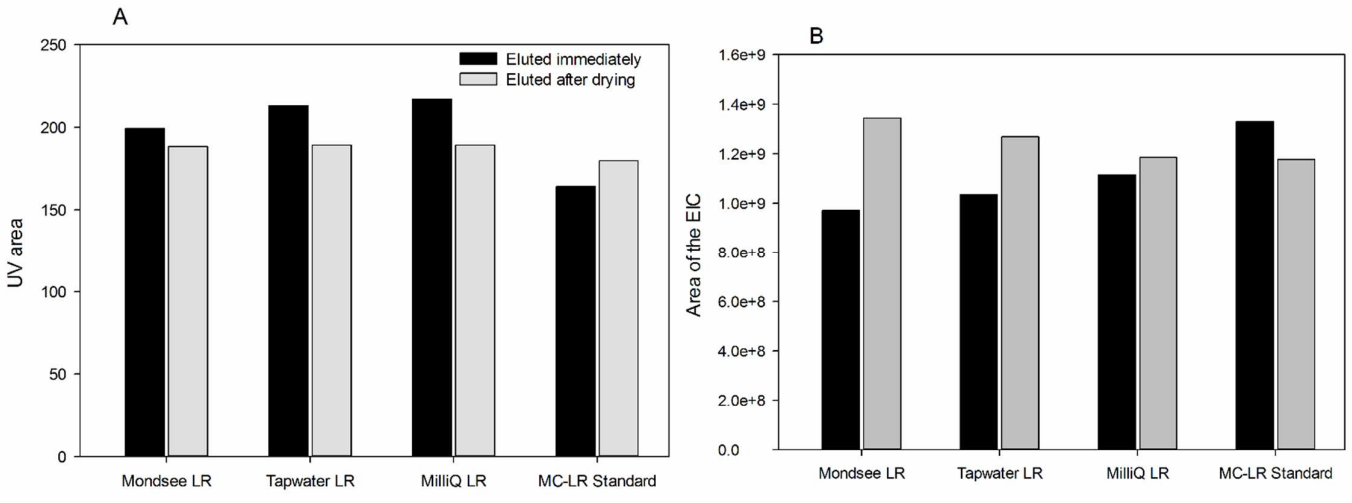

Figure 9: The UV chromatogram area (A) and MS chromatogram form of extracted ions peak (EIC) (B) showing the recovery rate of MC-LR from C_{18} SPE columns eluted immediately and dried before elution for analysis.

3.4.4 Extraction of intracellular microcystins

Intracellular microcystins were extracted according to Fastner *et al.* (1998). The dried filters with collected biomass were cut into small pieces and put into 2 ml self-lock Eppendorf tubes. Thereafter 750 µl of aqueous 75% (w/v) methanol was added and the filters grinded to aid the extraction of microcystins. The extracts were sonicated in a water bath (BANDELIN SONOREX Ultrasonic) for 10 minutes and then transferred to a shaker (Edmund Buhler 7400 Tubingen, KS 10) for 30 minutes. The tubes were centrifuged at 13,000 rpm for 10 minutes and 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 three times for each sample to ensure all the microcystins were extracted.

For injection into the Liquid Chromatography- Mass Spectrometry (LC-MS), the dried extracts were resuspended using 300 µl of 100% methanol, sonicated for 10 minutes and 100 µl of Millipore (MQ) water was added. The extracts were centrifuged at 13,000 rpm for 10 minutes and 500 µl of the clear supernatant were transferred into HPLC glass vials. The collected extracts were then injected into the HPLC-DAD and MS for analysis.

For HPLC

A LiChrosper® 100, ODS, 5µm LiChrosCART® 250-4 HPLC cartridge system (Merck, Darmstadt, Germany) using a linear gradient of aqueous acetonitrile (ACN) in 0.05% Trifluoroacetic acid (TFA) (30-70% CAN) during 45 minutes was used. The HPLC injection volume was set to 30-100 µl in the sample table, the separation temperature was 30°C and the flow rate was 1.0 ml per minute. According to Fastner

et al. (1999), the different variants of microcystins were identified by (i) characteristic Ultraviolet (UV) absorption spectra at 240 nm wavelength, (ii) retention time and the order of elution using analytical microcystin standards (MC-RR, YR and LR) and (iii) fragmentation patterns from the MS ionisation (AmaZon SL, Bruker).

The UV and MS spectra and peaks were manually integrated to obtain the peak area from which the concentrations were determined (**Figure 10**). The concentration of the different microcystin variants was determined as concentration equivalents of external analytical standards MC-RR, YR and LR (Cyano Biotech GmbH, Berlin). These were calculated from the regression curves i) MC-RR, y = 1735.9x-8.6716. (R^2 = 0.9994) ii) MC-YR, y = 1526.7x+7.5986. (R^2 = 0.9991) and iii) MC-LR, y = 1617.5x + 1.3696. (R^2 = 1); where y was the absorption (mAU) recorded at 240 nm wavelength (UV) and x was the concentration of the microcystin standards injected on the column (**Figure 11**).

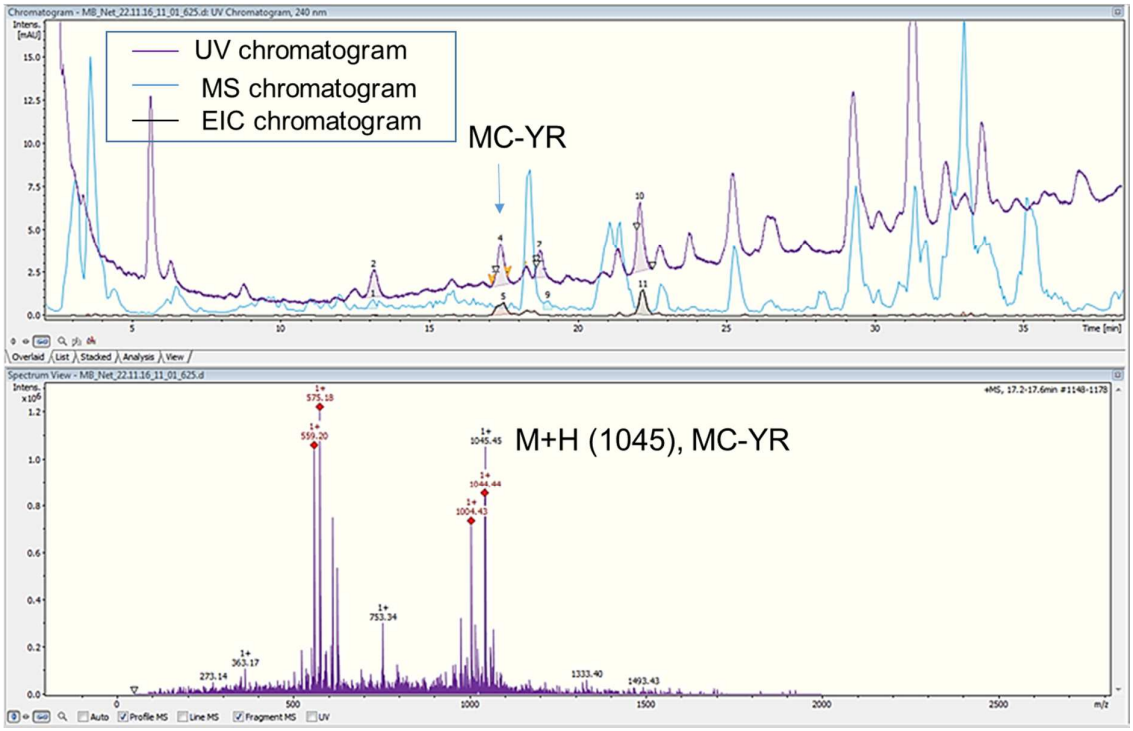

Figure 10: The UV and BPC mass spectra of Murchison Bay sample extract (22nd November 2016) whose peaks have been manually (above) integrated and the mass to charge ratio (M+H) of the selected peak for MC-YR shown (below).

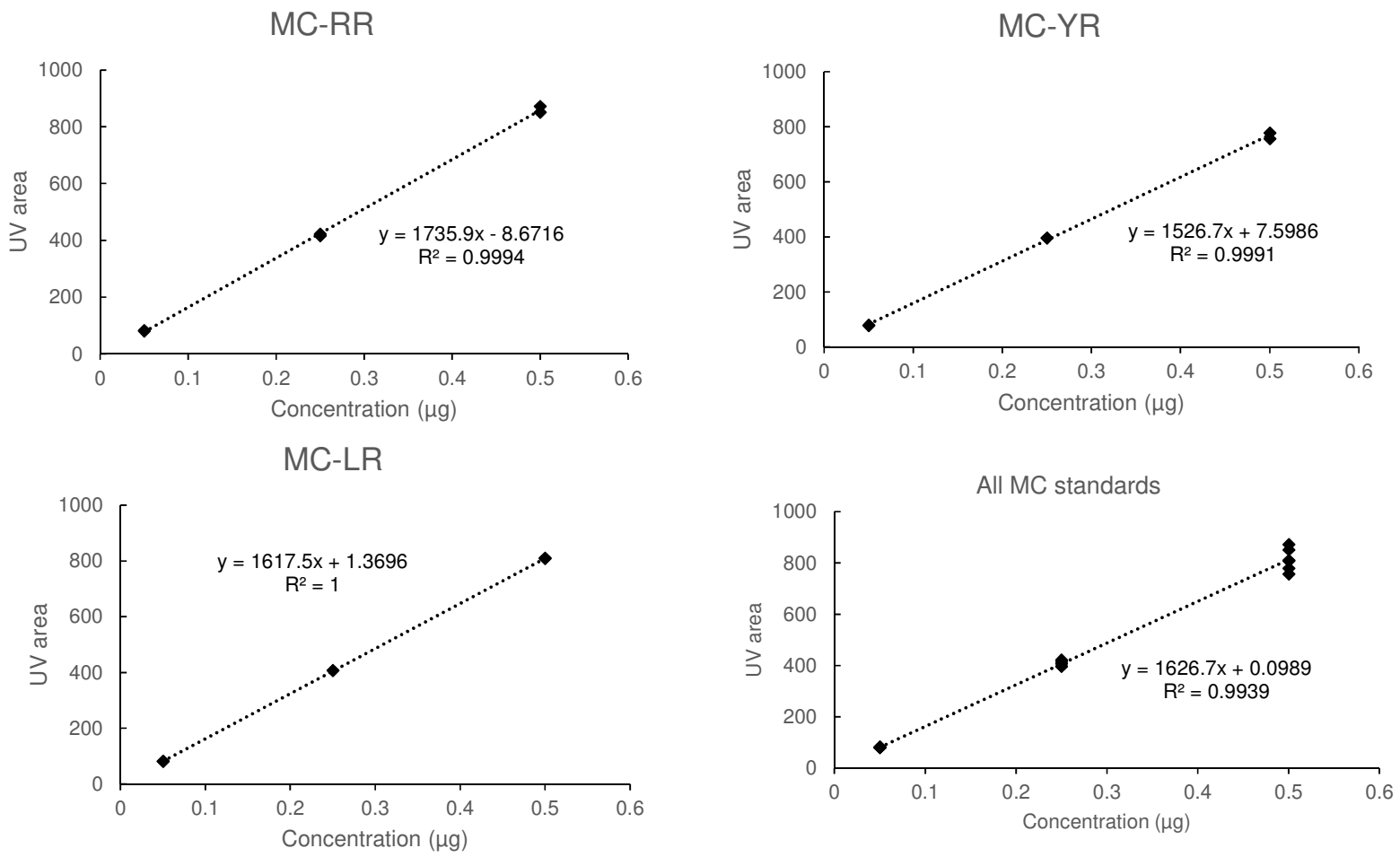

Figure 11: The UV area standard calibration series for structural microcystin standards MC-RR, MC-YR and MC-LR, and all the standards combined used to calculate the concentration of microcystins in water.

3.5 Data management and statistical analysis

For the physical-chemical characteristics for Napoleon Gulf and Murchison Bay, depth profiles were generated. For the physical-chemical characteristics in the water treatment plants, boxplots were derived. In general, the mean ± standard deviation (SD) were calculated for all the parameters. The efficiency of the treatment processes/steps was estimated based on the concentration of chlorophyll-a. Relationships among algal biomass, chlorophyll-a, phytoplankton biovolume, ELISA and HPLC-MS microcystins concentrations were explored using Pearson product moment correlations coefficient (r). All statistical tests and graphical outputs were produced using R and Sigmaplot software version 13.

CHAPTER FOUR

4.0 Results

4.1 Limnological characteristics of Napoleon Gulf and Murchison Bay

4.1.1 Water temperature, dissolved oxygen, pH and conductivity

The records of water temperature, dissolved oxygen, pH and conductivity with depth in Napoleon Gulf are shown in **Figure12 (a-d)** while those for Murchison Bay are shown in **Figure 13 (a-d)**. Between November 2016 and January 2017 in Napoleon Gulf, water temperature ranged between 26.3 - 28.0°C (27.1 ± 0.4) and in Murchison Bay between 25.4 - 26.8°C (26.1 ± 0.3). The surface temperature was slightly higher than the temperature at the bottom of the bays. In Napoleon Gulf, the bottom temperature ranged between 26.1 and 27.1°C while the surface temperature ranged between 26.4 and 28.0°C (**Figure 12a**). In Murchison bay, surface temperature and bottom ranged between 25.4 and 26.4°C (**Figure 13a**). In general Napoleon Gulf was warmer than Murchison Bay. For both sites, there was no evidence of physical stratification in the water column as the variation in temperature with depth was less than 1°C.

In Napoleon Gulf dissolved oxygen concentrations ranged from 5.8 to 11.9 mg/l at the surface while concentrations between 2.9 and 7.7 mg/l were recorded at the bottom (**Figure 12b**). In Murchison Bay, concentrations of dissolved oxygen ranged between 5.6 and 7.1 mg/l at the surface while concentrations of 2.3 to 5.0 mg/l were recorded at the bottom (**Figure 13b**). There is a clear trend in the decline in dissolved oxygen from the surface with depth tending to hypoxic conditions. The dissolved oxygen profile indicate occurring hypoxia (<2.0 mg/l) at the bottom in both bays. A closer look at the oxygen profile shows a moderate stratification at 6-8 m in Murchison Bay for the entire study period while in Napoleon Gulf, there was no distinct stratification in oxygen concentration.

The pH in both bays was generally high typical of productive freshwater systems and it declined with depth. In Napoleon Gulf, surface pH ranged from 8.6 to 9.6 while bottom pH was between 7.6 and 9.3 (**Figure 12c**). In Murchison Bay, the surface pH was between 7.8 and 8.9 while at the bottom the pH was between 7.3 and 7.8 (**Figure 13c**). It is also clear that the pH recorded in Napoleon Gulf was higher than that in Murchison Bay throughout the study period.

The conductivity in Napoleon Gulf ranged from 100.7 to 106.5 µS/cm at the surface and 99.7 to 103.5 µS/cm at the bottom. Typically, the conductivity decreased slightly with depth for most of the sampling days (**Figure 12d**). Conductivity in

Murchison Bay ranged from 113.0 to 119.0 µS/cm at the surface and 118.5 to 127.4 µS/cm at the bottom. The conductivity in Murchison Bay was higher and increased with depth in contrast to the profile observed in Napoleon Gulf.

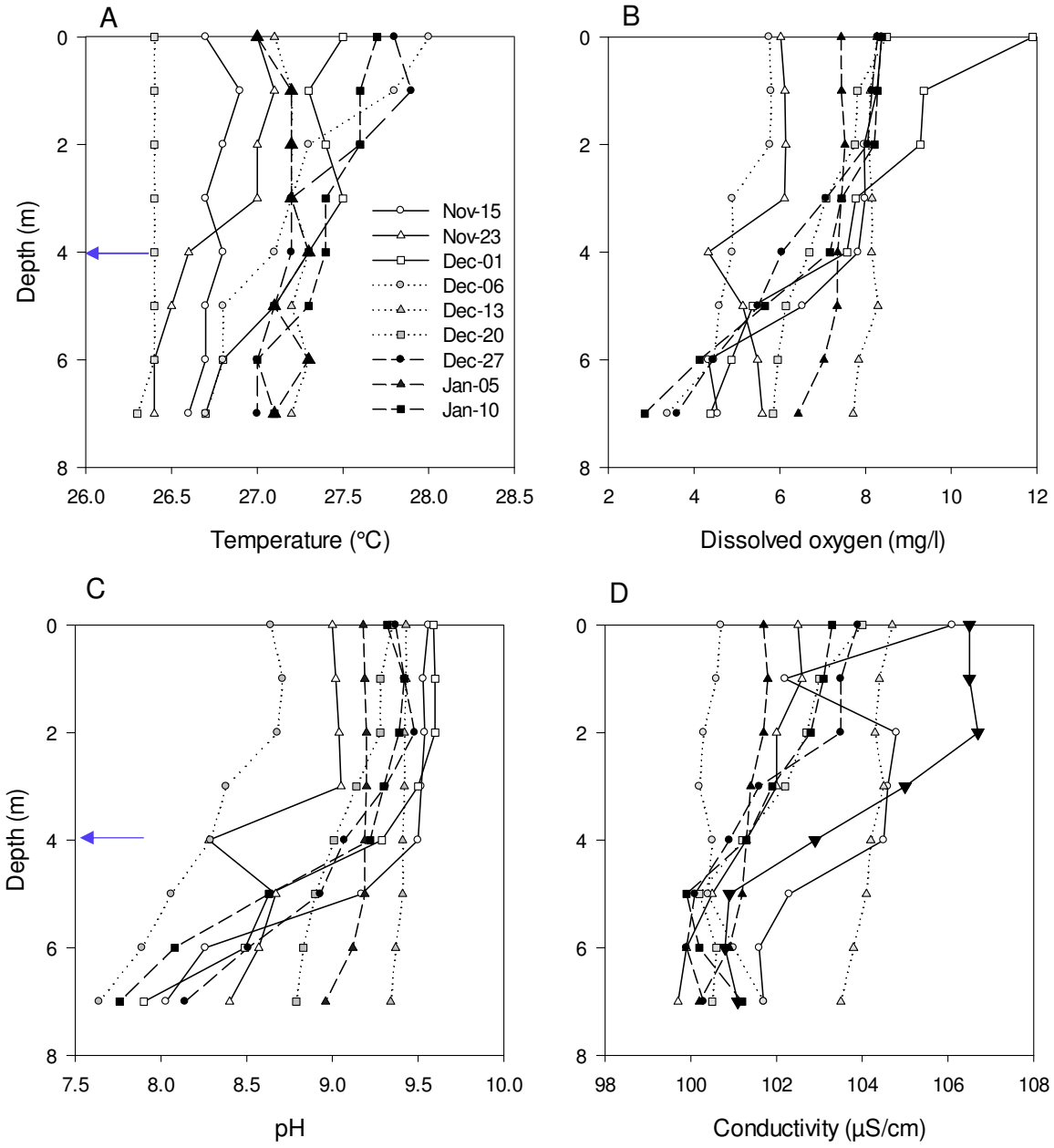

Figure 12: The depth profiles of (A) temperature (°C), (B) dissolved oxygen (mg/l), (C) pH, and (D) conductivity (µS/cm) measured between November 15th 2016 and January 10th 2017 at Masese in Napoleon Gulf, northern Lake Victoria. The arrow shows abstraction depth at 4 m.

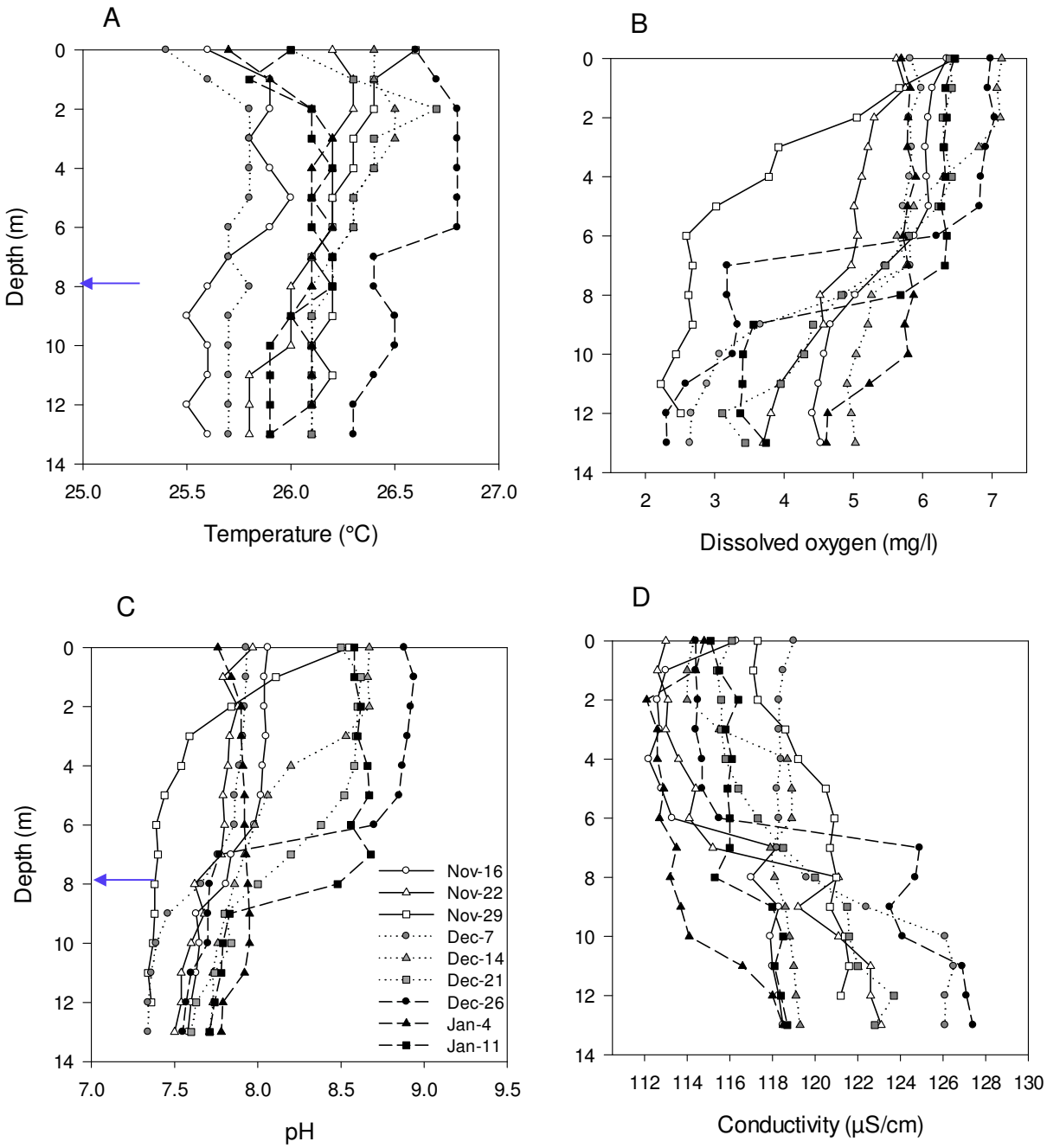

Figure 13: The depth profiles of (A) temperature (°C), (B) dissolved oxygen (mg/l), (C) pH, and (D) conductivity (µS/cm) measured between November 16th 2016 and January 11th 2017 at Gaba in Murchison Bay, northern Lake Victoria. The arrow shows abstraction depth at 8 m.

In summary, the stratification observed at the deeper sampling station in Murchison Bay was less by temperature but rather by biological processes as inferred from oxygen production, the differences in pH and conductivity in deeper waters.

4.1.2 Light transparency and turbidity in Napoleon Gulf and Murchison Bay

Water transparency of Napoleon Gulf was high with Secchi depth ranging between 0.7 and 1.8 m (1.2 ± 0.3 m) while that in Murchison Bay was generally lower 0.6 to 1.0 m (0.7 ± 0.12 m). On average the Secchi depth of Napoleon Gulf was almost two fold of that in Murchison Bay with a deeper euphotic zone (**Figure 14c**). The difference in Secchi depth between the two bays was also reflected in the turbidity where Murchison Bay was three fold as turbid as Napoleon Gulf. Turbidity in Murchison Bay ranged from 9.5 to 19 Nephelometric Turbidity Unit (NTU) at the surface and 6.7 to 15 NTU at the bottom (**Figure 14b**). In Napoleon Gulf, turbidity ranged from 2.8 to 6.7 NTU at the surface and between 1.9 and 4.2 NTU at the bottom (**Figure 14a**).

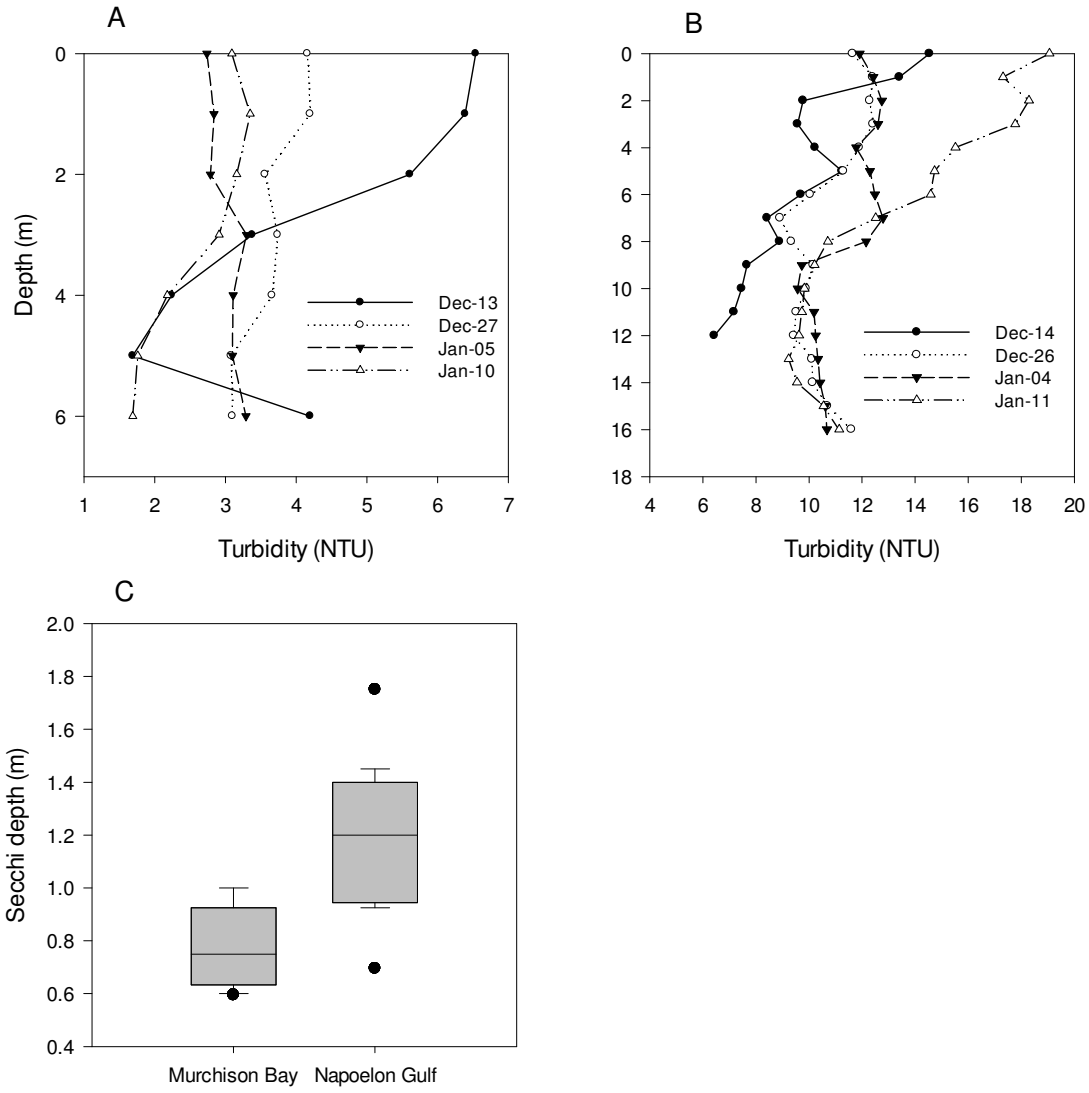

Figure 14: The Turbidity (NTU) of Napoleon Gulf (A) and Murchison Bay (B) and water transparency (Secchi depth) for both bays recorded between December 2016 and January 2017.

4.1.3 Water temperature, dissolved oxygen, pH and conductivity of water in Walukuba and Gaba III water treatment plants

Walukuba waterworks abstracts water at 4 m depth in Napoleon Gulf. At the abstraction depth of 4 m in Napoleon Gulf, the water temperature was between 26.4 and 27.4°C (27.1 ± 0.3°C), dissolved oxygen was between 4.3 and 8.1 mg/l (6.7 ± 1.3 mg/l), pH was between 8.3 and 9.5 (9.0 ± 0.4) and conductivity was between 100.5 and 104.5 µS/cm (102.0 ± 0.1 µS/cm). Generally, during the water treatment process in Walukuba waterworks, water temperature remained stable, dissolved oxygen concentration and conductivity decreased after sand filtration and then increased in the final water (**Figure 15**).

In general, the water temperature remained high throughout the water treatment processes. The median water temperature oscillated between 26.5 and 27.7°C with a slight decrease in temperature during sand filtration and increase in the final water (**Figure 15a**).

The concentration of dissolved oxygen in Walukuba waterworks in raw water, clarified water and sand filter was generally low (2.5-5.6 mg/l) but significantly increased in the final water (**Figure 15b**).

The median pH generally decreased during the treatment process (**Figure 15c**). Raw water pH was between 7.5 and 8.1 (7.8 ± 0.2) which decreased to between 7.3 and 7.9 (7.6 ± 0.2) in the final water.

The conductivity of lake water did not change in the raw water but increased in the final water. (**Figure 15d**). Raw water conductivity was between 99.4 and 119.4 µS/cm (104.4 ± 7.1 µS/cm) while the final water conductivity was 103.0 and 117.4 µS/cm (109.6 ± 4.1 µS/cm).

In summary, the relative stability of temperature indicated a high flow through and low retention time within the plant. Chemical treatment of final water with chlorine was linked to a significant increase in dissolved oxygen and conductivity.

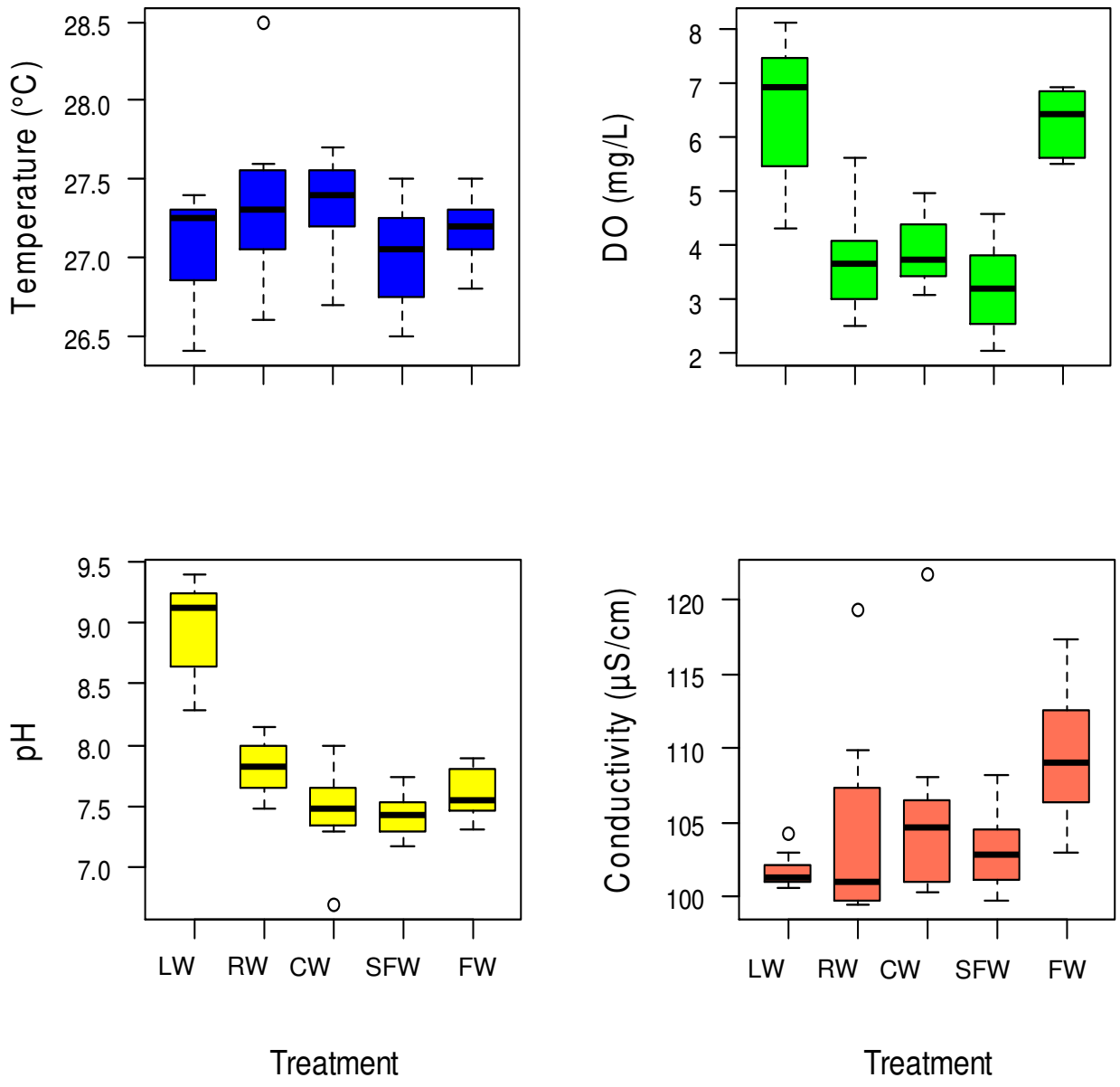

Figure 15: Boxplots of temperature (°C) (A), dissolved oxygen (mg/l) (B), pH (C) and conductivity (µS/cm) (D) of potable water during the treatment processes in Walukuba waterworks measured between November 23rd 2016 and January 10th 2017. Abbreviations LW = Lake water, RW = Raw water, CW = Clarified water, SFW = sand filtered water and FW = Final water.

On the other hand, Gaba III water treatment plant abstracts water from Murchison Bay at 8 m depth. At the abstraction depth of 8 m in Murchison Bay, the water temperature was between 25.7 and 26.4°C (26.1 ± 0.2°C), dissolved oxygen was between 2.6 and 5.9 mg/l (4.6 ± 1.1 mg/l), pH was between 7.4 and 8.5 (7.8 ± 0.3) and conductivity was between 113.2 and 124.8 µS/cm (118.8 ± 3.4 µS/cm).

Water temperature, dissolved oxygen concentration and conductivity in Gaba III water treatment plant increased after flocculation but pH decreased during the water treatment process (**Figure 16**). Water temperature remained stable until the sand filters and increased in the final water. This increase in temperature is probably due to incubation in the contact tank for chlorination treatment.

Dissolved oxygen and conductivity increased significantly before flocculation which is also probably due to the addition of chlorine gas (**Figure 16b, d**). The raw water oxygen levels were between 5.4 and 6.7 mg/l (5.9 ± 0.3 mg/l) while that in the final water were between 6.8 and 7.2 mg/l (7.1 ± 0.1 mg/l).

The median pH in Gaba III was also high in the lake water and generally decreased during the treatment process (**Figure 16c**). The raw water pH was between 7.4 and 7.8 (7.6 ± 0.1) which decreased to between 6.8 and 7.4 (7.0 ±0.2) in the final water.

The conductivity was considerably low in the raw water and high in the clarified, sand filtered and final water. Raw water conductivity was between 112.6 and 125.4 µS/cm (119 ± 3.7 µS/cm) while the final water conductivity was 125.3 and 138.8 µS/cm (131 ± 5.2 µS/cm).

In summary, there is a high flow through the water treatment plant as inferred in the small change in water temperature. The addition of chlorine as a pre-treatment process was linked to an increase in dissolved oxygen and conductivity.

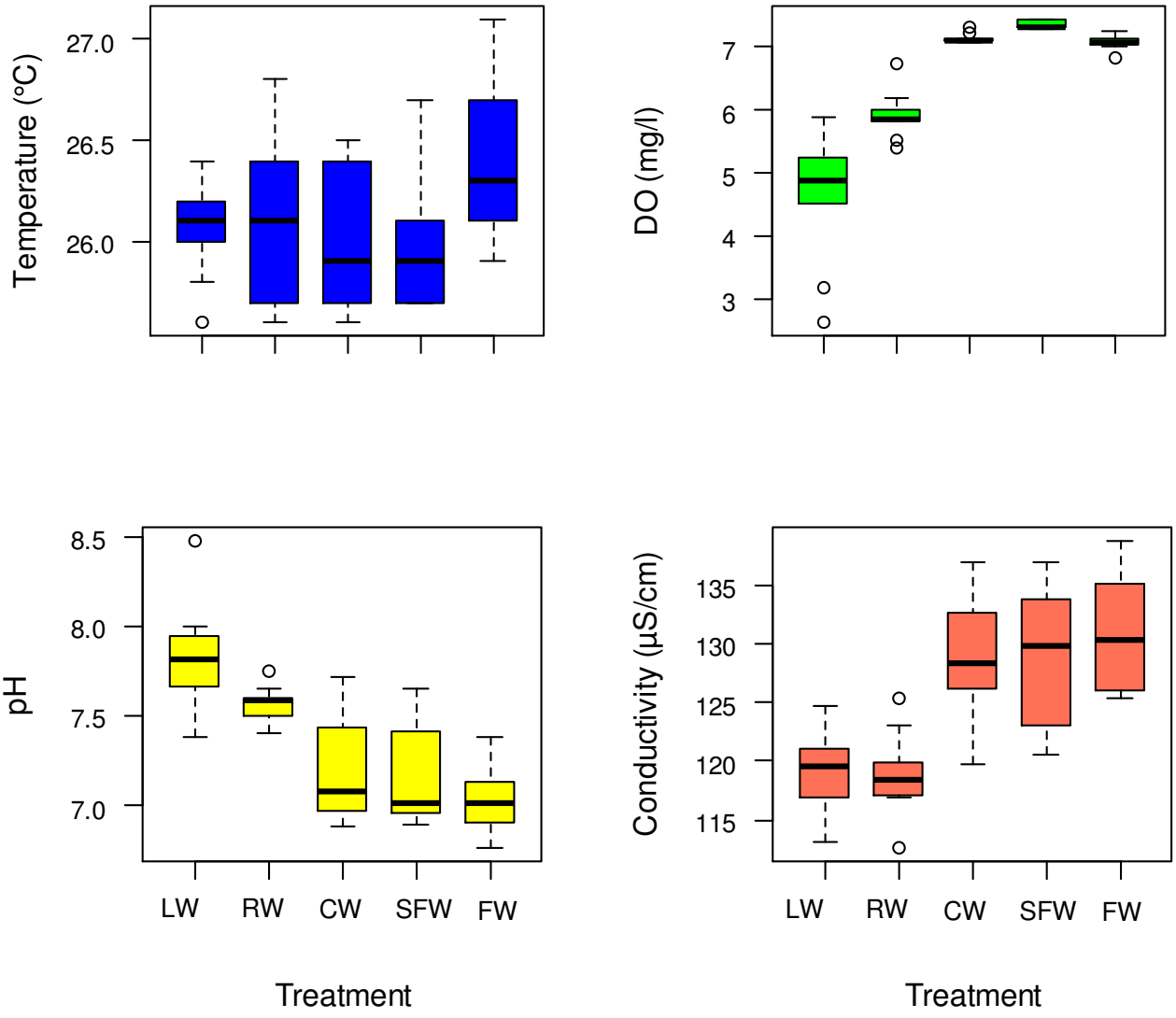

Figure 16: Boxplots of temperature (°C) (A), dissolved oxygen (mg/l) (B), pH (C) and conductivity (µS/cm) (D) of potable water during the treatment processes in Gaba III waterworks measured between November 16th 2016 and January 11th 2017. Abbreviations LW = Lake water, RW = Raw water, CW = Clarified water, SFW = sand filtered water and FW = Final water.

4.2 Phytoplankton community, biomass (dry weight) and chlorophyll-a in the lake and during the water treatment process

4.2.1 Phytoplankton biomass (dry weight) and chlorophyll-a in Napoleon Gulf and Murchison Bay

The depth-integrated chlorophyll-a concentration in Murchison Bay ranged from 42.3 to 77.0 µg/l (56.6 ± 9.1 µg/l) while that in Napoleon Gulf ranged between 13.9 to 34.1 µg/l (25.3 ± 6.4 µg/l). Therefore Murchison Bay had a significantly higher phytoplankton biomass ($t = 8.46$, $df = 16$, $p < 0.001$) (**Figure 17**). There was a temporal variation in algal biomass with the lowest Chlorophyll-a (13.9 µg/l) on November 23rd and the highest (34.1 µg/l) on December 20th 2016 in Napoleon Gulf. On the other hand, in Murchison Bay, the highest level of chlorophyll-a (77 µ/l) was on the 10th of January 2017 and the lowest (42.3 µg/l) on December 27th 2016. The dry weight however, showed little variation except on November 23rd when it was 4 folds higher in Murchison Bay than Napoleon Gulf. A positive correlation between algal biomass estimates (dry weight) and the measured chlorophyll-a was observed ($r = 0.32$, $p < 0.001$) (**Figure 18**).

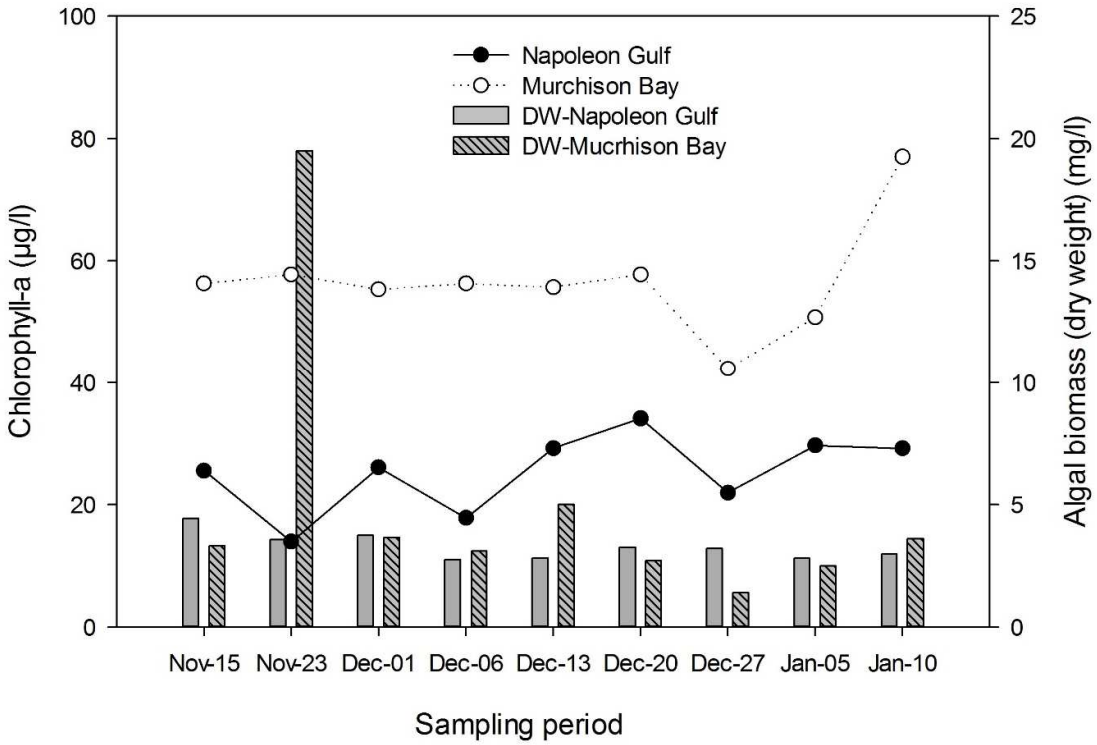

Figure 17: Temporal variation in chlorophyll-a (line graph) and algal biomass estimated as dry weight (bar graphs) of Napoleon Gulf and Murchison Bay measured between November 15th 2016 and January 10th 2017.

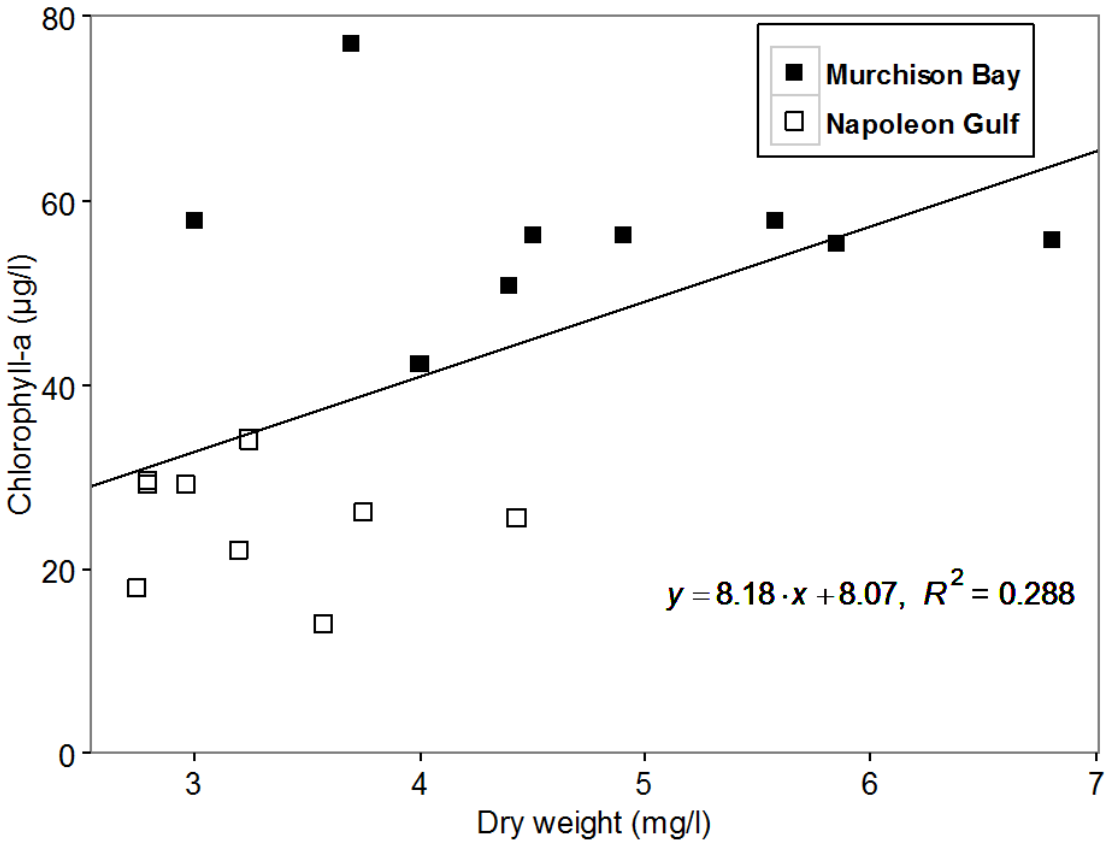

Figure 18: The correlation between chrolophyll-a (µg/l) and algal biomass (dry weight) (mg/l) in Napoleon Gulf and Murchison Bay, northern Lake Victoria measured between November 15th 2016 and January 10th 2017.

4.2.2 Phytoplankton biomass (dry weight) and chlorophyll-a during the water treatment process in Gaba III and Walukuba waterworks

The total phytoplankton dry weight (DW) and chlorophyll-a concentration were higher in lake water than in the raw water of both Gaba III and Walukuba waterworks. The algal biomass (dry weight) in Gaba III ranged from 1.4 to 19.4 mg/l (5.0 ± 5.2 mg/l) and it was between 2.4 and 9.2 mg/l (3.6 ± 1.9 mg/l) in Walukuba waterworks. During the treatment process, dry weight and chlorophyll-a decreased through flocculation and sand filtration **(Figure 19a-b)**. However, a higher dry weight of final chlorinated water in Gaba III than Walukuba waterworks was observed but was not reflected in the chlorophyll-a concentration. The dry weight and chlorophyll-a concentration of clarified and sand filtered water showed little variation throughout the study.

The chlorophyll-a concentration in the raw water ranged from 34.9 to 58.5 µg/l (47.6 ± 7.7 µg/l) in Gaba III and from 5.9 to 23.7 µg/l (12.3 ± 5.42 µg/l) in Walukuba waterworks. The raw water in Gaba III had significantly higher chlorophyll-a

concentration (t = 11.24, *df* = 16, p <0.001) than Walukuba water works. During the treatment process, chlorophyll-a decreased significantly in the final water. In Gaba III waterworks, flocculation reduced chlorophyll a by 89.9% to a mean of 4.8 ± 2.4 µg/l, while sand filtration by 94.4% to only 2.7 ± 1.8 µg/l in the final water. On the other hand, in Walukuba waterworks, flocculation reduced chlorophyll-a only by 63.5% to 4.5±1.1 µg/l after sand filtration by 81% 2.3 ± 0.4 µg/l in final water (**Figure 19b**).

In summary, the ratio of chlorophyll-a/dry weight decreased significantly from approximately 1:100 to 0.1:100 implying that algae were significantly removed during water treatment.

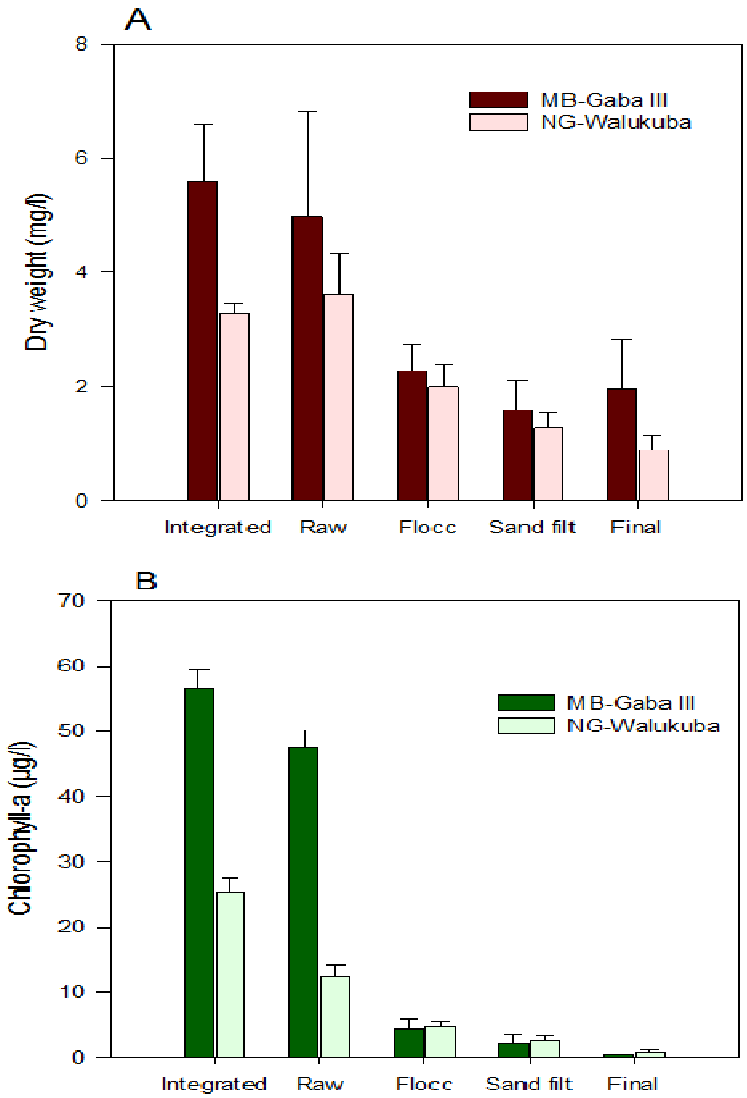

Figure 19: The overall mean algal biomass (dry weight) (mg/l) (A) and chlorophyll-a (µg/l) (B) measured in lake water in Murchison Bay and Napoleon Gulf and during water treatment process at Gaba III and Walukuba waterworks.

4.2.3 Relationship between phytoplankton biovolume chlorophyll-a, and dry weight

Data from this study show a linear relationship between chlorophyll-a and phytoplankton biovolume. The correlation between chlorophyll-a and phytoplankton biovolume was significant ($r = 0.7$, $p<0.001$). The dry weight data points, however, do not show a distinctive pattern with biovolume ($r=0.02$, $p=0.20$) (**Figure 20**) which is in correspondence to the weak relationship between chlorophyll-a and dry weight (**Figure 18**).

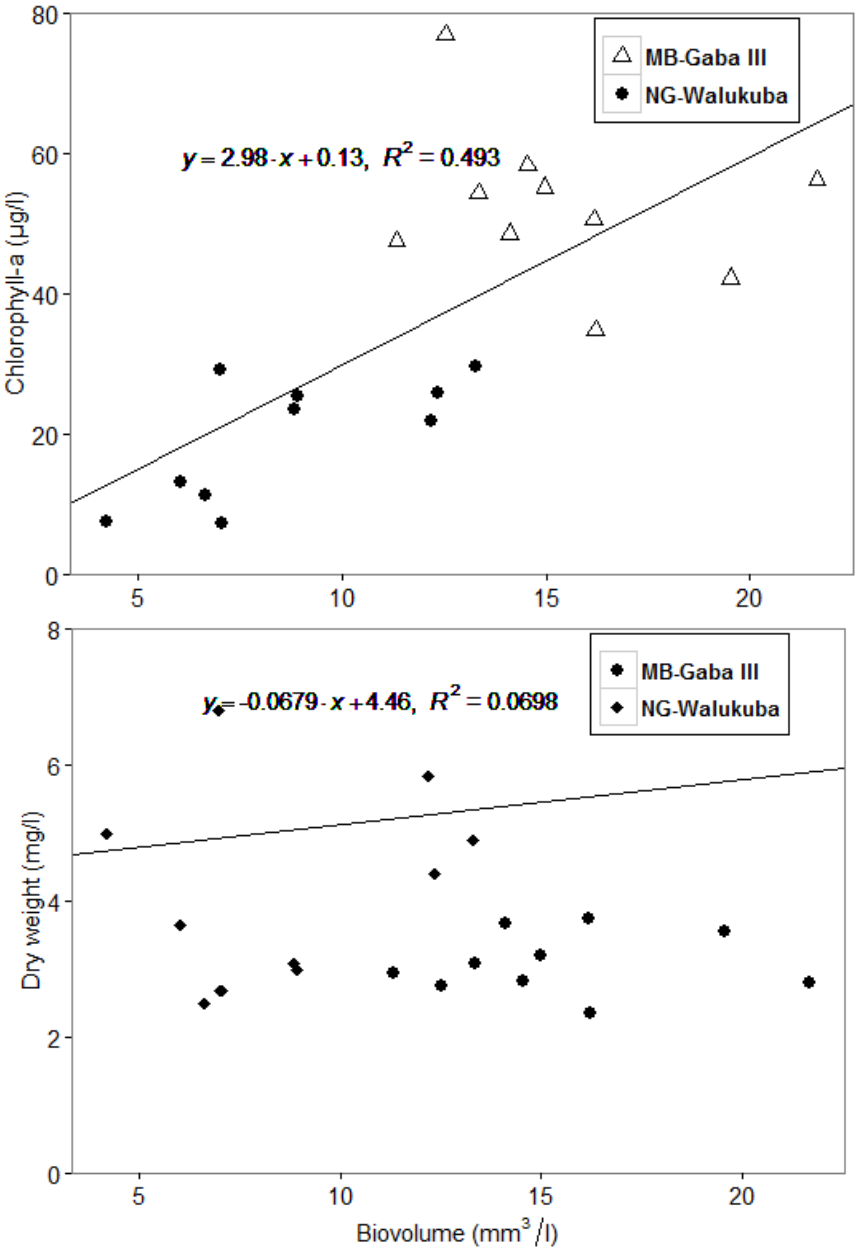

Figure 20: Relationship between chlorophyll-a and phytoplankton biovolume (upper panel) and algal biomass (dry weight) with phytoplankton biovolume (lower panel) in Napoleon Gulf and Murchison Bay.

4.2.4 Phytoplankton species composition in Napoleon Gulf, Murchison Bay, Gaba III and Walukuba waterworks

There were 22 genera of phytoplankton belonging to four major taxonomic groups identified from both lake water in Napoleon Gulf and Murchison Bay and in the raw water in Walukuba and Gaba III water treatment plants. The four identified phytoplankton families were Bacillariophyceae (diatoms), Chlorophyceae (green algae), Cryptophyceae (cryptomonads) and Cyanophyceae (Blue green algae). Both cyanobacteria and green algae had eight genera; diatoms had four and only one genus of Cryptomonas was recognised (**Table 2**). The cyanobacteria *Aphanizomenon* was recorded only Napoleon Gulf while *Actinastrum* was only found in Murchison Bay. Diatoms *Nitzschia* sp., *Aulacoseira* sp. and "centric diatom", were present both in the lake water of the two bays and the raw water of the water treatment plants.

Table 2: The phytoplankton taxonomic checklist, species composition and distribution of phytoplankton in the two bays (Napoleon Gulf and Murchison Bay) and in the raw water of the subsequent water treatment plants. *√* denotes presence.

Class	Genera	Napoleon Gulf	Walukuba Waterworks	Murchison Bay	Gaba III Waterworks
Blue-green	*Anabaena*	√	√	√	√
	Anabaenopsis	√	√	√	√
	Aphanizomenon	√			
	Aphanocapsa	√	√	√	√
	Chroococcus	√	√	√	√
	Merismopedia	√	√	√	√
	Microcystis	√	√	√	√
	Planktolyngbya	√	√	√	√
Cryptomonads	*Cryptomonas*	√	√	√	√
Diatoms	*Aulacoseira*	√	√	√	√
	"Centric diatom"	√	√	√	√
	Navicula	√	√	√	√
	Nitzschia-large	√	√	√	√
	Nitzschia-small	√	√	√	√
Green algae	*Actinastrum*			√	√
	Ankistrodesmus	√	√	√	√
	Closterium			√	√
	Coelastrum	√	√	√	√
	Pediastrum	√	√	√	√
	Scenedesmus	√	√	√	√
	Staurastrum	√	√	√	√
	Tetraedron	√	√	√	√
Total genera		**20**	**19**	**21**	**21**

4.2.5 Phytoplankton species composition and contribution to biovolume

Generally, the phytoplankton biovolume in the lake water was higher than that in the water treatment plants. Cyanobacteria were the most dominant phytoplankton group in both the lake water in Napoleon Gulf and Murchison Bay and the raw water of the respective water treatment plants in Walukuba and Gaba III (**Figure 21 and 22**).

Total phytoplankton biovolume in Napoleon Gulf, ranged from 7.0 to 13.3 mm^3/l (10.8 ± 2.7 mm^3/l) while that in Walukuba water treatment plant ranged from 4.2 to 8.9 mm^3/l (6.6 ± 1.7 mm^3/l) with cyanobacteria contributing most to the biomass (**Figure 21a and b**).

Cyanobacteria contribution varied between 6.7 and 12.4 mm^3/l (9.8 ± 2.6 mm^3/l) in lake water and 3.8 to 8.1 mm^3/l (5.9 ± 1.5 mm^3/l) in Walukuba raw water (**Figure 21c and d**). Cyanobacteria genera *Microcystis*, *Planktolyngbya*, and *Aphanocapsa* contributed most to the cyanobacteria biovolume both lake water and raw water. *Microcystis* and *Planktolyngbya* contributed equitably with each making nearly 45% of the biovolume (**Figure 21c and d**).

In Napoleon Gulf, *Planktolyngbya* contributed highest with biovolume ranging from 2.4 to 5.9 mm^3/l (4.5 ± 1.3 mm^3/l), followed by *Microcystis* contributing between 2.2 and 6.8 mm^3/l (4.4 ± 1.7 mm^3/l) and *Aphanocapsa* contributed between 0.6 and 1.0 mm^3/l (0.8 ± 0.2 mm^3/l) (**Figure 21c**). The cyanobacteria biovolume in the raw water in Walukuba waterworks were lower than the biovolume in the lake water in Napoleon Gulf but followed the sample pattern. During the study *Microcystis* contribution ranged from 1.9 to 4.3 mm^3/l (2.7 ± 1.0 mm^3/l), *Planktolyngbya* was between 1.5 and 3.6 mm^3/l (2.7 ± 0.8 mm^3/l) while *Aphanocapsa* contribution was between 0.3 and 0.7 mm^3/l (0.5 ± 0.1 mm^3/l) (**Figure 21d**). Other genera such as *Anabaenopsis*, *Chroococcus* and *Merismopedia* contributed only ≤ 0.1% of the biovolume.

In Murchison bay, phytoplankton biovolume ranged from 12.5 to 21.6 mm^3/l (17.0 ± 3.6 mm^3/l) and from 11.3 to 16.2 mm^3/l (14.0 ± 1.8 mm^3/l) in raw water in Gaba III water treatment plant with cyanobacteria contributing >90% of the total biomass (**Figure 22a and b**). Cyanobacteria contribution was generally high, ranging from 11.9 to 20.4 mm^3/l (15.9 ± 3.5 mm^3/l) in Murchison Bay lake water (**Figure 22c**) and from 10.1 to 15.0 mm^3/l (12.7 ± 1.8 mm^3/l) in raw water at Gaba III water treatment plant (**Figure 22d**). *Microcystis* dominated the cyanobacteria biovolume contributing to >70% with total biomass ranging from 9.4 to 16.3 mm^3/l (12.3 ± 2.6 mm^3/l) in Murchison Bay lake water (**Figure 22c**) and ranging from 5.7 to 10.8 mm^3/l (8.2 ± 1.8

mm³/l) in the raw water at Gaba III water treatment plant (**Figure 22d**). Total *Planktolyngbya* sp. biovolume ranged from 0.5 to 4.5 mm³/l (2.8 ± 1.4 mm³/l) in the lake water and from 3.2 to 4.1 mm³/l (3.6 ± 0.3 mm³/l) in raw water at Gaba III water treatment plant, which made less ≤15% of the total cyanobacteria biomass (**Figure 22c and d**). Other genera/species such as *Aphanocapsa* sp., *Anabaena* sp., *Chroococcus* sp. and *Merismopedia* sp. contributed <10% of the cyanobacteria biovolume.

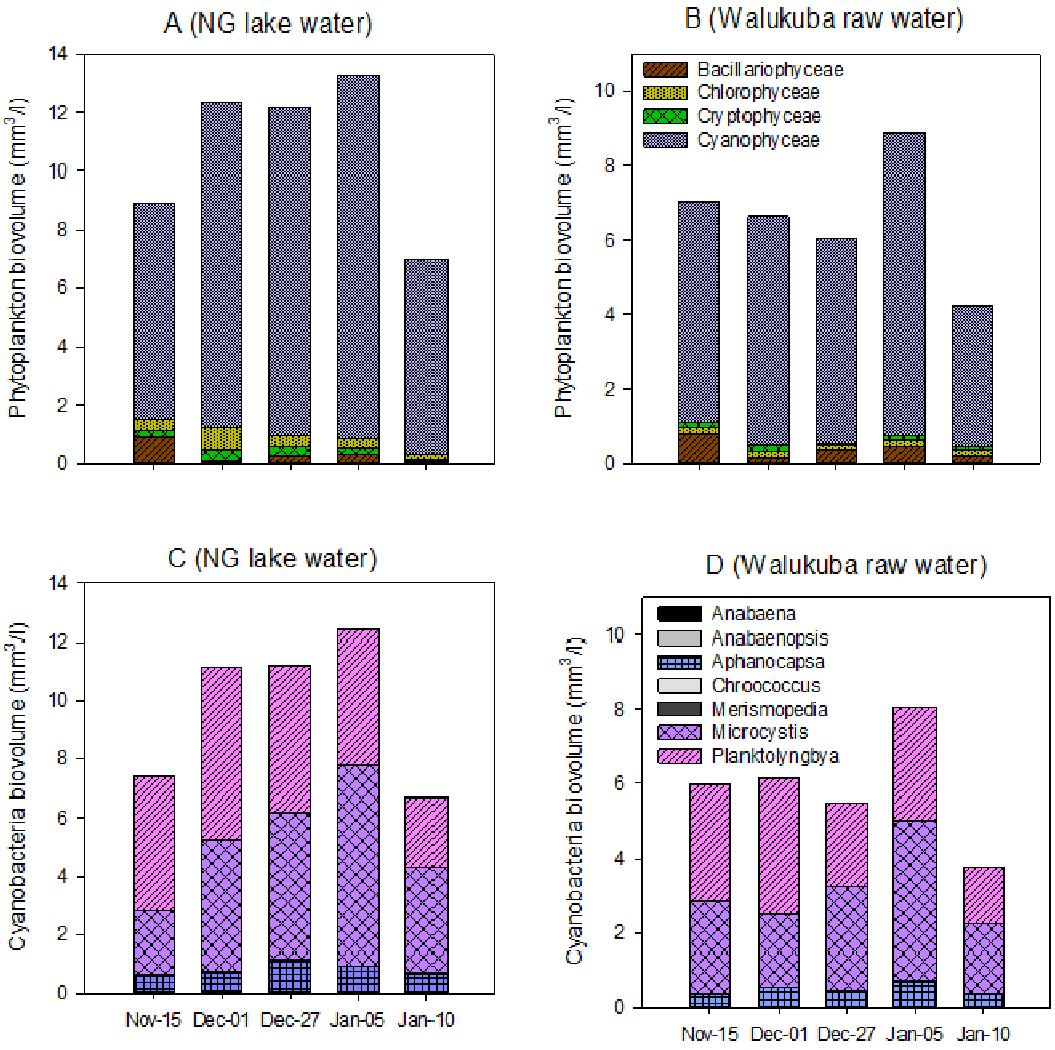

Figure 21: Temporal variation in total phytoplankton biovolume composition by algal classes (above panel) (A & B) and cyanobacterial composition (lower panel) (C & D) of depth integrated water from Napoleon Gulf (NG) and the subsequent raw water at Walukuba waterworks.

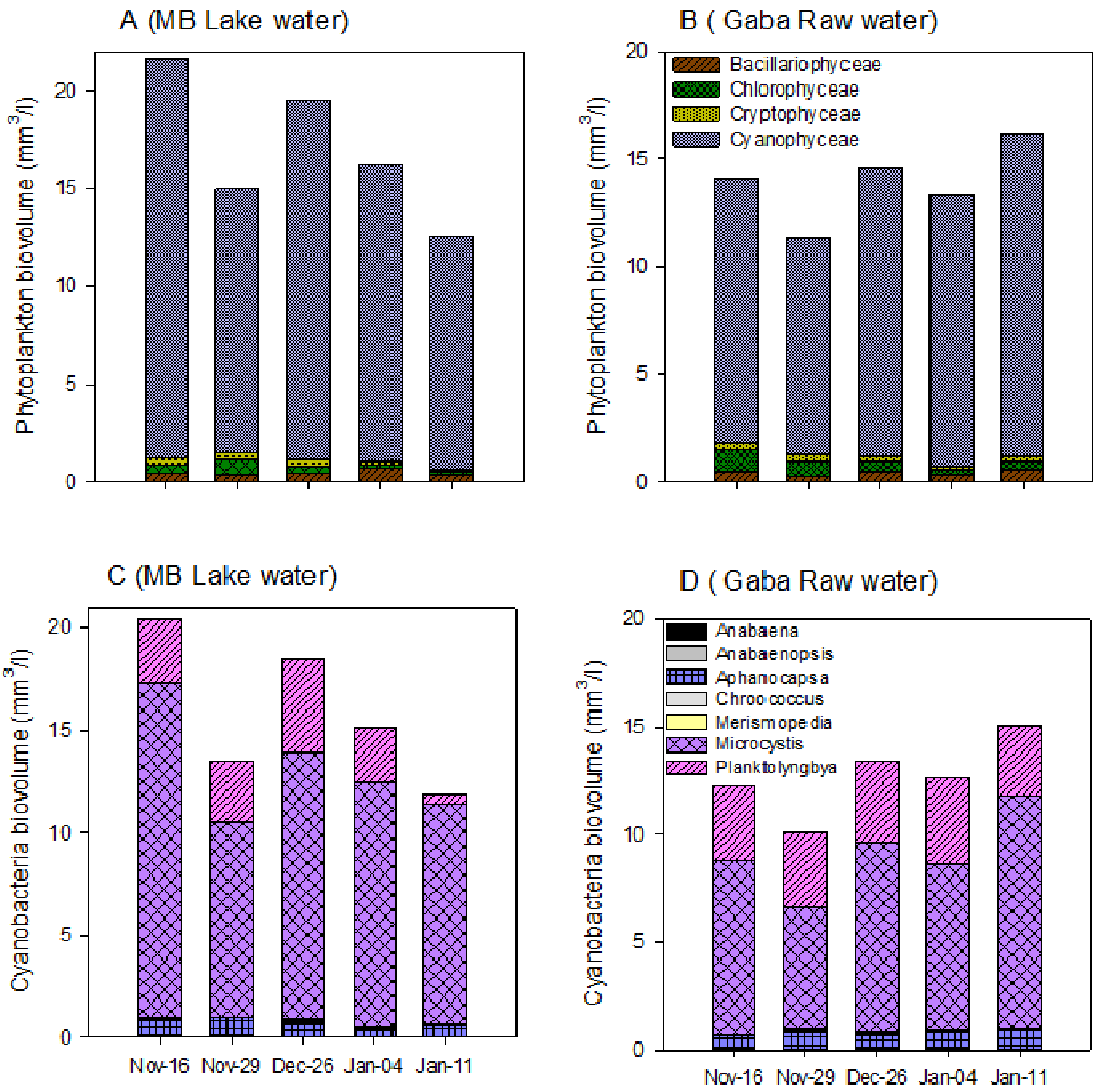

Figure 22: Temporal variation in total phytoplankton biovolume composition in upper panel (A). Depth integrated water from Murchison Bay and raw water from Gaba III waterworks (B). Lower panel shows the cyanobacterial composition in integrated lake water from Murchison Bay (C) and raw water from Gaba III waterworks (D).

In summary, the phytoplankton community in both Napoleon Gulf and Murchison Bay and the respective water treatment plants was dominated by cyanobacteria contributing >70% of the total phytoplankton biomass. The relative cyanobacteria composition were similar i.e. *Microcystis* was dominant, followed by *Planktolynbya* and *Aphanocapsa*. Despite the similarity in relative composition, there was a significant difference in the biovolume between the two confirming the higher phytoplankton biomass in Murchison Bay. The decrease in biomass from the lake water to raw water probably resulted from the abstraction of water from the deeper part of the water column.

4.3 Occurrence and concentration of microcystins

4.3.1 Concentration of analytical microcystin standards used as controls

All the analytical microcystin structural variants (MC-RR, YR and LR) used as positive controls were recovered from C_{18} SPE columns. The concentrations determined by the HPLC-MS were close to 1.0 µg/l as expected from the MC standards (**Table 3**). Occasionally MC-RR and MC-YR concentrations greater than 1.0 µg/l were detected in the lake water from Murchison Bay and raw water at Gaba III water treatment plant while that of MC-YR were ≥ 1.0 µg/l in Napoleon Gulf and Walukuba waterworks.

Table 3: Concentrations of the analytical standard structural microcystin variants used as controls for SPE recovery. MB denotes Murchison Bay and NG is Napoleon Gulf. The parentheses "-" means "not spiked"

	MB-Gaba III (µg/l)			NG-Walukuba (µg/l)		
Water sample	MC-RR	MC-YR	MC-LR	MC-RR	MC-YR	MC-LR
Lake	1.60	1.63	1.45	1.43	1.75	1.91
Raw	-	2.09	-	1.49	-	1.04
Flocc	1.03	1.31	1.35	-	-	-
Sand filter	-	-	-	1.49	1.63	1.78
Final	-	-	-	-	-	1.29

4.3.2 Structural microcystin variants from Murchison Bay and Napoleon Gulf, Gaba III and Walukuba waterworks

The HPLC-MS analysis detected and identified six microcystins variants from both the lake water and raw water of the subsequent water treatment plants (**Table 4**). These variants were determined by their characteristics retention time and mass to charge ratio from the MS.

Table 4: The structural microcystin variants from both lake water and raw water detected by the HPLC-MS

MC variant	**[M+H$^+$]**	**Retention time (min)**
MC-RR	1038.5	13.1-13.3
[NMeSer^7]-MC-RY[a]	1063.5	15.7-16.1
MC-YR	1045.5	17.3-17.5
MC-LR	995.5	18.6-18.9
[Asp^3]-MC-RY[a]	1031.5	20.8
[MeAsp^3]-MC-RY[a]	1045.5	22.1-22.2

[a]Described by Okello et al. (2010a & b)

4.3.3 Total of intracellular (cell bound) concentration and proportion of structural MC variants

All the six structural MC variants were detected in Murchison Bay plankton net samples, integrated water samples and Napoleon Gulf plankton net samples. Throughout the study period, total MC-LR equivalents in both depth integrated and plankton net samples were higher in Murchison Bay than Napoleon Gulf (**Figure 23**). In Murchison bay, [MeAsp^3]-MC-RY contributed most to the total MC-LR equivalent concentration followed by MC-RR and MC-YR in both depth integrated and plankton net samples. In Napoleon Gulf, the structural variants [NMeSer^7]-MC-YR dominated the MC recorded in depth-integrated samples (**Figure 23, Upper panel**) while MC-YR contributed most of the MC in the plankton net samples (**Figure 23, lower panel**).

In summary, most important structural MC variants were the rare [NMeSer^7]-MC-YR, [MeAsp^3]-MC-RY, and MC-YR.

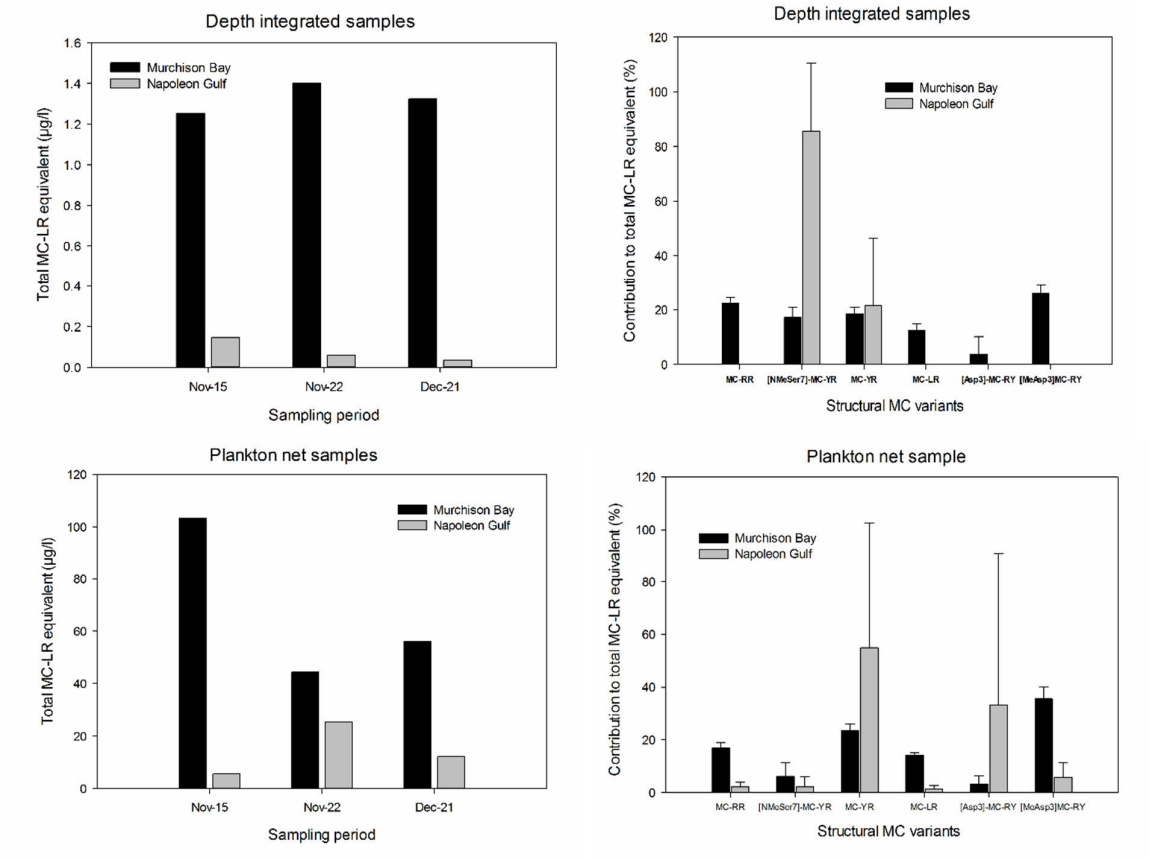

Figure 23: Total MC-LR equivalent (µg/l) and the relative proportions of the structural MC variants (%) in depth integrated samples (Upper panel), and plankton net samples (Lower panel) collected from Murchison bay and Napoleon Gulf. Note: the scales for total MC-LR equivalent are different.

4.3.4 Total concentration of dissolved microcystins detected by ELISA

Total dissolved microcystins were detected by ELISA above the 0.15 µg/l Level Of Detection (LOD) in 62.2% of samples from lake water in Murchison Bay and in Gaba III waterworks (n = 45), 26.6% (n=45) in Napoleon Gulf and Walukuba waterworks (**Table 5**). Concentrations of detection varied between bays and within water treatments plants (**Figure 24**). In Murchison bay, lake water concentrations of detection ranged from 0.1 to 0.4 µg/l (0.3 ± 0.09 µg/l), which increased in the raw water in Gaba III detected between 0.6 and 0.7 µg/l (0.6 ± 0.2 µg/l). In Gaba III waterworks, 100% of the sand filtered water had detectable microcystins concentrations ranging from 0.1 to 2.3 µg/l (1.05 ± 0.8 µg/l). The detectable concentration in final water in Gaba III was below 0.1 µg/l, however, quenched water samples generally revealed a higher microcystin concentration > 1.0 µg/l (**Table 5**).

In Napoleon Gulf and Walukuba waterworks, only lake water (0.13 ± 0.02 µg/l) and raw water (0.12 ± 0.09 µg/l) had concentrations detected above 0.1 µg/l (**Table 5**).

Table 5: The concentration of dissolved microcystins determined as MC-LR equivalent (µg/l) from lake water and during the drinking water treatment process. The parentheses "-" indicate no detection, LOD = level of detection, MB denotes Murchison Bay while NG is Napoleon Gulf.

Station	Date sampled	Lake water	Raw water	Flocc[b] water	Sand filter water	Final water	Quenched* Final water
MB - Gaba	Nov-16	-	-	0.9	1.0	-	
	Nov-22	-	-	-	1.4	-	-
	Nov-29	0.2	0.2	-	1.1	<LOQ	
	Dec-07	0.3	0.5	-	0.1	-	
	Dec-14	0.1	0.3	<LOQ	0.1	-	-
	Dec-21	0.3	0.5	0.1	0.5	-	1.3
	Dec-26	0.3	0.5	0.2	0.6	<LOQ	1.1
	Jan-04	-	0.7	1.0	2.3	-	2.0
	Jan-11	-	-	0.9	2.1	-	-
NG-Walukuba	Nov-15	-	-	-	-	-	
	Nov-23	-	-	-	-	-	-
	Dec-01	-	-	<LOQ	-	-	
	Dec-06	-	<LOQ	<LOQ	<LOQ	-	
	Dec-13	0.1	0.2	0.1	0.1	-	
	Dec-20	0.1	0.2	<LOQ	-	<LOQ	
	Dec-27	-	-	-	-	-	-
	Jan-05	-	-	-	-	-	-
	Jan-10	-	-	-	-	-	-

[b]= clarified water, * = sample treated with ascorbic acid

Generally, the mean total dissolved microcystin detected by ELISA were higher in Murchison Bay and Gaba III waterworks than Napoleon Gulf and Walukuba waterworks (**Figure 24**).

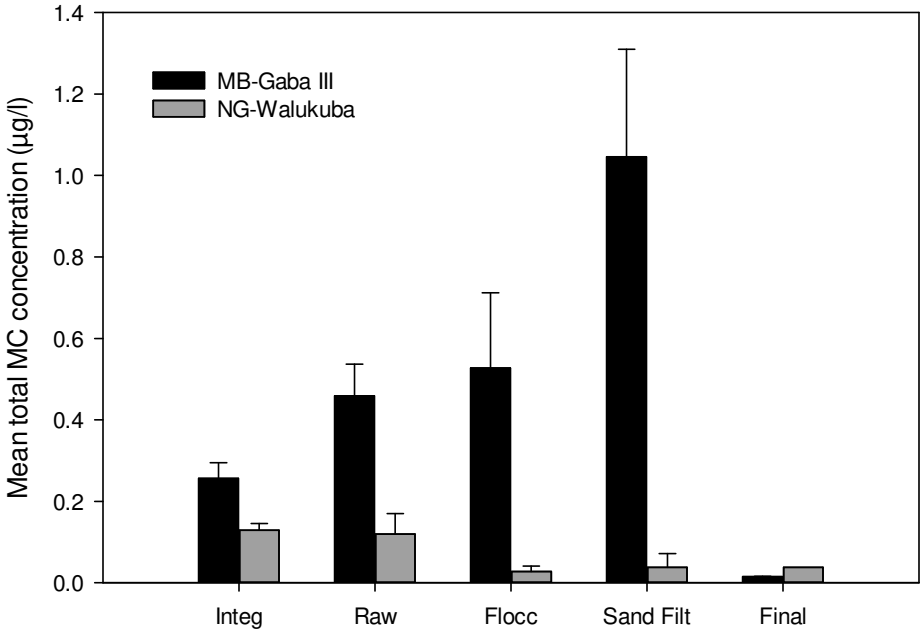

Figure 24: The mean total concentration of dissolved MCs as determined by ELISA in lake water in the two bays and during the water treatment process. MB denotes Murchison bay, NG = Napoleon Gulf, Integ = depth integrated water, Raw = raw water, Flocc = flocculated water, Sand Filt = Sand filtered water, Final = final water

4.3.4 Qualitative correspondence in microcystin detection by ELISA and HPLC-MS

All the C_{18} SPE columns of final water from both Gaba III and Walukuba waterworks were analysed by LC-MS. In addition, samples collected between November 15th-16th and December 20th -21st 2016 were analysed to validate the ELISA results (**Table 7**). Qualitatively, there was high (80%) correspondence in detection between the two methods (**Table 6 and 7**). Both methods showed positive and negative detection. There were four false positive and two false negative detections by ELISA. The false positive detections were in final treated water collected from Gaba III waterworks on November 29th and December 26th 2016. Another false positive detection was in lake water and raw water collected on December 21st 2016. On the other hand, false negatives were in final water collected from Gaba III on January 4th 2017 and sand filtered water collected from Walukuba waterworks on December 20th 2016.

Table 6: Total dissolved microcystins as detected by ELISA, "-"denotes negative, "F-" denotes false negative and <LOQ below level of quantification according to the standard calibration series

Station	Date sampled	Lake water	Raw water	Flocc[b] water	SandFilter water	Final water
MB - Gaba	Nov.16	-	-	0.9	1.0	-
	Nov.22	-	-	-	1.4	-
	Nov.29	0.2	0.2	-	1.1	***<LOQ***
	Dec-07	0.3	0.5	-	0.1	-
	Dec-14	0.1	0.3	*<LOQ*	0.2	-
	Dec-21	**0.3**	**0.5**	0.1	0.5	-
	Dec-26	0,3	0.5	0.2	0.7	***<LOQ***
	Jan-04	-	0.7	1.0	2.3	**F-**
	Jan-11	-	-	0.9	2.2	-
NG-Walukuba	Nov.15	-	-	-	-	-
	Nov.23	-	-	-	-	-
	Dec-01	-	-	*<LOQ*	-	-
	Dec-06	-	*<LOQ*	*<LOQ*	*<LOQ*	-
	Dec-13	0.1	0.2	0.1	0.1	-
	Dec-20	0.1	0.2	*<LOQ*	**F-**	*<LOQ*
	Dec-27	-	-	-	-	-
	Jan-05	-	-	-	-	-
	Jan-10	-	-	-	-	-

Table 7: Total dissolved microcystins concentrated using C18 SPE columns and independently re-analysed using HPLC-MS, the parentheses "-" denotes negative and <LOQ = below level of quantification. Blank spaces – data was not analysed by HPLC.

Station	Date sampled	Lake water	Raw water	Flocc[b] water	SandFilter water	Final water
MB - Gaba	Nov.16	-	-	0.2	0.3	-
	Nov.22					-
	Nov.29					-
	Dec-07					-
	Dec-14					-
	Dec-21	-	-	0.2	0.5	-
	Dec-26					-
	Jan-04					0.1
	Jan-11					-
NG-Walukuba	Nov.15	-	-	-	-	-
	Nov.23					-
	Dec-01					-
	Dec-06					-
	Dec-13					-
	Dec-20	*<LOQ*	*<LOQ*	*<LOQ*	*<LOQ*	0.1
	Dec-27					-
	Jan-05					-
	Jan-10					-

[b] = clarified water

4.3.5 Quantitative correspondence in the total concentration of microcystin detected by ELISA and HPLC-MS

The mean concentrations of dissolved microcystins in the raw water in Gaba III was 0.5 ± 0.2 µg/l (ELISA) but nothing was detected by the HPLC-MS. However, in the final water collected from Gaba III, MCs were detected at 0.02 ± 0.0 µg/l (ELISA) and 0.14 ± 0.0 (HPLC-MS) with the structural variant [NMeSer^{7}]-MC-YR as the major microcystin. Clearly, ELISA did not detect any microcystins in pre-chlorinated water at Gaba III water treatment plant but HPLC-MS detected concentrations of 0.1 ± 0.06 µg/l majorly from MC-YR and [NMeSer^{7}]-MC-YR.

In Napoleon Gulf and Walukuba waterworks, HPLC-MS detected and identified microcystins that were reported by ELISA in all the samples. For instance, in the raw water, concentrations were found at 0.12 ± 0.07 µg/l (ELISA) and 0.03 ± 0.0 µg/l (HPLC-MS) from [NMeSer^{7}]-MC-YR. In final water microcystins were reported at 0.04 ± 0.0 µg/l (ELISA) and 0.06 ± 0.0 µg/l (HPLC-MS) from [MeAsp^{3}]-MC-RY (**Table 8**).

Table 8: The concentration of dissolved microcystin lake water in the two bays (Murchison Bay and Napoleon Gulf) and from the water treatment processes as analysed by ELISA (n =45) and HPLC (n =6*). Only a few samples have been analysed by HPLC-MS.

Station/Site	Treatment	Microcystins (µg/l) (mean ±SE)		Major microcystin variant
		ELISA	HPLC-MS	
MB-Gaba III	Lake water	0.26±0.08	n.d.	-
	Raw water	0.46±0.17	n.d.	-
	Pre-chlorinated water	n.d.	0.11±0.06	MC-YR; [NMeSer^{7}]-MC-YR
	Flocc^{b} water	0.53±0.41	0.12 ±0.02	MC-RR; MC-LR; [NMeSer^{7}]-MC-YR
	Sand filter	1.05±0.75	0.11±0.03	MC-RR; MC-LR; [NMeSer7]-MC-YR
	Final water	0.02±0.00	0.14±0.00	[NMeSer^{7}]-MC-YR
NG-Walukuba	Lake water	0.13±0.02	0.04±0.00	[NMeSer^{7}]-MC-YR
	Raw water	0.12±0.07	0.03±0.00	[NMeSer^{7}]-MC-YR
	Flocc^{b} water	0.03±0.02	0.04±0.00	[NMeSer^{7}]-MC-YR
	Sand filter	0.04±0.03	0.02±0.00	[Asp^{3}]-MC-RY
	Final water	0.04±0.00	0.06±0.00	[MeAsp^{3}]-MC-RY

^{b} = clarified water, *Concentrated by C_{18} SPE columns.

Both the ELISA and HPLC-MS were in correspondence for low concentrations of dissolved microcystins especially in Napoleon Gulf and Walukuba waterworks. There was a moderate positive correlation between the ELISA and HPLC-MS analyses of the dissolved microcystins in water (r = 0.079, p = 0.018) (**Figure 25)**.

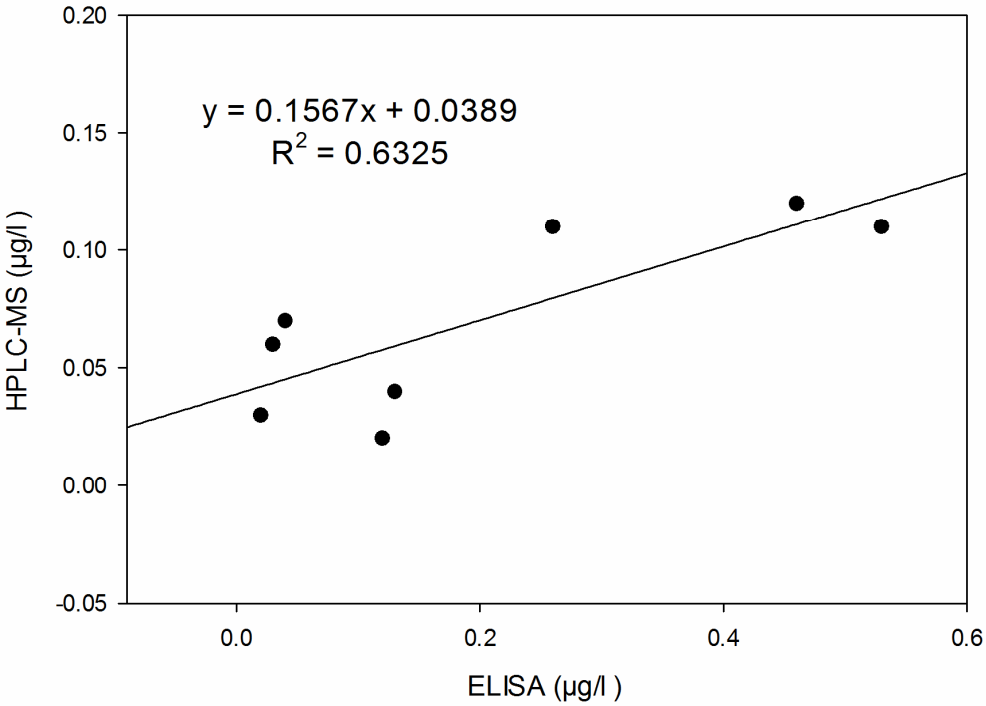

Figure 25: Correlation between the results of ELISA and HPLC analyses of total dissolved microcystin content from Napoleon Gulf, Murchison Bay, Gaba III and Walukuba waterworks, n= 45 (ELISA), and n= 12 (HPLC). Only data positive detection was used.

4.3.6 Relationship between total MC detected by ELISA and cyanobacteria abundance

There was a linear relationship between the concentration of dissolved MC detected, cyanobacteria biovolume and the potential producers of microcystin (**Figure 26**). In general, the relationship between the detected dissolved microcystins and cyanobacteria was significant (r = 0.52, *df* = 18, p = 0.01). From all the potential producers of MCs, only *Microcystis* biovolume was significantly correlated to the levels of MCs detected (r =0.44, df = 18, p = 0.05). The other taxa i.e. *Planktolyngbya* (r = 0.3, df = 18, p= 0.19) and *Aphanocapsa* (r = 0.2, df = 18, p = 0.3) were not significantly correlated to the MCs concentration. Therefore the levels of MCs detected vary dependently with the cyanobacteria composition.

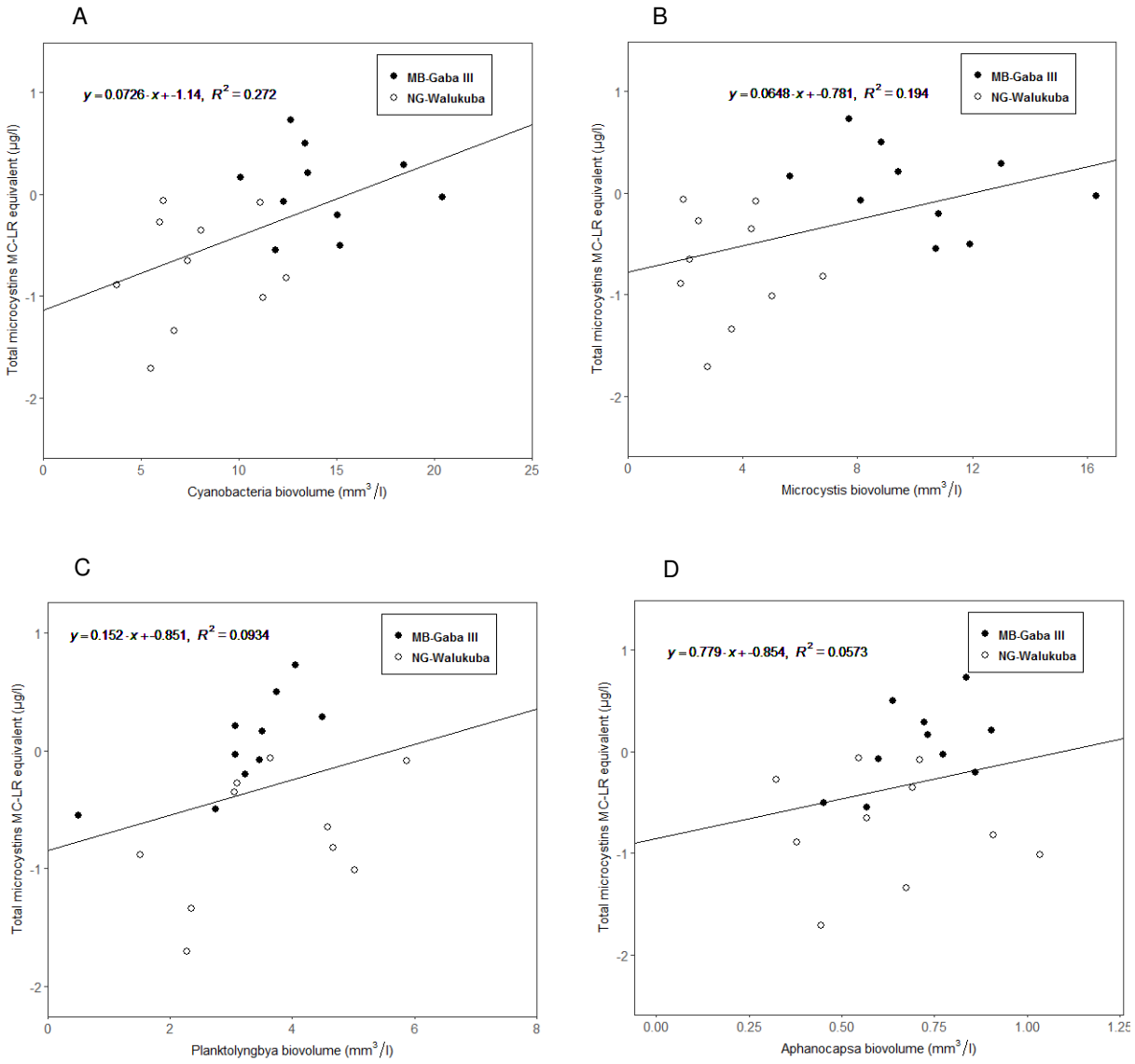

Figure 26: The relationship between dissolved MC concentrations (µg/l) of MC-LR equivalents and A. cyanobacteria biovolume, B. *Microcystis* biovolume, C. *Planktolyngbya* biovolume and D. *Aphanocapsa* biovolume in (mm^3/l) from lake water and raw water during November 2016 to January 2017.

CHAPTER FIVE

5.0 Discussion

5.1.1 Limnological characteristics of Napoleon Gulf and Murchison Bay

Both sampling sites, Napoleon Gulf and Murchison Bay are classified as eutrophic. Eutrophication in both bays and generally the whole Lake Victoria have been reported and emphasised over the past decades (Kling *et al.*, 2001; Haande *et al.*, 2011; Okello & Kurmayer, 2011; Sitoki *et al.*, 2012). This study did not observe any pronounced thermal stratification in Murchison bay and Napoleon Gulf which is in line with Semyalo *et al.* (2009) who reported thermal stratification exceptionally during the wet season (November and March). During the other months, the stratification was not visible in Murchison bay. The absence of thermal stratification suggests that the Napoleon Gulf and Murchison Bay are constantly mixing which is supported by the observed seiches and water exchange in Murchison Bay and the main basin of Lake Victoria (Haande *et al.*, 2011; Luyiga *et al.*, 2015). Both bays have a high temperature (>23°C) which is typical for the tropics. Several studies undertaken in Murchison Bay and Napoleon Gulf have reported water temperature between 23.3-27.8°C (Okello *et al.*, 2010b; Haande *et al.*, 2011), dissolved oxygen between 4 and 9 mg/l (Semyalo *et al.*, 2009), pH ranges from 6 to 9 and conductivity between 94 to 105 (Okello *et al.*, 2010b; Luyiga *et al.*, 2015) which are all in correspondence with this present study. High dissolved oxygen at the surface and close to hypoxia towards the bottom is an indication of eutrophication.

The pH profile in Napoleon Gulf and Murchison Bay are generally high but the decline is prominent in Murchison Bay. The decline in pH can be attributed to bacterial metabolism at the bottom that consumes dissolved oxygen, and thus the hypoxia. This process releases carbon dioxide and enhances the formation of HCO_3 and CO_3 depending on the pH and thus a gradual pH decline is observed with depth (Wetzel, 2001). This suggests that there is more bacterial activity in Murchison Bay than in Napoleon Gulf.

Conductivity in the two bays shows a distinct pattern, as it increases towards the bottom in Murchison Bay and decreases in Napoleon Gulf. Generally, higher conductivity means higher ions (dissolved molecules) present in the water. The higher conductivity is also due to remineralization processes within the water column. There is a decreasing pollution gradient from Nakivubo wetland from the inner Murchison Bay to the more open Murchison Bay (Luyiga *et al.*, 2015) of which the sampling point

in this study is along the gradient. Generally, there is a pronounced biologically stratification depicted in the pH, dissolved oxygen and conductivity due to algal growth.

Water transparency was lower in Murchison Bay than Napoleon Gulf from the Secchi disk because of higher algal biomass and turbidity observed in Murchison Bay. Turbidity measurements from the bays declined with depth and slightly increased towards the bottom on some occasions. The turbidity at the surface might be from algal biomass down to 6 m in Murchison Bay and 3 m in Napoleon Gulf while that at the bottom is possibly from suspended solids and organic matter. Murchison Bay and Napoleon gulf are also is dominated by cyanobacteria that can escape shading by vertical migration.

5.1.2 Water quality in Gaba III and Walukuba waterworks

The raw water temperature, dissolved oxygen, pH and conductivity in both Gaba III and Walukuba were similar to those in the lake water in Murchison Bay and Napoleon Gulf at the depth of abstraction. This is mainly because water is pumped straight to the water treatment plant and thus low retention time. There is generally a high flow through since there is little change in water temperature during water treatment process.

There is a clear influence of chlorine addition to final water in both water treatment plants. Chlorine is an oxidising agent and thus there is a rise in dissolved oxygen. In Gaba III which uses pre-chlorination, the dissolved oxygen concentration increased much earlier.

The influence of the addition of alum/PAC is depicted in the decline in pH after flocculation in both waterworks. , There is a considerable decline in pH of the water and thus requires chemical pH correction of the final water to reach pH 6.5- 8.5 according to WHO (WHO, 2004). The pH in the final water on Gaba III waterworks is therefore achieved by automatic pH correction with the addition of calcium carbonate (Ca_2CO_3) during chlorination. The decrease in pH during water treatment has been reported in a study by Chow *et al.* (1999) in Australia where pH of natural lake water dropped from 8.7 to 6.9 and 7.2 after dosage with 4.8 m/l and 2.8 m/l of alum [$Al_2(SO_4)_3$].

In contrast to the pH, conductivity in final water increased by at least 30 µS/cm in Gaba III and 15 µS/cm in Walukuba waterworks during the treatment process. The increase in conductivity is associated with residual sulphate ions (SO_4^2) in the final water. This has been reported in a number of studies where the ionic strength of final

water increased and pH decreased with increased dosage of Alum (Ndabigengesere & Subba Narasiah, 1998)

5.2 Phytoplankton community, biomass (dry weight) and chlorophyll-a in the lake and during the water treatment process

The phytoplankton community in Murchison Bay and Napoleon Gulf is dominated by cyanobacteria. There is a clear trend towards increasing cyanobacteria dominance in northern Lake Victoria. The increase in the phytoplankton biovolume and biomass might be due to increased nutrient loading (Mugidde, 1993; Kling *et al.*, 2001; Verschuren *et al.*, 2002; Okello *et al.*, 2010b; Sitoki *et al.*, 2012).

The cyanobacteria, *Microcystis* sp. was the dominant species followed by *Planktolynbya* sp. and *Aphanocapsa* sp. in Murchison Bay which is in agreement with earlier studies (Okello *et al.*, 2010b; Semyalo *et al.*, 2010; Haande *et al.*, 2011). In Napoleon Gulf, both the dominance of *Microcystis* sp. and *Aphanocapsa* sp. have been reported by Okello *et al.* (2010b). Despite the dominance of cyanobacteria, diatoms and green algae were recorded throughout the study. Diatoms such as *Nitzschia* sp. and *Aulacoseira* sp. present during this study were the dominant phytoplankton during the 1960s in the nearshore areas (Mugidde, 1993). The frequent occurrence of these former dominant diatom taxa suggests a connection with the main basin and a mixing influence. Nevertheless, the dominance of *Microcystis* sp. in Murchison Bay and other eutrophic lakes in Uganda (Okello *et al.*, 2010b) can be considered stable. Therefore, both Gaba III and Walukuba waterworks produce potable water from cyanobacteria (*Microcystis*) contaminated lake water.

The raw water in the Gaba III and Walukuba waterworks had a similar phytoplankton composition and dominance as in the lake water because the water is pumped directly from the lake. In the early 2000s, the raw water at Gaba II waterworks that abstract water from the inner Murchison bay nearly the same depth as the current Gaba III was dominated by green algae contributing over 420 cells/ml (Kanyesigye *et al.*, 2003). After the commissioning of Gaba III water treatment plant in 2007, an additional treatment process of pre-chlorination to aid coagulation of the algae was recommended. Pre-chlorination is reported to cause cell lysis and release of microcystins during drinking water production (Zamyadi *et al.*, 2012; Zamyadi *et al.*, 2013) unlike coagulation with alum alone (Chow *et al.*, 1999). This was evident in this study when concentrations of about 0.11 µg/l of MC-YR and [NMeSer^{7}]-MC-YR were

detected in pre-chlorinated water at Gaba III waterworks. Although there were detectable levels of microcystins, this was below the WHO guideline.

5.3 Occurrence of microcystins in lake water and drinking water

This study reports the occurrence of dissolved (extracellular) and cell-bound (intracellular) microcystins in depth-integrated water and plankton net samples. Total dissolved microcystins were detected with ELISA and six structural microcystin variants confirmed by HPLC-MS. Coinciding with the dominance with *Microcystis* sp. in the lake water and raw water, co-occurring microcystins were detected.

High levels of total intracellular MC concentrations increase the chances of breakthrough and accumulation of microcystins within Gaba III and Walukuba waterworks.

Flocculation and rapid sand filtration water treatment processes established at Gaba III and Walukuba waterworks have been efficient in removal of algal biomass (chlorophyll-a and dry weight). This is in agreement with reports from Gaba II (Kanyesigye *et al.*, 2003), Gaba and Masaka (Kalibbala *et al.*, 2006) to meet the drinking water quality standards. The algal biomass (dry weight) of final water had consistently higher biomass (weight) but less chlorophyll-a in Gaba III waterworks which could be attributed to solids (compounds) formed after pH correction or formation of chlorination by-products. (Merel *et al.*, 2009; Merel *et al.*, 2010; Westrick *et al.*, 2010).

Chorus & Bartram (1999) indicated that slow sand filtration effectively removed microcystins. This study detected microcystins from rapid gravity sand filtered water in both ELISA and HPLC-MS. The higher amounts of microcystins detected in the sand filter water could be associated with filter clogging and the frequent backwashing due to the high algal biomass in the raw water.

For instance, the levels of microcystins from Gaba III waterworks were occasionally >1.0 µg/l in sand filters which further decreased to about 0.02 µg/l (ELISA) in the final water after chlorination. This decline is probably caused by chlorination since it is the final oxidation step before distribution. Gaba III WTP maintains a residual chlorine of 0.5 mg/l in the final water with a contact time of about 50 minutes in the holding tanks. With this residual chlorine and a holding time of about 30 minutes, microcystins should be effectively removed (Chorus & Bartram, 1999). However, samples quenched with ascorbic acid revealed higher microcystin concentration suggesting that chlorination is not always effective in eliminating

microcystins (Westrick *et al*., 2010). According to Carmichael (2001), the Total Daily Intake (TDI) for microcystin variant MC-LR is 0.04 µg/kg Body weight (BW) or 1.0 µg/l. In this study, this threshold has not been exceeded. However due to the dominance of *Microcystis* sp. and consistent detection of microcystins in both raw water and final water, a potential microcystin breakthrough from increased algal biomass cannot be excluded.

6.0 Conclusion and recommendations

Cyanobacteria were the dominant algal group at both sites. Although there were significant quantitative differences in phytoplankton and cyanobacteria biovolume, the relative composition was similar between the study sites. The cyanobacteria were dominated by *Microcystis* sp. followed by *Planktolyngbya* sp. and *Aphanocapsa* sp. Only cyanobacteria *Microcystis* sp. had a significant linear relationship with MCs detected and thus probably constitute the MC producers. Throughout the study period cyanobacteria (*Microcystis* sp.) producing MCs occur in high number at both sites resulting in MC detection in lake water and raw water. The increased dominance of *Microcystis* sp. is expected to have a higher chance of MC production. Six structural MC variants detected. The variants MC-YR and [MeAsp^{3}]-MC-RY were the most abundant. The rare MC structural variants [NMeSer^{7}]-MC-RY and [MeAsp^{3}]-MC-RY occurring in Gaba and Walukuba final water are generally considered less toxic than MC-LR.

There was generally high flow through the water treatment processes (low retention) with a clear influence of chlorination used as an oxidant on dissolved oxygen concentration and conductivity. The ratio of chlorophyll-a/dry weight decreased significantly from ~1% in raw water to 0.1% in the final water implying that algae were significantly removed from the raw water. There was higher MCs detection in Murchison Bay and Gaba III waterworks by both ELISA and HPLC. However, most of the positive detections were very low concentrations and below Level of Quantification (LOQ).

The more frequent presence of low amounts of dissolved MCs in final water in Gaba III (ELISA and LC-MS) requires a routine monitoring program. Daily consumption of this water, might be a health risk and therefore efforts should be towards the reduction of *Microcystis* sp. in the raw water. During high blooms with levels as high as those reported in the plankton net samples, the treatment procedures applied in Gaba and Walukuba might be insufficient to provide safe drinking water.

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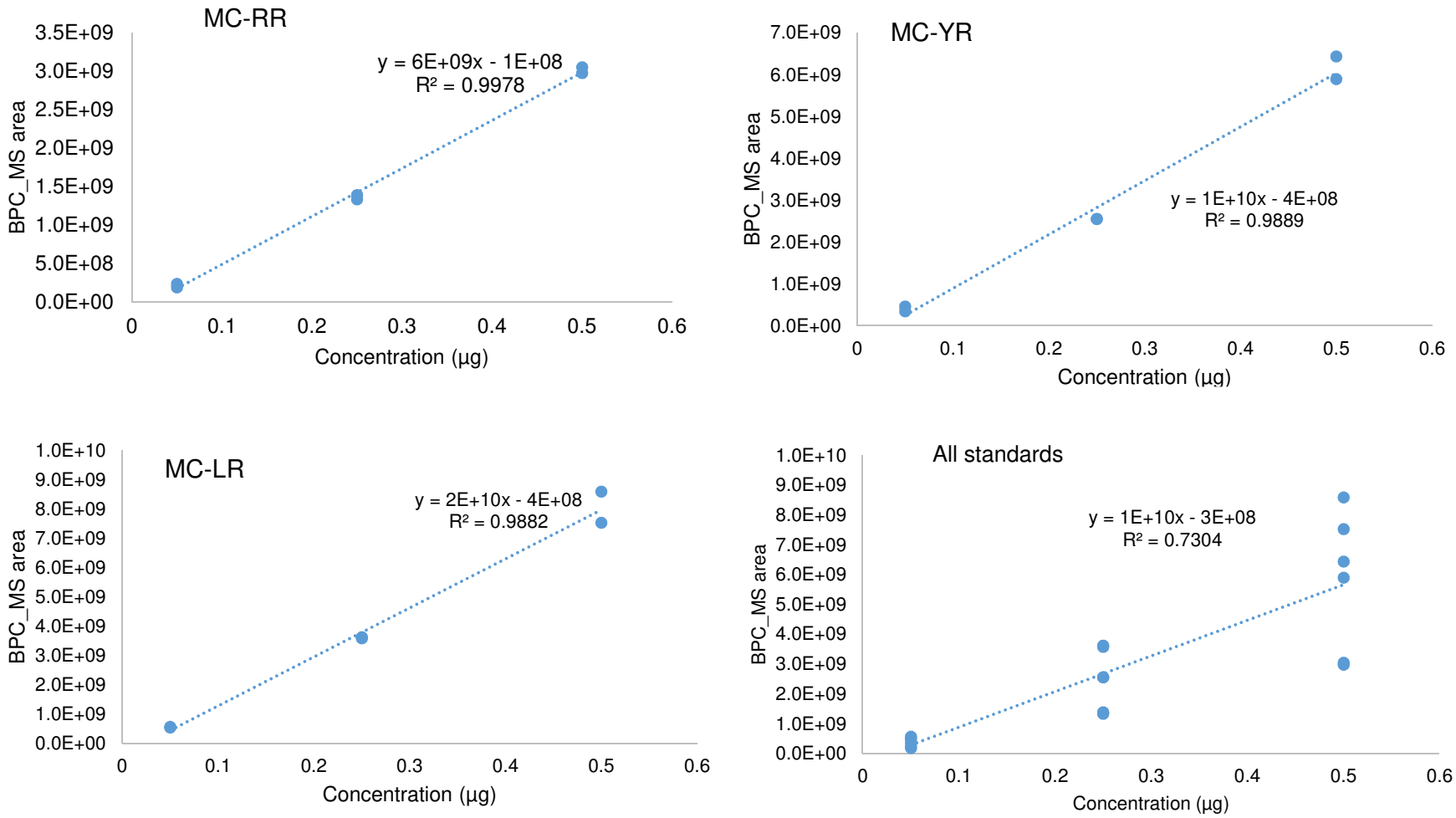
Appendices

Appendix 1: The time plan and schedule for the MSc study

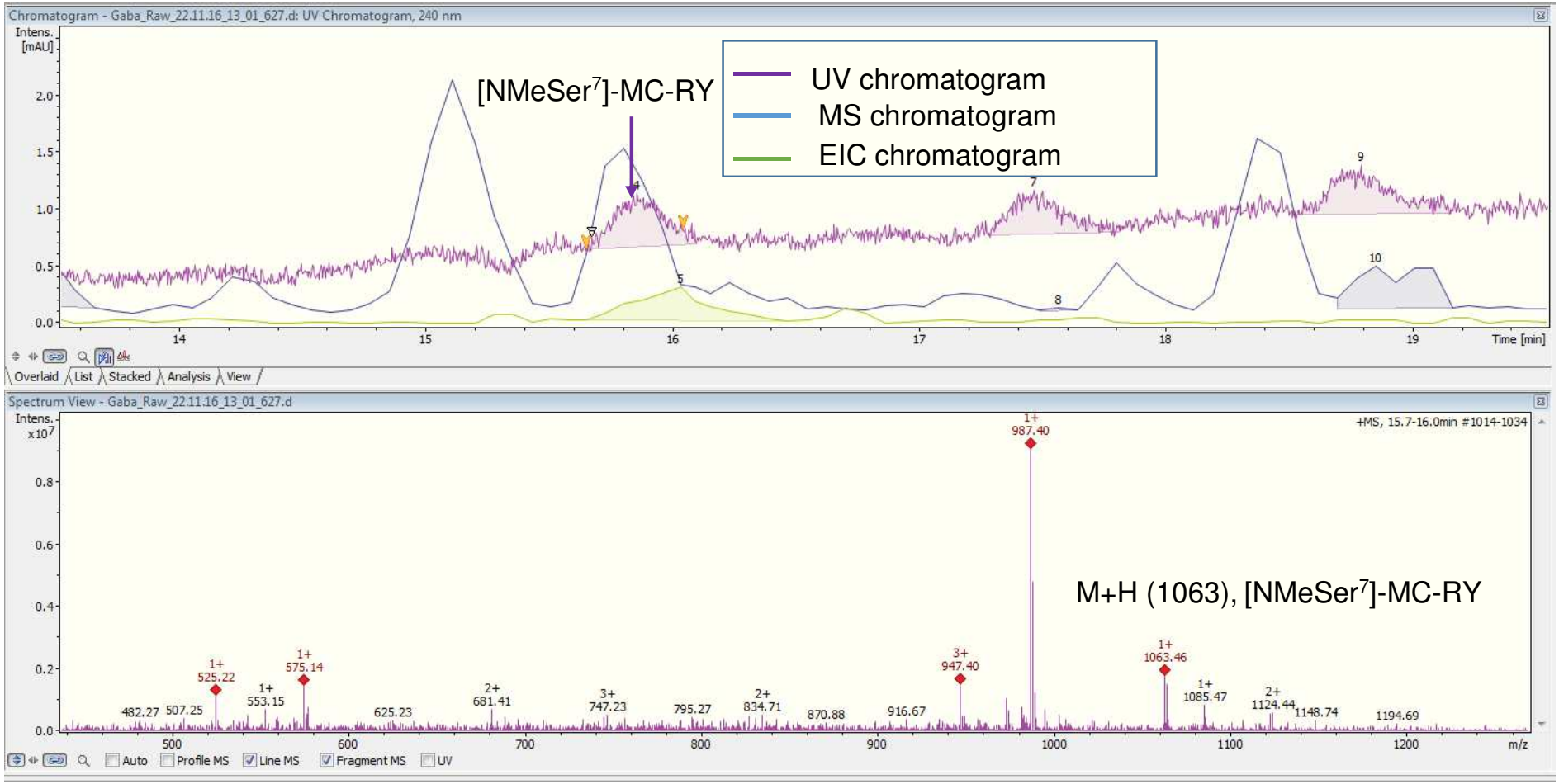
Year	Month	Date	Activity	Location/Notes/Remarks
2016	Sept	5th	Travelling UNESCO-IHE, Delft (Amsterdam) to Vienna, then Mondsee	
	Sept/Oct	5-24th	Finalising draft proposal, development of analytical protocol (hands on Lab training)	Research Institute for Limnology, Mondsee. University of Innsbruck - AUSTRIA
			Training in sample collection and preparation	
	Oct	17th	Travel to Uganda from Vienna to Entebbe to start data collection	
	Oct		Site reconnaissance and Logistics arrangement	Napoleon Gulf, Murchison Bay, NWSC- Gaba (Kampala) & NWSC-Jinja (UGANDA)
			Data collection	Sample preparations (SPE); Chlorophyll-a analysis
	Nov		Data collection	Algal cell counts (Microscopy)
	Dec		Data collection	
2017	Jan		Data collection	
	Jan	17th 18th	Travelling to Austria from Entebbe to Vienna. Proceed to Mondsee for Lab work	
	Jan/Feb		Lab analysis	Research Institute for Limnology, Mondsee. University of Innsbruck
	March		Lab analysis/Write up	
	March	15th	Master thesis seminar	
	March	24th	Submission of draft thesis	University of Natural Resources and Life Sciences, Vienna
	April	5th-27st	Submission defence application, graduation documents and Defense (viva)	

Appendix 2: Budget for fieldwork and sampling for 35 days in Murchison Bay, Gaba III, Napoleon Gulf, and Walukuba WTPs

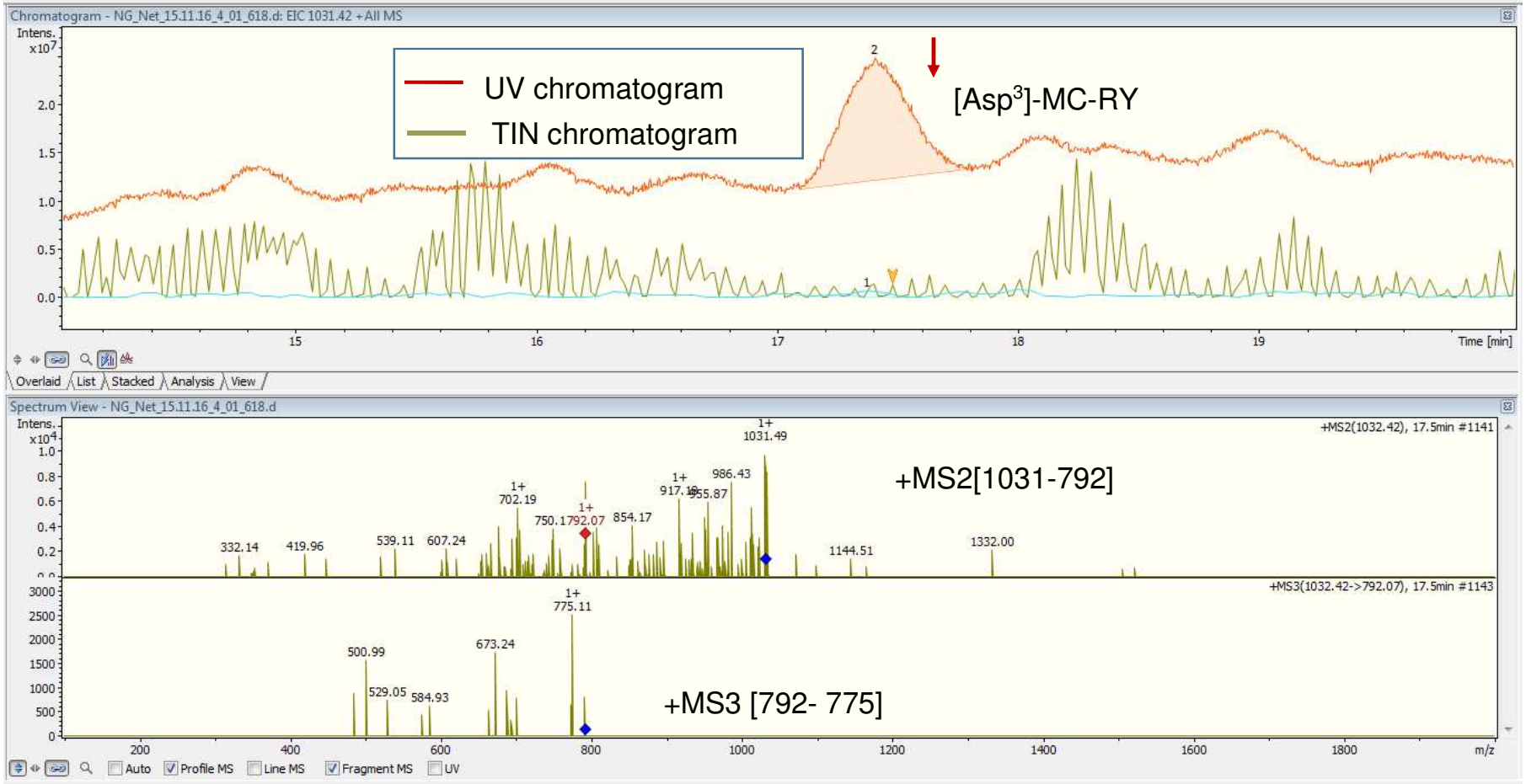
Item	Requirement	Unit cost (UGX)	Description	Total units	Total cost (UGX)	Total cost (EURO) Rate 1 €=3700 UGX
Car-Fuel	24ltrs	3,250	35 days	840	2,730,000	780.00
Car Hire*	1	150,000	35 days	35	5,250,000	1,500.00
Driver	1	12,000	35 days	35	420,000	120.00
Boat	2	40,000	35 days	70	2,800,000	800.00
Petrol-Boat	10ltrs	3,450	35 days	350	1,207,500	345.00
2T-engne oil	1.5ltrs	6,000	35 days	52.5	315,000	90.00
Field assistant	2	12,000	35 days	70	840,000	240.00
Cooler Box (70ltrs)	2	380,000	NA	2	760,000	217.14
Lab- assistant	1	110,000	4 nights/month	12	1,320,000	377.14
Total costs					**15,642,500**	**4,469.29**



Appendix 3: The BPC_MS area standard calibration curves for analytical microcystin standards MC-RR, MC-YR, MC-LR and all the analytical standards combined used to calculate the concentration of MCs in water



Appendix 4: The UV, BPC_MS and Extracted Ion Chromatograph (EIC) of raw sample from Gaba III (November 22nd 2016) that has been manually integrated (above) and the mass to charge ratio (M+H+) of the selected peak for a rare microcystin variant [NMeSer7]-MC-RY



Appendix 5: The UV and Total Ion Chromatogram (TIN) of plankton net sample from Napoleon Gulf (November 15th 2016) that has been manually integrated (above) and the fragmentation pattern of the ($M+H^+$) values of the selected peak for a microcystin variant [Asp^3]-MC-RY

Appendix 6: The maximum concentration of extracellular (cell bound) microcystin variants detected by the HPLC-MS from plankton net samples, depth integrated samples of Murchison Bay (MB) and Napoleon Gulf (NG) and raw water of Gaba III and Walukuba waterworks. Dates: November 2016 and January 2017

			Concentration of cell bound MCs (µg/l)					
MC structural variant	**[M+H$^+$]**	**Retention Time (min)**	**MB - plankton Net**	**MB- Integrated**	**Gaba-Raw water**	**NG - plankton Net**	**NG- Integrated**	**Walukuba Raw water**
MC-RR	1038.5	13.1-13.3	34.6	0.9	1.0	1.2	*n.d.*	<LOQ
[NMeSer^7]-MC-RY	1063.5	15.7-16.1	14.7	0.7	0.7	0.8	0.2	0.1
MC-YR	1045.5	17.3-17.5	47.0	0.7	0.6	31.2	0.1	<LOQ
MC-LR	995.5	18.6-18.9	29.4	0.5	0.6	0.7	*n.d.*	<LOQ
[Asp^3]-MC-RY	1031.5	20.8	7.8	0.1	0.2	5.6	*n.d.*	*n.d.*
[MeAsp^3]MC-RY	1045.5	22.1-22.2	70.3	1.0	1.1	3.6	*n.d.*	*n.d.*

n.d. = not detected, LOQ = level of quantification ~0.1 µg/l

Appendix 7: The minimum, maximum and mean concentration of microcystin variants extracted from plankton net sample (net), integrated water sample (integrated) from the Murchison Bay (MB), Napoleon Gulf (NG) (n = 6) and raw water (n = 7) from the two water treatment plants as detected by HPLC. Dates: November 2016 and January 2017

MC variant	[M+H$^+$]	Retention time (min)	Concentration of cell bound microcystins (µg/l), range (Mean ± Std. Error)					
			MB net	MB integrated	Gaba III raw water	NG net	NG integrated	Walukuba raw water
MC-RR	1038.5	13.1-13.3	8.26-18.02 (11.54±3.24)	0.28-0.30 (0.29±0.01)	0.12-0.38 (0.25±0.05)	0.00-0.75 (0.39±0.22)	-	0.00-0.02 (0.01±0.006)
[NMeSer^7]-MC-RY[a]	1063.5	15.7-16.1	5.23-9.49 (7.35±2.13)	0.20-0.27 (0.23±0.02)	0.08-0.27 (0.19±0.04)	0.00-0.82 (0.28±0.28)	0.04-0.08 (0.06±0.01)	0.01-0.04 (0.03±0.008)
MC-YR	1045.5	17.3-17.5	11.67-22.39 (15.65±3.39)	0.22-0.30 (0.25±0.02)	0.07-0.26 (0.16±0.04)	0.00-21.4 (10.40±6.18)	0.00-0.06 (0.02±0.02)	0.00-0.02 (0.008±0.008)
MC-LR	995.5	18.6-18.9	6.29-15.81 (9.81±3.01)	0.13-0.21 (0.17±0.02)	0.08-0.22 (0.16±0.03)	0.00-0.42 (0.25±0.12)	-	0.00-0.01 (0.003±0.003)
[Asp^3]-MC-RY[a]	1031.5	20.8	3.10-4.66 (3.88±0.77)	0.00-0.15 (0.15±0.0)	0.06-0.13 (0.09±0.03)	0.00-5.55 (2.77±2.77)	-	-
[MeAsp^3]-MC-RY[a]	1045.5	22.1-22.2	18.05-33.02 (23.43±4.80)	0.30-0.40 (0.35±0.03)	0.16-0.41 (0.29±0.05)	0.00-2.86 (1.21±0.85)	-	-

[a]Described by Okello et al. (2010a & b)

Appendix 8: Descriptive statistics for the pH, conductivity (µS/cm), Temperature (°C), dissolved oxygen concentration (mg/l) and saturation (%), for each depth measured in both Napoleon Gulf and Murchison Bay, northern Lake Victoria.

Depth (m)	Station	Parameter	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
.0	Murchison Bay	pH	9	1.12	7.76	8.88	8.3222	.13148	.39445
		Cond. (µS/cm)	9	6.0	113.0	119.0	115.589	.6006	1.8017
		Temp (ºC)	9	1.2	25.4	26.6	26.056	.1444	.4333
		DO (mg/L)	9	1.52	5.62	7.14	6.3244	.17844	.53533
		DO (%)	9	21.9	79.8	101.7	89.567	2.6792	8.0376
	Napoleon Gulf	pH	9	.95	8.64	9.59	9.2711	.09923	.29768
		Cond. (µS/cm)	9	5.8	100.7	106.5	103.711	.6382	1.9147
		Temp (ºC)	9	1.6	26.4	28.0	27.244	.1788	.5364
		DO (mg/L)	9	6.15	5.75	11.90	8.1011	.59062	1.77186
		DO (%)	9	66.0	82.2	148.2	115.178	6.7902	20.3706
1.0	Murchison Bay	pH	9	1.15	7.79	8.94	8.2789	.14085	.42254
		Cond. (µS/cm)	9	5.9	112.6	118.5	114.989	.6297	1.8891
		Temp (ºC)	9	1.1	25.6	26.7	26.144	.1192	.3575
		DO (mg/L)	9	1.41	5.66	7.07	6.2344	.16872	.50616
		DO (%)	9	20.1	80.9	101.0	88.711	2.5090	7.5270
	Napoleon Gulf	pH	9	.89	8.71	9.60	9.2889	.09328	.27984
		Cond. (µS/cm)	9	5.9	100.6	106.5	103.078	.5580	1.6739
		Temp (ºC)	9	1.5	26.4	27.9	27.267	.1546	.4637
		DO (mg/L)	9	3.56	5.80	9.36	7.7044	.37218	1.11655
		DO (%)	9	53.1	83.0	136.1	111.278	5.5100	16.5299
2.0	Murchison Bay	pH	9	1.08	7.84	8.92	8.2656	.14255	.42764
		Cond. (µS/cm)	9	6.2	112.1	118.3	114.878	.7207	2.1620
		Temp (ºC)	9	1.0	25.8	26.8	26.289	.1148	.3444
		DO (mg/L)	9	2.07	5.05	7.12	6.0911	.23425	.70275
		DO (%)	9	29.6	72.2	101.8	87.800	3.6704	11.0112
	Napoleon Gulf	pH	9	.92	8.68	9.60	9.2922	.09606	.28817
		Cond. (µS/cm)	9	6.4	100.3	106.7	103.200	.6305	1.8914
		Temp (ºC)	9	1.2	26.4	27.6	27.167	.1291	.3873
		DO (mg/L)	9	3.51	5.77	9.28	7.6400	.35843	1.07528
		DO (%)	9	52.3	82.7	135.0	110.256	5.3645	16.0936
3.0	Murchison Bay	pH	9	1.31	7.59	8.90	8.2111	.14979	.44937
		Cond. (µS/cm)	9	6.0	112.6	118.6	115.167	.7478	2.2433
		Temp (ºC)	9	1.0	25.8	26.8	26.233	.1067	.3202
		DO (mg/L)	9	2.99	3.92	6.91	5.9022	.30300	.90901
		DO (%)	9	43.5	56.0	99.5	84.111	4.3700	13.1100
	Napoleon Gulf	pH	9	1.14	8.38	9.52	9.2022	.11555	.34665
		Cond. (µS/cm)	9	4.8	100.2	105.0	102.600	.5600	1.6800
		Temp (ºC)	9	1.1	26.4	27.5	27.089	.1148	.3444
		DO (mg/L)	9	3.26	4.89	8.15	7.1056	.34237	1.02710
		DO (%)	9	47.7	70.6	118.3	103.067	5.0202	15.0605
4.0	Murchison Bay	pH	9	1.33	7.54	8.87	8.1667	.14832	.44497
		Cond. (µS/cm)	9	7.0	112.2	119.2	115.700	.8818	2.6453
		Temp (ºC)	9	1.0	25.8	26.8	26.233	.0986	.2958
		DO (mg/L)	9	3.06	3.78	6.84	5.8400	.30283	.90850
		DO (%)	9	44.5	54.0	98.5	83.189	4.3494	13.0483
	Napoleon Gulf	pH	9	1.22	8.28	9.50	9.0289	.14938	.44815
		Cond. (µS/cm)	9	4.0	100.5	104.5	102.011	.4926	1.4777
		Temp (ºC)	9	1.0	26.4	27.4	27.044	.1192	.3575
		DO (mg/L)	9	3.81	4.33	8.14	6.6689	.44187	1.32560
		DO (%)	9	56.4	61.9	118.3	96.611	6.4932	19.4795
5.0	Murchison Bay	pH	9	1.41	7.44	8.85	8.1256	.15310	.45930
		Cond. (µS/cm)	9	7.7	112.8	120.5	116.078	.8953	2.6860

Depth (m)	Station	Parameter	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
		Temp (ºC)	9	1.0	25.8	26.8	26.200	.0913	.2739
		DO (mg/L)	9	3.80	3.02	6.82	5.6456	.36666	1.09999
		DO (%)	9	55.2	43.0	98.2	80.422	5.2671	15.8013
	Napoleon Gulf	pH	9	1.35	8.06	9.41	8.8433	.13403	.40209
		Cond. (µS/cm)	9	4.2	99.9	104.1	101.067	.4500	1.3500
		Temp (ºC)	9	.9	26.4	27.3	26.911	.1073	.3219
		DO (mg/L)	9	3.70	4.59	8.29	6.0600	.38757	1.16270
		DO (%)	9	54.4	65.9	120.3	87.611	5.7068	17.1203
6.0	Murchison Bay	pH	9	1.31	7.39	8.70	8.0633	.13697	.41091
		Cond. (µS/cm)	9	8.2	112.7	120.9	116.333	.9162	2.7486
		Temp (ºC)	9	1.1	25.7	26.8	26.189	.1006	.3018
		DO (mg/L)	9	3.76	2.59	6.35	5.4489	.37724	1.13173
		DO (%)	9	53.7	36.8	90.5	77.544	5.3956	16.1867
	Napoleon Gulf	pH	9	1.48	7.89	9.37	8.5689	.15893	.47680
		Cond. (µS/cm)	9	3.9	99.9	103.8	100.967	.3990	1.1969
		Temp (ºC)	9	.9	26.4	27.3	26.856	.1107	.3321
		DO (mg/L)	9	3.71	4.13	7.84	5.3922	.43721	1.31162
		DO (%)	9	54.0	59.7	113.7	77.222	6.3265	18.9794
7.0	Murchison Bay	pH	9	1.28	7.40	8.68	7.9200	.11789	.35366
		Cond. (µS/cm)	9	11.4	113.5	124.9	118.122	1.1005	3.3014
		Temp (ºC)	9	.7	25.7	26.4	26.078	.0778	.2333
		DO (mg/L)	9	3.64	2.68	6.32	5.0489	.42032	1.26096
		DO (%)	9	52.0	38.1	90.1	71.678	5.9570	17.8710
	Napoleon Gulf	pH	9	1.70	7.64	9.34	8.3289	.19527	.58582
		Cond. (µS/cm)	9	3.8	99.7	103.5	101.100	.3775	1.1325
		Temp (ºC)	9	.9	26.3	27.2	26.789	.1086	.3257
		DO (mg/L)	9	4.86	2.85	7.71	4.9256	.52950	1.58850
		DO (%)	9	70.7	41.1	111.8	72.189	7.7194	23.1581
8.0	Murchison Bay	pH	9	1.10	7.38	8.48	7.8289	.10238	.30714
		Cond. (µS/cm)	9	11.5	113.2	124.7	118.889	1.1444	3.4331
		Temp (ºC)	9	.8	25.6	26.4	26.067	.0799	.2398
		DO (mg/L)	9	3.25	2.62	5.87	4.6511	.36219	1.08658
		DO (%)	9	46.3	37.2	83.5	66.033	5.1321	15.3964
9.0	Murchison Bay	pH	9	.57	7.38	7.95	7.6933	.06083	.18248
		Cond. (µS/cm)	9	9.8	113.7	123.5	119.544	.9723	2.9168
		Temp (ºC)	9	1.0	25.5	26.5	26.011	.0949	.2848
		DO (mg/L)	9	3.06	2.68	5.74	4.2044	.32417	.97251
		DO (%)	9	43.4	38.1	81.5	59.633	4.5841	13.7523
10.0	Murchison Bay	pH	9	.58	7.37	7.95	7.6722	.06502	.19505
		Cond. (µS/cm)	9	12.0	114.1	126.1	120.400	1.1857	3.5570
		Temp (ºC)	9	.9	25.6	26.5	26.011	.0873	.2619
		DO (mg/L)	9	3.35	2.44	5.79	4.0144	.35279	1.05836
		DO (%)	9	47.8	34.6	82.4	56.844	5.0466	15.1397
11.0	Murchison Bay	pH	9	.58	7.34	7.92	7.6267	.06397	.19190
		Cond. (µS/cm)	9	10.3	116.6	126.9	121.256	1.2313	3.6940
		Temp (ºC)	9	.8	25.6	26.4	25.989	.0857	.2571
		DO (mg/L)	9	3.01	2.22	5.23	3.7356	.34850	1.04550
		DO (%)	9	42.8	31.6	74.4	53.189	4.9627	14.8882
12.0	Murchison Bay	pH	9	.45	7.34	7.79	7.5889	.05298	.15894
		Cond. (µS/cm)	9	9.1	118.0	127.1	121.611	1.1572	3.4715
		Temp (ºC)	9	.8	25.5	26.3	25.956	.0835	.2506
		DO (mg/L)	9	2.67	2.30	4.97	3.5300	.32593	.97779
		DO (%)	9	37.9	32.6	70.5	49.944	4.6079	13.8238

Depth (m)	Station	Parameter	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
13.0	Murchison Bay	pH	8	.44	7.34	7.78	7.5963	.04946	.13989
		Cond. (µS/cm)	8	8.9	118.5	127.4	121.800	1.2690	3.5893
		Temp (ºC)	8	.7	25.6	26.3	25.925	.0818	.2315
		DO (mg/L)	8	2.72	2.31	5.03	3.7500	.33782	.95550
		DO (%)	8	38.4	33.0	71.4	52.850	4.6578	13.1743

Appendix 9: Descriptive statistics for the pH, conductivity (µS/cm), Temperature (°C), dissolved oxygen concentration (mg/l) measured throughout the water production chain in Gaba III and Walukuba water works

Treatment plant	Water sample	Parameter	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
Gaba III	Clarified water	Temp (ºC)	9	0.9	25.6	26.5	26.033	0.1225	0.3674
		DO (mg/L)	9	0.25	7.07	7.32	7.1411	0.02721	0.08162
		pH	9	.84	6.88	7.72	7.2011	0.09752	0.29255
		Cond. (µS/cm)	9	17.2	119.7	136.9	128.811	1.8315	5.4944
	Final water	Temp (ºC)	9	1.2	25.9	27.1	26.411	0.1389	0.4167
		DO (mg/L)	9	0.42	6.82	7.24	7.0689	0.03914	0.11741
		pH	9	0.62	6.76	7.38	7.0211	0.06242	0.18725
		Cond. (µS/cm)	9	13.5	125.3	138.8	130.989	1.7557	5.2670
	Raw water	Temp (ºC)	9	1.2	25.6	26.8	26.111	0.1448	0.4343
		DO (mg/L)	9	1.35	5.39	6.74	5.9156	0.12959	0.38878
		pH	9	0.35	7.40	7.75	7.5678	0.03366	0.10097
		Cond. (µS/cm)	9	12.8	112.6	125.4	118.978	1.2394	3.7181
	Sand filters	Temp (ºC)	9	1.0	25.7	26.7	25.978	0.1090	0.3270
		DO (mg/L)	9	0.18	7.27	7.45	7.3578	0.02350	0.07049
		pH	9	0.76	6.89	7.65	7.1700	0.09332	0.27996
		Cond. (µS/cm)	9	16.4	120.5	136.9	129.211	1.9671	5.9014
Walukuba	Clarified water	Temp (ºC)	8	1.0	26.7	27.7	27.338	0.1133	0.3204
		DO (mg/L)	8	1.86	3.10	4.96	3.9038	0.24194	0.68431
		pH	8	1.32	6.68	8.00	7.4537	0.13612	0.38500
		Cond. (µS/cm)	8	21.5	100.3	121.8	105.788	2.4696	6.9850
	Final water	Temp (ºC)	8	0.7	26.8	27.5	27.175	0.0750	0.2121
		DO (mg/L)	8	1.44	5.50	6.94	6.2863	0.22675	0.64134
		pH	8	0.59	7.30	7.89	7.5988	0.07638	0.21603
		Cond. (µS/cm)	8	14.4	103.0	117.4	109.563	1.5994	4.5239
	Raw water	Temp (ºC)	8	1.9	26.6	28.5	27.362	0.1981	0.5605
		DO (mg/L)	8	3.13	2.50	5.63	3.7113	0.34455	0.97454
		pH	8	0.66	7.48	8.14	7.8175	0.08040	0.22739
		Cond. (µS/cm)	8	20.0	99.4	119.4	104.363	2.4932	7.0518
	Sand filters	Temp (ºC)	8	1.0	26.5	27.5	27.013	0.1231	0.3482
		DO (mg/L)	8	2.53	2.04	4.57	3.2163	0.30303	0.85710
		pH	8	0.57	7.17	7.74	7.4250	0.06568	0.18578
		Cond. (µS/cm)	8	8.5	99.7	108.2	103.100	0.9579	2.7092

Appendix 10: Descriptive statistics for turbidity (NTU) for each depth measured in both Napoleon Gulf and Murchison Bay, northern Lake Victoria.

Site	Depth	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
MB	.0	5	7.41	11.65	19.06	14.9840	1.50137	3.35716
	1.0	5	4.91	12.40	17.31	13.6200	.94107	2.10429
	2.0	5	8.51	9.78	18.29	12.9000	1.43981	3.21952
	3.0	5	8.20	9.57	17.77	12.7400	1.36767	3.05820
	4.0	5	5.29	10.23	15.52	12.1120	.90168	2.01623
	5.0	5	4.39	10.34	14.73	11.9840	.75332	1.68447
	6.0	5	4.90	9.69	14.59	11.4380	.92640	2.07150
	7.0	5	4.37	8.42	12.79	10.4780	.91334	2.04230
	8.0	5	3.26	8.89	12.15	10.1620	.58058	1.29822
	9.0	5	2.55	7.66	10.21	9.5940	.49184	1.09979
	10.0	5	2.79	7.46	10.25	9.4000	.49699	1.11131
	11.0	5	4.03	7.17	11.20	9.5620	.66442	1.48569
	12.0	5	7.03	6.43	13.46	9.8380	1.12065	2.50585
	13.0	4	5.81	9.23	15.04	11.1800	1.30871	2.61742
NG	.0	4	3.80	2.74	6.54	4.1325	.85745	1.71490
	1.0	4	3.55	2.84	6.39	4.1950	.78359	1.56717
	2.0	4	2.82	2.79	5.61	3.7800	.62993	1.25987
	3.0	4	.83	2.91	3.74	3.3325	.17026	.34053
	4.0	4	1.48	2.18	3.66	2.7975	.35750	.71500
	5.0	4	1.41	1.69	3.10	2.4075	.39432	.78864
	6.0	4	2.51	1.69	4.20	3.0700	.51886	1.03772

Appendix 11: Descriptive statistics for chlorophyll-a and algal biomass (dry weight) measured in Napoleon Gulf, Murchison bay, Gaba III and Walukuba waterworks between November 2016 and January 2017.

Treatment	Station	Parameter	N	Range	Minimum	Maximum	Mean	Std. Error	Std. Deviation
Final	Gaba III	Dry weight	9	0.0023	0.0002	0.0025	0.0009	0.00025	0.00076
		Chlorophyll-a	9	1.184	.000	1.184	.2962	.1209	.362
	Walukuba	Dry weight	9	0.0083	0.00010	0.0084	0.001955	0.00086	0.0025
		Chlorophyll-a	9	2.36	.000	2.36	.822	.260	.78
Flocc	Gaba III	Dry weight	9	0.0030	0.00060	0.0036	0.001996	0.00039	0.001171
		Chlorophyll-a	9	12.73	.888	13.62	4.51	1.35	4.07
	Walukuba	Dry weight	9	0.0045	0.00030	0.0048	0.002266	0.00046	0.001396
		Chlorophyll-a	9	7.10	1.18	8.29	4.79	.814	2.44
Integrated	Gaba III	Dry weight	9	0.0016	0.00275	0.0044	0.003280	0.00018	0.000555
		Chlorophyll-a	9	34.72	42.29	77.01	56.56	3.031	9.09
	Walukuba	Dry weight	9	0.0101	0.00300	0.0131	0.005583	0.00101	0.003037
		Chlorophyll-a	9	20.14	13.92	34.06	25.25	2.12	6.36
Raw	Gaba III	Dry weight	9	0.0068	0.00235	0.0091	0.003608	0.00070	0.002119
		Chlorophyll-a	9	23.61	34.88	58.50	47.56	2.55	7.67
	Walukuba	Dry weight	9	0.0181	0.00140	0.0195	0.004972	0.00184	0.005534
		Chlorophyll-a	9	17.77	5.92	23.69	12.32	1.81	5.43
Sand Filter	Gaba III	Dry weight	9	0.0024	0.00040	0.0028	0.001288	0.00025	0.000779
		Chlorophyll-a	9	11.27	.59	11.86	2.25	1.20	3.62
	Walukuba	Dry weight	9	0.0044	0.00010	.0045	0.001577	0.00053	.0015943
		Chlorophyll-a	9	5.92	0.592	6.516	2.66	0.61	1.83