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# Cyanotoxins occurrence in drinking waters and risk of exposure to human in Ukerewe district Mwanza, Tanzania

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**CYANOTOXINS OCCURRENCE IN DRINKING WATERS AND RISK  
OF EXPOSURE TO HUMAN IN UKEREWE DISTRICT MWANZA,  
TANZANIA**

**Geofrey Joseph Mchau**

**A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of  
Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of  
Science and Technology**

**Arusha, Tanzania**

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## ABSTRACT

There is global concern regarding the increase of cyanobacteria and cyanotoxins in freshwater and their potential effects on human health. This study was conducted to determine the occurrence of cyanotoxins and assessed their risk of exposure to human. A cross sectional study of 432 subjects was conducted to assess related health risk due to cyanobacteria and cyanotoxins exposure in selected villages of the Ukerewe District in Mwanza, Tanzania. A total of 138 water samples and 432 serum samples were collected in two phases (February and December). Thirteen cyanotoxins namely; Microcystins (-LA, -LF, -LR, -LY, -LW, -RR, -YR, -WR, dm MC-RR and dm MC-LR), anatoxin-a (AT-A), nodularin (NOD) and cylindrospermopsin (CYN) were assessed in water and in human serum by UPLC-MS/MS. Cylindrospermopsin was the most abundant cyanotoxin detected in the lake water samples in both phases. Microcystin (MC) congeners; -RR, -LR and -YR were detected in phase I while MC-RR and MC-LR were detected in phase II. No cyanotoxins were detected in wells and treated pipe water samples. Furthermore, phycocyanin concentration detected in Lake Victoria ranged from 5 to 58.4 µg/L which is above the WHO limit. The concentrations of cyanobacteria cells were beyond WHO acceptable limits. Species of *Microcystis aeruginosa* and *Anabaena* spp were identified as the most abundant cyanobacteria. Acute illnesses such as throat, eye, skin irritation and gastrointestinal illnesses were highly reported by lake water users as compared to wells and pipe water ( $P<0.001$ ). Cyanotoxins of CYN, NOD and MCs congener (-LR, -RR and dmMC-LR) were detected in human serum. The concentration of CYN detected in humans ranged from 0.02 to 0.15 ng/mL and MCs ranged from 0.2 to 0.11 ng/mL. Concentration of cyanotoxin detected in human serum and liver biochemistry indices elevation, shows an association between the two with correlation coefficient of 0.33 for MC-LR while for combined cyanotoxins of MC-LR, CYN and NOD is 0.78. This is the first study to report CYN, dm MC-LR and NOD in human serum, and CYN and NOD in freshwater of Lake Victoria. This study indicates the potential health risk of using lake water without any treatment for human consumption.

**DECLARATION**

I Geoffrey Joseph Mchau, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my own work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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The supervisor(s) should certify that they have read the thesis, and found it to be in a form acceptable for examination. The statement is for the initial submission; at the final submission, the supervisor should sign again and certify for acceptance.

The undersigned certify that they have read and hereby recommend for acceptance for the thesis entitled “Cyanotoxins occurrence in drinking waters and risk of exposure in Ukerewe district Mwanza, Tanzania.” In fulfilment of the Award of Doctoral of Philosophy in Food and Nutrition Science at The Nelson Mandela African Institution of Science and Technology

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## **DEDICATION**

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## LIST OF ACRONYMS AND ABBREVIATIONS AND SYMBOLS

ATX-A	Anatoxin-a
AEIA	Acetylcholine Esterase Inhibition Assay
ALP	Alkaline Phosphate
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BGAS	Blue Breen Algae Supplements
BIL-D	Direct Bilirubin
BIL-T	Total Bilirubin
BMAA	Beta-Methylamino-L-alanine
Cfas	Calibrator for Automated Systems
cOR	Crude Odds ratio
CYN	Cylindrospermopsin
ELISA	Enzyme-Linked Immunosorbent Assay
HAB	Harmful Algal Bloom
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
IFCC	International Federation of Clinical Chemistry
LOD	Limit of Detection
LOQ	Limit of Quantification
LV	Lake Victoria
MC	Microcystin
MC-LA	Leucine and Alanine in the Positions of X and Z of Microcystin
MC-LF	Leucine and Phenylalanine in the Positions of X and Z of Microcystin
MC-LR	Leucine and Arginine in the Positions of X and Z of Microcystin
MC-RA	Arginine and Alanine in the Positions of X and Z of Microcystin
MC-RR	Arginine and Arginine in the Positions of X and Z of Microcystin
MC-RY	Arginine and Tyrosine in the Positions of X and Z of Microcystin
MC-YA	Tyrosine and Alanine in the Positions of X and Z of Microcystin
MC-YR	Tyrosine and Arginine in the Positions of X and Z of Microcystin

MS/MS	Mass Spectrometry Mass Spectrometry
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucleotide (reduced form)
NHL-QATC	National Health Laboratory Quality Assurance and Training center
NIMR	National Institute for Medical Research
NM-AIST	Nelson Mandela-Africa Institute of Science and Technology
NOD	Nodularin
OATPS	Organic Anion-Transporting Polypeptides
ODK	Open Data Kit
PC	Phycocyanin
PP1 and PP2A	Protein Phosphatases type 1 and 2A
QUB	Queens University of Belfast
STX	Saxitoxins
TDI	Tolerable Daily Intake
TP	Total Protein
TQ-MS	Triple Quadruple Mass Spectrometer
UPLC-MS/MS	Ultra Performance Liquid Chromatography Mass Spectrometry
US-EPA	United State – Environmental Protection Agency
WHO	World Health Organization



# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of the problem

Cyanobacteria have been present on the earth for more than three billion years. Subsequently, they have colonized almost all terrestrial and aquatic ecosystems. Cyanobacteria, also known as blue-green algae, are ancient Gram-negative prokaryotes with fossil records of 3500 million years of the Earth's history (Falconer & Humpage, 2005). They are most abundant in aquatic habitats as part of the plankton and benthos, and some can be found on surfaces of plants, rocks and in extreme environments e.g. hot springs and arctic ice (Whitton & Potts, 2007). Cyanobacteria causes a number of effects including being nuisance in water, change of water taste and odour due to compounds including geosmin and methyl isoborneol, discoloration, increased turbidity, foul smell and foam formation. Cyanobacterial blooms also interfere with water treatment work. Of particular concern, some cyanobacteria (toxic cyanobacteria) are known to produce cyanobacterial toxins (cyanotoxins) that can affect a variety of organisms, including humans, domestic animals and wildlife (Codd *et al.*, 1999).

Toxic cyanobacteria produce secondary metabolites known as cyanotoxins, which have adverse impacts on aquatic ecosystems and human health (Tomitani *et al.*, 2006). Cyanotoxins have now been reported in several parts of the world including Africa, United Kingdom, USA, China, and Australia (Roegner *et al.*, 2014). Toxic cyanobacteria produce a wide range of secondary metabolites (cyanotoxins), the leading producers being *Microcystis*, *Cylindrospermopsis*, *Anabaena*, *Lyngbya*, *Nostoc*, and *Plantothrix* (Boopathi & Ki, 2014). The most researched cyanobacteria are *Microcystis aeruginosa*, which produce over 90 different types of toxins and are one of the most widespread occurring cyanobacteria worldwide (Merel *et al.*, 2013).

Cyanobacteria including the harmful algal bloom *Microcystis aeruginosa* are found in lakes, ponds, rivers and other surface waters, which are used as sources of drinking water and other domestic activities. Harmful algal blooms (HABs) such as *Microcystis aeruginosa*, *Anabaena*, and *Cylindrospermopsis* in freshwater are of general concern owing to their ability to produce cyanotoxins that can potentially cause health problems. Cyanotoxins are classified based on their modes of action in invertebrates. They comprise of hepatotoxins such as microcystins (MC), nodularin (NOD) and cylindrospermopsin (CYN), neurotoxins

such as anatoxin-a (ATX-A), anatoxin-a (S) (ATX-a (S) and saxitoxins (STX), lastly dermatotoxins includes; lipopolysaccharide (LPS) endotoxins and marine toxin (Carmichael *et al.*, 2001). Among the cyanotoxins, MC is the most common one with the following congener MC-LR, MC-RR and MC-YR, which are potential contaminants of drinking water. Therefore, the World Health Organization (WHO) has proposed a provisional guideline for the acceptable limit of concentration of 1.0 µg/L for Microcystin-LR in drinking water (WHO, 2008). The International Agency for Research on Cancer (IARC) has categorized MC-LR as one of the possible human carcinogens in group 2B with strong evidence supporting that can exhibit tumor promotion mechanism (IARC, 2010). MC exposure can cause acute toxicity leading to several poisoning outbreaks. The most evident case of MC poisoning outbreak involved 131 patients at the kidney dialysis centre in Brazil. The report showed that 56 patients died and 44 presented symptoms related to MC intoxication such as acute neurotoxicity and hepatotoxicity symptoms (Pouria *et al.*, 1998). Enlarged liver due to acute hepatitis and raised levels of blood bilirubin and alkaline phosphatase, are the indicators of MC (YR, LR and AR) in serum and liver tissues of the exposed patients (Yuan *et al.*, 2006). Cyanotoxins related exposure illnesses to human vary according to the type of toxins and the modes of exposure, including swimming, bathing, inhalation, haemodialysis and ingestion. Several acute effects such as nausea, vomiting, diarrhoea, mouth blisters, irritation of skin, throat, and eye are all related to exposure to cyanobacteria and cyanotoxins (Kibria, 2016). Through absorption, microcystin is transported to the liver by organic anion transport proteins where they exert their toxicity via inhibition of protein phosphatases 1 and 2A and promote tumour formation (Runnegar *et al.*, 1991). Cylindrospermopsin can also inhibit protein synthesis and is a potential carcinogen due to its cytotoxicity and genotoxicity (Moreira *et al.*, 2013).

Globally, few countries have set up surveillance systems that monitor incidences of HABs related illnesses as well as establishing country-specific tolerable limits for the concentration of cyanobacteria cell density in drinking water for humans. Many countries without such surveillance systems use the WHO general guidelines, which are not country-specific. The World Health Organization (WHO, 2003) has proposed guidelines for different cell densities of cyanobacteria bloom levels and related illnesses. Guideline for recreational waters cyanobacteria cell densities of 20 000 cells/mL is associated with risk of short-term illnesses, and at a higher cell density of 100 000 cells/mL an additional risk for long-term illness exists. Toxic cyanobacteria scum in bathing areas is associated with severe health outcomes such as

throat and skin irritation, headache, fever and gastrointestinal illness (WHO, 2003). Due to the non-specific nature of the symptoms of HABs related illnesses and lack of specific guidelines for surveillance and diagnosis, it is difficult for health-care providers to identify and report HABs associated illnesses. This causes a severe public health omission in the identification of HABs related effects in humans. Therefore, health care providers should be aware of the HABs and cyanotoxins similar symptoms as illness may present otherwise from other recreational water-associated illnesses, and onset may occur soon after exposure.

Lake Victoria has been reported to face eutrophication for the last four decades, resulting in elevation of toxin-producing cyanobacteria levels in all seasons of the year (Ngupula *et al.*, 2011). Eutrophication is the process of increased primary production of a water body as it gets enriched with nutrients especially phosphorus (P) and nitrogen (N) (Mbonde *et al.*, 2004). Toxin producing cyanobacteria such as *Microcystis* and *Anabaena* which, produce microcystin (MC) and other toxins, has been identified and documented in all countries surrounding the Lake Victoria in East Africa. Studies conducted in Tanzania, Kenya and Uganda revealed the existence of HABs and cyanotoxins (Sekadende *et al.*, 2005; Okello *et al.*, 2010; Sitoki *et al.*, 2012a). Rising temperatures have been considered to be a contributing factor to the increase in algal bloom globally and since the continent of Africa is heating up faster than the rest of the world, its expected that the consequences of an increase in HABs and cyanotoxins in its freshwaters will be higher in most of African countries along the tropical (Liu *et al.*, 2011). This emphasizes the importance of strengthening water safety surveillance on cyanobacteria bloom, especially by monitoring the concentration of existing and emerging cyanotoxins.

In Tanzania, studies conducted in Lake Victoria reported the presence of cyanobacteria bloom and cyanotoxins which persist for more than one decade, the presence of cyanotoxins might cause livestock, human health effects and endanger aquatic ecosystem (Miles *et al.*, 2013; Mbonde *et al.*, 2015). There is a documented and observed seasonal variation of the concentration of the algal bloom in the rainy and dry seasons in Lake Victoria. This seasonal variation in algal bloom concentration is due to the availability of nutrient and temperature changes (Okello *et al.*, 2010). The variation of water quality parameters concentrations in water may enhance cyanobacteria growth and increase the availability of toxins. These water quality parameters include phosphorus and nitrogen, pH, temperature, electrical conductivity (EC), and dissolved oxygen (DO) (Marion *et al.*, 2012). Increase in human activities and

environmental contamination of the lake has led to the eutrophication of water and thus negatively affecting the water quality of the lake water. Portable water is essential for daily human activities worldwide; most of the world's population depends on surface freshwaters as their primary source for drinking, food preparations, and other domestic use. Little information is available on the extent of exposure of the lake residents in Tanzania on the cyanobacteria and cyanotoxin. The aim of this study was therefore, to assess the occurrence of cyanotoxins and to assess the risk of exposure in human subjects through contaminated drinking water among Lake Victoria populations.

## **1.2 Statement of the problem**

Studies conducted on Lake Victoria reported presence of cyanobacteria bloom for the past one decade (Miles *et al.*, 2013). Despite this fact, very little is known on the occurrence of a wide range of cyanotoxins and their risk of exposure to human residing around Lake Victoria. This is due to the limited extensive epidemiological data regarding route and circumstances of exposure that can establish the linkage of exposure and human health effect, to date there is only one study conducted in China which detail health related risk due to exposure of cyanotoxins globally (Chen *et al.*, 2009). Diseases related to cyanobacteria and cyanotoxins are largely ignored health problem hence. On other hand diagnosis of chronic exposure to cyanotoxins by estimating the consumed toxins in the human body is complicated and nearly impossible due to metabolic activities that take place in the body when exposed to cyanotoxins (Heussner *et al.*, 2014). In this regard, development of cyanotoxins detection in human blood (serum) after exposure is critical for understanding the relationship between illness and toxin exposure. Therefore, this study determined the occurrence of cyanotoxins and assessed risk of exposure in human subjects through contaminated drinking water among Lake Victoria populations.

## **1.3 Rationale of the study**

Cyanotoxins occurrence, exposure and its impact on human health are new research areas globally, which need scientists' attention. Illness related to exposure to cyanobacteria and cyanotoxins is an ignored health problems in developing and developed countries. The problem might be more prominent in developing countries due to lack of well-established HABs-related illness surveillance systems to capture epidemiological data. Based on the nature of exposure and cumulative effect of low dose of cyanotoxins exposure to human it may take years to realize the chronic manifestation of the diseases such as liver and colon

cancer. Acute effects of cyanotoxins present similar symptoms like many other diseases, which make it difficult to be identified and recorded for proper assessment of the magnitude of HAB related illness in the country. Findings from this study have provided baseline information on the magnitude of cyanobacteria species diversity and human health effects. Information gathered in this study can be used as a baseline to improve water quality surveillance system in the country. Estimates for increased cancer cases around Lake Victoria can also be investigated from this perspective and establish evidence of cyanotoxins contribution to cancer cases by other researchers.

## **1.4 Objectives**

### **1.4.1 Main objective**

To determine the occurrence of cyanotoxins in drinking water and assess their risk of exposure to human at Ukerewe District, Tanzania.

### **1.4.2 Specific objectives**

- (i) To determine cyanotoxins occurrences in surface waters of Ukerewe District
- (ii) To assess the influence of water quality parameters on cyanotoxins occurrences
- (iii) To identify cyanobacteria species and associated health risks among users of Lake Victoria freshwater
- (iv) To validate cyanotoxins detection method with comparison to liver biochemical indices

## **1.5 Research question**

The main question was what are the risks of exposure in humans through cyanotoxin contaminated drinking water?

This question will be answered through the answering of the following research questions:

- (i) What is the magnitude of cyanotoxins contamination in surface waters of Ukerewe district?
- (ii) What are the human illnesses associated with cyanobacteria/cyanotoxins exposures?
- (iii) What are proxy indicators for cyanotoxins existence?
- (iv) What are the species of cyanobacteria that exist in Ukerewe freshwater?
- (v) How does cyanotoxins exposure relate to biochemical liver indices

## **1.6 Significance of the study**

This study provides an excellent opportunity for understanding the cyanotoxins occurrence in Lake Victoria and the health effect related to toxins exposure. The study documents a statistical prediction model that is a proxy indicator of the presence of cyanotoxins, which can easily be used by the local authorities to notice the potential increase of cyanotoxins. The proxy indicator can be used without any extra cost or high-end technological equipment. This is the first study to assess multiple toxins existence other than microcystin in the lake whereby thirteen toxins were assessed, the study reports potential existence of multiple toxins, which are hepatotoxins.

Moreover, acute health risks are well documented and the information gathered can be used to set up toxic cyanobacteria related illness surveillance systems in the country. Based on the fact that toxic cyanobacteria are expected to increase in tropical areas including Tanzania, it's high time now for the country to set up standards for cyanotoxins limits in water used for drinking and recreational activities. Water and health surveillance systems are critical to be established to gather epidemiological data that indicate the magnitude of acute and chronic effect due to cyanotoxin exposure. The information documented in this study bridged knowledge gaps about cyanobacteria and cyanotoxins, it informs further studies to be directed in this new research area of public health concerns that will highlight the cyanotoxins contribution to observed cancer increase in lake zone.

## **1.7 Delineation of the study**

Due to global concern on the increase of cyanobacteria and cyanotoxins in freshwater and their potential effects on human health, there was a need to conducted a study to determine the occurrence of cyanotoxins and assessed their risk of exposure to human. This study present for first time in Tanzania the co-occurrence of cyanotoxins such as cylindrospermopsin and nodularin in the freshwater of Lake Victoria and in human serum. This study did not assess other causative agents that could have contributed to the same health effect on human such as other bacterial that can lead to acute health effect and other toxins that can damage liver cells rather focused only on cyanotoxins.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 What are the cyanobacteria

The names "cyanobacteria" and "blue-green algae" (Cyanophyceae) are valid and compatible systematic terms. This group of micro-organisms comprises unicellular to multicellular prokaryotes that possess chlorophyll *a* and perform oxygenic photosynthesis associated with photosystems I and II (Castenholz & Waterbury, 1989). Cyanobacteria are one of the most diverse groups of gram-negative photosynthetic prokaryotes. Cyanobacteria are prokaryotes have a simple cell structure with a real nucleus Prokaryotic. Their body is made from a single cell, often clustered cells as colonies of different shapes. Cyanobacteria are typically much larger than bacteria in size, it contains many types of pigments such as carotenoids and phycocyanin. A characteristic of water-soluble pigment in cyanobacteria gives the group of cyanobacteria their blue green coloration. Cyanobacteria living in individuals places in fresh and salt water, and some other types live in moist soil. The water distinctive bluish colour is results for cyanobacteria blooms when it dies. Researchers found that only about 10% of all blooms types are considered toxins producer.

Morphologically, cyanobacteria may be unicellular or their cells arranged in colonies while others form filaments in single trichomes or filaments with or without branching. The cyanobacterium cell diameter ranges from 2  $\mu\text{m}$  to 40  $\mu\text{m}$  (Osswald *et al.*, 2007). Some species of cyanobacteria can form specialized cells like heterocyst's, which are used for  $\text{N}_2$  fixation, and akinetic, as resting cells that enhance the species to survive during unfavourable conditions. Most of the bloom forming species of cyanobacteria possesses gas vesicles. The, gas vesicles are the components of gas vacuoles, which provide buoyancy to cyanobacteria allowing them to position in the water column in response to physical and chemical factors (Walsby, 1994). The bloom-forming cyanobacteria, which also possess gas vesicles mainly belong to the genera *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Arthrospira*, *Cylindrospermopsis*, *Oscillatoria*, *Nodularia*, and *Microcystis* (Oliver & Ganf, 2000). Several filamentous cyanobacteria also form short fragments of the filament called hormogonia, used for asexual reproduction and dispersal (Hoffmann *et al.*, 2005)

## **2.2 Classification of cyanobacteria**

Due to their dual characteristics of plant that perform oxygenic photosynthesis and bacterium, there are two taxonomical classifications of cyanobacteria, the botanical and the bacteriological classification systems (Wilmotte, 1994). Cyanobacteria is a Phylum ascribed to the Bacterial Domain this is according to Bergey's Manual of Systematic Bacteriology (Guerrero, 2001) . The phylum Cyanobacteria is classified in five major divisions, namely I – V (Table 1) (Guerrero, 2001). Cyanobacteria classification based on botanical, can be categorized into five orders namely Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales (Van Apeldoorn *et al.*, 2007).



**Table 1: Botanical classification of cyanobacteria**

<b>Botanical Classification</b>	<b>Bacteriological classification</b>	<b>Common characteristic features</b>	<b>Examples of cyanobacteria genera</b>
Order Chroococcales	Subsection I	Unicellular, aggregate in colonies, embedded in sheaths, capsules or slime, reproduce by budding or binary fission, may or may not possess gas vacuoles, they are planktonic and benthic species and some species have a potential to produce cyanotoxins like MCs	<i>Microcystis, Synechococcus, Gloeotheca, Gloeobacter, Gloeocapsa, Synechocystis, Chroococcus, Aphanocapsa, Merismopedia, Woronichinia, Snowella</i>
Order Pleurocapsales	Subsection II		<i>Pleurocapsa, Dermocarpa, Xenococcus, Dermocarpella, Myxosarcina, Chroococciopsis</i>
		vacuoles or not, cells are straight, loosely curved or tightly helical, reproduce by binary fission, some have gliding motility and some species have a potential to produce cyanotoxins like MCs, anatoxin-a and saxitoxins	<i>Leptolyngbya, Tychonema Pseudoanabaena, Planktolyngbya</i>
Order Nostocales	Subsection IV	Filaments, no true branching, straight, curved, spherical colonies, possess specialized cells: heterocysts and akinets, hormogonia, may have gas vesicles or not, they are ecologically diverse (planktonic, benthic, periphyton, terrestrial) and most species have a potential to produce cyanotoxins like MCs, nodularins, cylindrospermopsin, anatoxin-a and saxitoxins	<i>Anabaena, Anabaenopsis, Aphanizomenon, Nodularia, Cylindrospermum, Nostoc, Scytonema, Calothrix</i>

<b>Botanical Classification</b>	<b>Bacteriological classification</b>	<b>Common characteristic features</b>	<b>Examples of cyanobacteria genera</b>
Order Stigonematales	Subsection V	Filaments, false/true branching, have heterocysts and akinets, divide in more than one plane, occur in aquatic and terrestrial habitats, usually not as part of the phytoplankton and some species have a potential to produce cyanotoxins like MCs.	Chlorogloeopsis, Hapalosiphon, Mastigocladopsis, Nostochopsis, Symphyonema, Westiellopsis

### 2.3 Factors influencing cyanobacteria occurrence and cyanotoxins production

Nutrient enrichment of freshwater has been associated with an increase of cyanobacteria bloom and hence cyanotoxins promotion, expansion and persistence. Moreover, the global rise in temperature due to climate change postulates an additional catalyst for more cyanobacteria proliferation (Paerl & Otten, 2013).

Daily human activity around water bodies, including agricultural runoff, inadequate sewage treatment, and runoff from roads can cause excessive fertilization (eutrophication) that might lead to cyanobacterial proliferation (de Figueiredo *et al.*, 2004). There numerous factors that enhance cyanobacterial growth and increasing the possibilities for cyanotoxins production. The factors include chemical and physical properties of water quality, weather changes such as temperature increase and light intensity (Marion *et al.*, 2012). Some more water quality parameters can further enhance cyanobacterial growth and these include pH, redox potential, dissolved oxygen, EC, total dissolved solids (TDS), total chlorophyll (total chl), nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ), nitrite nitrogen ( $\text{NO}_2\text{-N}$ ), phosphate ( $\text{PO}_4^{3-}$ ) and reactive phosphorus (P).

Growth of cyanobacteria is fevered by warmer temperature whereby maximal growth rate occurs at an optimal temperature between  $24^\circ\text{C}$  and  $28^\circ\text{C}$ . Temperature has been reported to have a direct relationship with algal blooms (Davis *et al.*, 2009). Temperature increase is thought to be a factor contributing to the global rise in algal bloom globally, Africa is heating faster than the rest of the world hence the increase of cyanobacteria is expected to be higher than the rest of the world (Liu *et al.*, 2011). Relative neutral to higher pH range favour cyanobacteria increase in water bodies (Ndlela *et al.*, 2016; Dalu & Wasserman, 2018). Natural increases in nitrogen and phosphorus concentrations lead to eutrophication, causing algal proliferation and increase cyanotoxin production (Yang *et al.*, 2008). Nitrogen and phosphorus ratio is vital in cyanobacteria growth, N:P ratio below 15 is considered as favorable on cyanobacterial growth and increased PC concentrations, as well as cyanotoxins (Lee *et al.*, 2000; Harke *et al.*, 2016). Other positive factors that influence cyanobacteria growth includes; sufficient iron, higher dissolved organic matters, low turbulence and higher light (Paerl & Otten, 2013).

Like higher plants all phytoplankton need light for photosynthesis. However, geographically light intensity as well as the requirements of different phytoplankton species is not the same. In the tropics light availability is more predictable when compared with the temperate climate

and this enhances dense growth of phytoplankton in the epilimnion under eutrophic conditions. Due to this dense growth and other suspended matters, light will then become steeply attenuated vertically through the water column and phytoplankton growth will become light limited. Under light limiting conditions species with low light requirements will have competitive advantage in areas with high light attenuation over those with higher light requirements. Cyanobacteria have different light requirements. Some species like *Planktothrix agardhii* have a low critical light intensity and are considered to be better light competitors and therefore can dominate in various shallow eutrophic lakes. Other strongly buoyant cyanobacteria like *Aphanizomenon* and *Microcystis* are poorer light competitors but can tolerate highest irradiance at the surface (Huisman *et al.*, 1999).

Lake water may mix vertically or horizontally due to wind action. This wind induced mixing may affect nutrient distribution and changes in phytoplankton species composition. Vertical mixing may re-suspend nutrients deposited in the lake sediments like silicate and phosphorus into the water column, therefore enhancing the growth of diatoms. During mixing conditions buoyant cyanobacteria cannot efficiently accumulate at the surface and may become light limited resulting in poor growth. During calm conditions, buoyant cyanobacteria like *Anabaena* and *Microcystis* (potential toxin producers) concentrate on the water surface. Winds may sweep the bloom near the shore, resulting in an increase in cell densities by several orders of magnitude (Chorus & Bartram, 1999). The intensity of vertical mixing therefore is one of the major factors structuring a phytoplankton community.

Apart from vertical mixing, wind-induced horizontal water mixing is also known to occur in Lake Victoria, especially in bays or gulfs (Haande *et al.*, 2011). Like ocean tides which cause the water level to rise and fall within a day, in large lakes seiches are formed by the same principle although their amplitude is only a few cm. Seiches are defined as standing waves that move water down and up (Ji & Jin, 2006). At the shore these waves will cause water movement in and out of shallow bays (Haande, 2008). Studies conducted in Ugandan water of Lake Victoria (Murchison Bay) showed that seiches diluted phytoplankton density in the bay and thus influenced water quality at the bay due to horizontal mixing with the main lake.

#### **2.4 Cyanotoxins production and water contamination**

Cyanobacteria are considered to be the most widespread organisms of the first group of photosynthetic prokaryotes (Chorus *et al.*, 2000). They are ubiquitous and can live in every

conceivable habitat ranging from aquatic to terrestrial environments. They occur primarily in surface water, where they can thrive on a variety of ecological niches of freshwaters, salinity, lower light intensity, high turbidity, in hot spring and ice-cold water (Rastogi *et al.*, 2015). Cyanobacteria can grow massively in a favorable condition of high nutrient availability, light and temperature; this will give rise to bloom accumulation (Hilborn *et al.*, 2014). These masses of excessive collections of blooms may contain Harmful Algal Blooms (HABs) that are capable of producing secondary metabolites known as cyanotoxins. Harmful Algal Blooms can occasionally occur and produce visible scam of algal on the surface of water bodies. Bloom mass occurrences in freshwaters are rapidly increasing globally and attracting the attention of human and animal health organizations, water and environmental agencies because cyanobacterial blooms pose water quality and treatment challenges (Chorus *et al.*, 2000).

Cyanobacterial blooms cause significant problems to water quality surveillance and treatment management of lakes, rivers and water reservoirs. Cyanobacterial blooms and cyanotoxins have adverse impacts on human health and aquatic ecosystems leading further to ecological and economic effects (Carey *et al.*, 2012). Same bloom forming cyanobacteria produce a diverse of secondary metabolites know as cyanotoxins, which are toxic and a threat to drinking water and recreation activities, these blooms produce stinking compounds and can disrupt food web in the ecosystem (Cheung *et al.*, 2013).

Cyanotoxins are produced during and after cyanobacteria bloom occurrence, potentially dangerous concentrations of toxins may be present in water even when there are no visible blooms. Cyanotoxins can be enclosed in the cell walls, exist intracellular in the cytoplasm, or be released to become extracellular cyanotoxins. Their release can occur during the cell life nevertheless the release occurs predominantly after cell death, leading to a massive outflow of the cellular content (Chorus *et al.*, 2000). Besides, there is a possibility of cyanotoxin accumulation in aquatic organisms because, as they live in water bodies they accumulate a small amount of cyanotoxin concentration that exceeds the surrounding environment over time (Sanchez *et al.*, 2014). The toxin can be transported to higher levels of food webs with higher toxicity levels, ultimately endangering animal and human health (Peng *et al.*, 2010). A study conducted by Chen *et al.* (2009) shows that the toxins observed in fish contribute significantly to the daily computation of toxins to the fisherman hence in-depth study of human exposure to cyanotoxin from aquatic organisms is important.

Cyanotoxins are classified based on their modes of action in invertebrates and on chemical composition. In their mode of action in invertebrate they comprise of hepatotoxins, a neurotoxin, and dermatotoxins. Hepatotoxins include; microcystin (MCs), cylindrospermopsin (CYN) and nodularin (NODs). Neurotoxin includes; anatoxin-a (ATX-A), homoanatoxin-a (hATX-a), anatoxin a-(s) (ATX-a(S)) and paralytic shellfish poison (PSP) toxins. Finally are dermatotoxins, which are gastrointestinal and irritants toxins include; Lipopolysaccharidic (LPS) endotoxins and marine toxin lyngbyatoxin (Funari & Testai, 2008). Based on their chemical composition, cyanotoxins are grouped into alkaloids such as (ATXs), saxitoxins (STXs), CYN, aplysiatoxin, lyngbiatoxin-a), the second group is cyclic peptides such as MCs and NODs, the last group is lipopolysaccharides (Kotak & Zurawell, 2007; Kaplan *et al.*, 2012). Most of the cyanotoxins are intracellular except CYN, which are extracellular they release a very high concentration of toxins after lyses, which exhibit allopathic properties that have effects to aquatic microorganisms and surrounding environment (Boopathi & Ki, 2014).

Cyanotoxins are geographically distributed worldwide in freshwater systems, cyanotoxins have been found in Europe (Denmark, Norway, Finland, France, England), USA, Egypt, Japan, Australia, China and Brazil (Chorus & Bartram, 1999). In Africa, MCs have been reported in the Nhlanguzane Dam, Kruger National Park, South Africa (Oberholster *et al.*, 2009). Studies conducted in East Africa around the Lake Victoria and other like Manyara reported the presence of cyanotoxins in same surface water (Nonga *et al.*, 2011; Miles *et al.* 2013). Seasonality variation of bloom and production of cyanotoxins in the freshwater has been reported in some studies conducted in Uganda, whereby massive blooms are observed during February and August (Okello *et al.*, 2010). Observed seasonal variations of cyanotoxins concentrations in water bodies during the rainy and dry season have been reported elsewhere in Africa (Ndebele-Murisa *et al.*, 2010). The difference may be contributed by the nutrient content of freshwater and weather parameters, which have a substantial impact on the frequency and severity of blooms occurrence and cyanotoxin productions (Paerl & Paul, 2012).

## **2.5 Cyanotoxins occurrence and classification**

There are number of reasons as to why cyanotoxins are produced by cyanobacteria that lead to bioactivation of secondary metabolites. Studies have shown that the possible functions of cyanotoxins include: (a) inducing alteration of population structures to gain ecological

advantage, (b) avoidance of grazing on cyanobacteria by other organisms such as zooplankton and higher animals, and (c) mediating cell signaling allelopathy and chemotaxy to establish trophic relationships with other cyanobacteria or other organisms (Wiegand & Pflugmacher, 2005). Several types of cyanotoxins are produced by different species of cyanobacteria as detailed in the following subsection

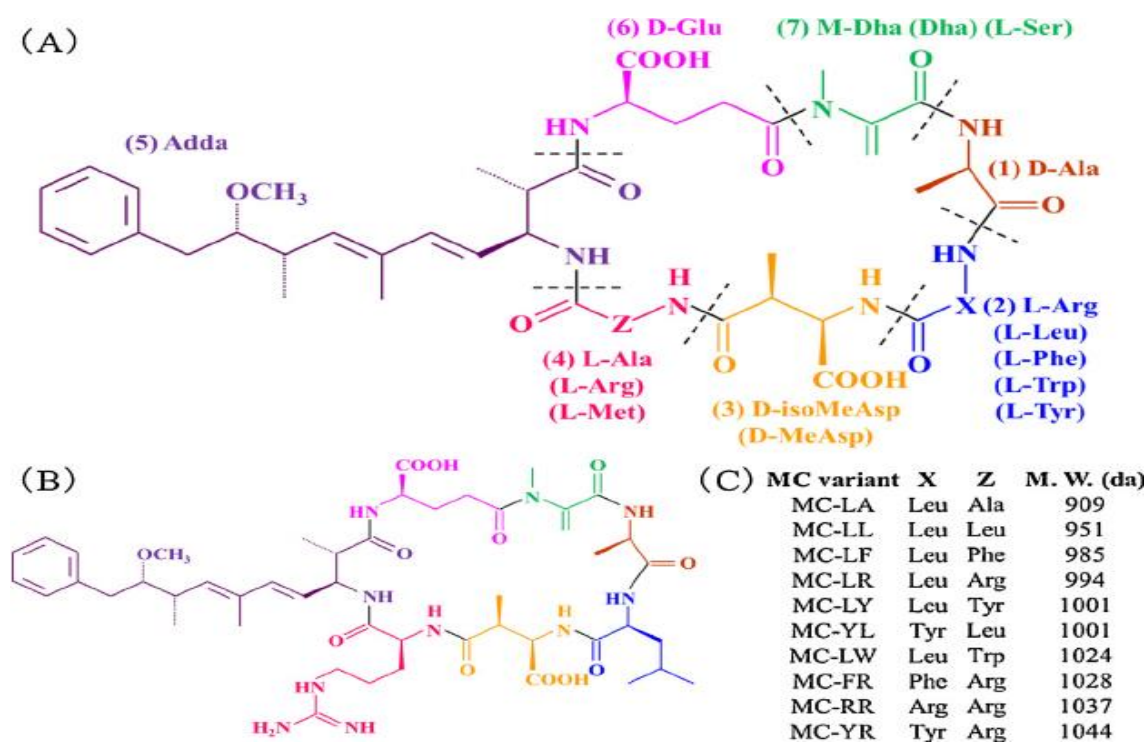
### **2.5.1 Microcystins (MC) and Nodularins (NODs)**

Microcystins and Nodularins are both cyclic peptides and hepatotoxins found to be similar in structure and mode of action as discussed above; therefore, they will be discussed together in this section. Microcystins and NODs hepatotoxins have a common characteristic that is amino acid Adda (Fig. 1-2), which is unique for cyanobacteria and responsible for molecules toxicity (Funari & Testai, 2008; Greer *et al.*, 2018). Microcystins are produced by cyanobacteria of genera *Microcystis* spp, *anabaena*, *Merismopedia*, *Limnothrix redekei*, *Phormidium formosum*, *Hapalosiphon hibernicus*, *Planktothrix* spp, *Nostoc*, *Synechocystis* and *Cyanobium bacillare*. The structure of MC and NOD varies by changing two positions of amino acid(s) such as leucine and arginine variants, and these changes include other small side groups, as shown in Fig 1. Microcystin congeners includes; microcystin (-LA, -LF, -LR, -LY, -LW, -RR, -YR, -WR, dm MC-RR and dm MC-LR). This changes in different small side group result in more than 6 NOD and 70 MC variants (Sivonen & Jones, 1999). MCs are reported in varied regions like Africa, Asia, Europe, North America and Scandinavian countries while NODs appear to be confined to Australia, New Zealand and the Baltic Sea (Gehring & Wannicke, 2014). The most common MC congener is MC-LR characterized by the presence of leucine (L) and arginine (R) as L-amino acids in positions 2 and 4 (Fig. 1). The positioning of the amino acid determines acute toxicity that why MC-LR is considered among the most potent hepatotoxins within the different variants due to amino acid profile attached to its adda and is by far the most studied (Mekebri *et al.*, 2009). Based on this regards the World Health Organization (WHO) established a 1 µg/L guideline value for drinking water and a Tolerable Daily Intake (TDI) of 0.04 µg/kg body weight per day for MC-LR in contaminated water (WHO, 2006).

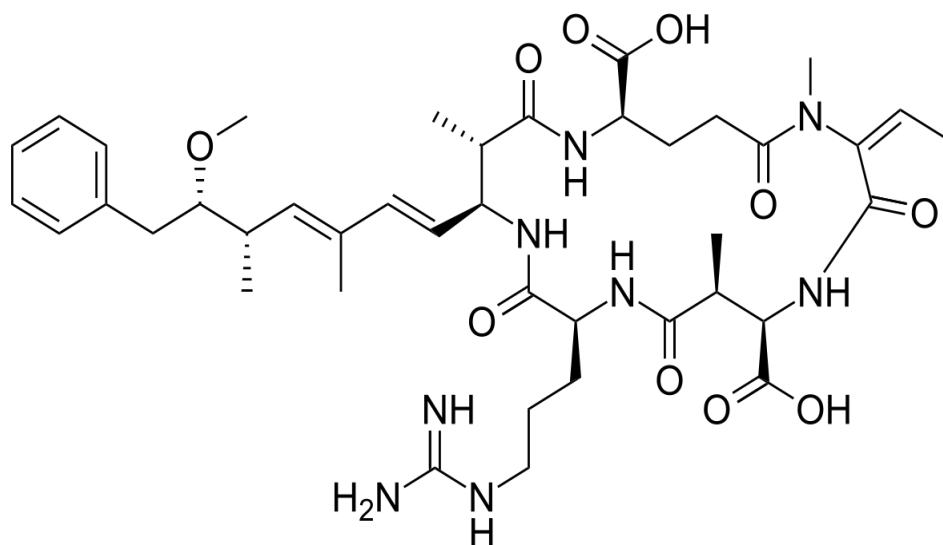
Microcystins and Nodularins were found to target brain and liver cells by inhibiting the protein phosphatase that will result in the accumulation of phosphorylated proteins type 1 and 2A in the liver cells, which triggers cell death through necrosis and tumor formation (Feurstein *et al.*, 2009). As discussed previously, MCs and NODs bioaccumulation in tissues

and do this as unrestricted or bound through their conjugation to protein phosphatases (1 and 2) in specific muscles. Binding of MCs to protein phosphatases is permanent and the damage can occur even in a small dose of exposure of which the effect will be realized after a very long time (Neffling *et al.*, 2010). MCs transportation and uptake into the liver through the bile acid transport system in interaction with Organic Anion-Transporting Polypeptides (OATPS) that, expressed in liver cells. Additionally, OATPs can be revealed in other organs such as the stomach, kidney, brain, small and large intestines (Greer *et al.*, 2018). On the other hand, MC considered affecting other organs as well based on the ability to damage DNA and promote tumours due to genotoxic characteristics (Žegura *et al.*, 2011). The evidence of the possible primary liver cancer (PLC) and promotion after exposure to MC-LR contaminated surface water as compared to well water have been reported in China and Asia (Chen *et al.*, 2009). Furthermore, evidence of renal function implication and promotion of migration and invasion of colorectal cancer also was reported in China (Ren *et al.*, 2017). More effect of MC-LR has been useful in mice studies that can lead to apoptosis and changes to the absorptivity of the mitochondrial membrane and suppress haemotopoiesis function (He *et al.*, 2017). There is only one human study that provides epidemiological evidence of human exposure of cyanotoxin that has subsequent health effect in China as a result of prolonged exposure of 5-10 years (Chen *et al.*, 2009).





**Figure 1: Chemical structure of MC and its congener**

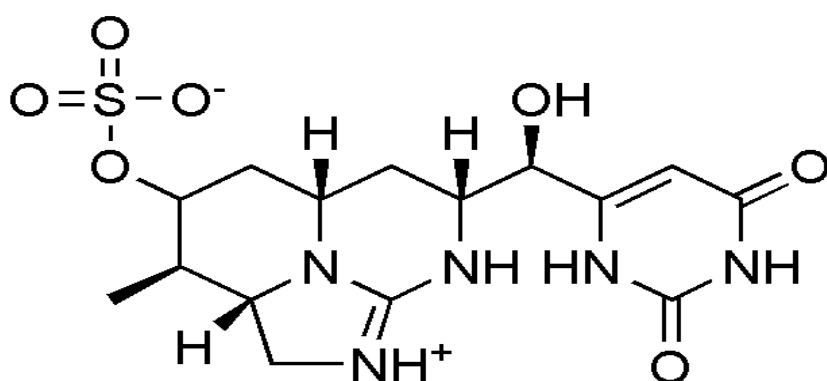


**Figure 2: Chemical structure of nodularin**

### 2.5.2 Cylindrospermopsin

Cylindrospermopsin (CYN) is alkaloid (Fig. 3) that is capable of producing both hepatotoxic and nephrotoxic effects and also can effect on other organs in the body such as brain (Chorus *et al.*, 2000). The CYN toxins are produced by filamentous cyanobacteria includes; *Cylindrospermopsis raciborskii*, *Raphidiopsis curvata*, *Anabaena bergii*, *Sphaerospermopsis*

*aphanizomenoides*, *Aphanizomenon ovalisporum*, *Umezakia natans*, *Aph. flos-aquae*, *Oscillatoria* sp. PCC6506 and *M. aeruginosa*. In European countries, occurrence of CYN was reported in Germany and France by Fastner *et al.* (2003) as well as in Ireland by Greer *et al.* (2016). The detection of CYN toxin in freshwaters is a global concern as it is a new phenomenon and has similar mechanisms of action as MC, as demonstrated by Yoshizawa *et al.* (1990). The two toxins CYN and MC have been reported as potent protein phosphatase 1 and 2A inhibitors which has been shown to have long term cumulative toxic effect for potential tumour formation (Rastogi *et al.*, 2015). Cyindrospermopsin inhibits protein P450 and glutathione synthesis, which lead to cell death of lung, intestine, liver and kidney, this occurs through irreversible inhibition of protein synthesis (Bernard *et al.*, 2003). Additionally, CYN other effects reported are chromosome loss, micronucleus induction and tumour initiation (Humpage *et al.*, 1994; Froscio *et al.*, 2003).

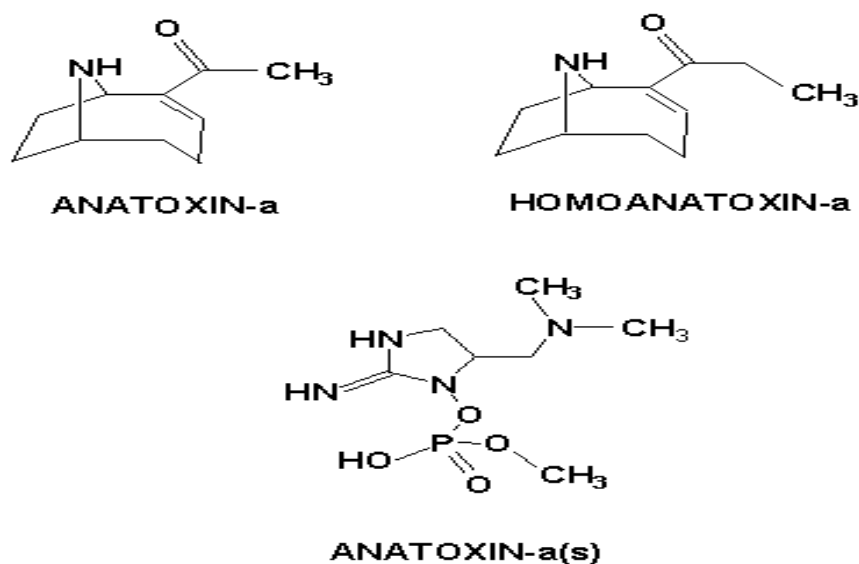


**Figure 3: Cyindrospermopsin chemical structure**

### 2.5.3 Anatoxins (ATXs)

Anatoxins are one of the cyanotoxins produced by cyanobacteria including, *Anabaena flos aquae*, *Microcystis*, *Oscillatoria* and *Aphanizomenon* (Cadel-Six *et al.*, 2009). There are three main variants of anatoxins (Fig. 4), namely anatoxin-a (ATX-a), homoanatoxin-a (hATX-a) and anatoxin-a(s) –(ATX-a (s)). Anatoxin-a is the common one and widely reported in Asia, Europe and Africa while ATX-a (s) is observed to be in same areas like Brazil, Denmark, USA and Scotland (Merel *et al.*, 2010). Anatoxin-a have also been reported in kenyan saline lakes and was involved in mortalities of lesser flamingos in the lakes (Ballot *et al.*, 2004).

Anatoxin-a can potentially lead to neurotoxicity and imitation of the neurotransmitter acetylcholine. Anatoxin-a can cause several health effects, including tingling, burning, numbness, drowsiness, incoherent speech, and respiratory paralysis leading to death (Boopathi & Ki, 2014).

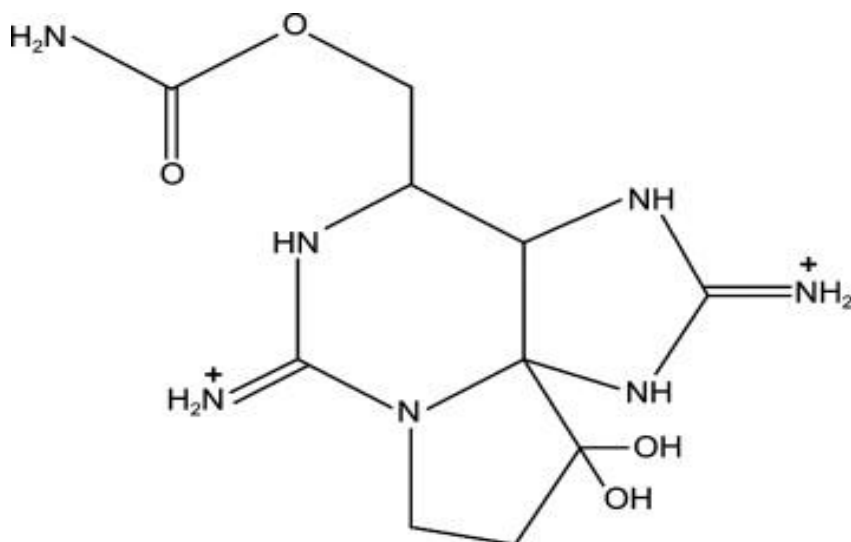


**Figure 4: Anatoxins chemical structures**

#### 2.5.4 Saxitoxins (STX)

There are several types of paralytic shellfish poisoning (PSP) including the common one that is saxitoxin and neosaxitoxin (Fig. 5). Many toxicological reports of STX are from marine organisms, and limited information is available for STX produced by cyanobacteria of freshwater. Nevertheless, the toxicological profile and a chemical structure of marine and freshwater STX are the same (Funari & Testai 2008). Saxitoxin mainly produced by cyanobacteria genera of *Raphidiopsis*, *Scytonema*, *Anabaena*, *Aphanizomenon*, *Lyngbya*, *Planktothrix* and *dinoflagellates* (Neilan *et al.*, 2013).

Saxitoxins are known to cause neurotoxicity and blockage of voltage-gated Na<sup>+</sup> channels in humans and other vertebrates. The toxins also lead to numbness, burning, tingling, drowsiness, incoherent speech and respiratory paralysis resulting in death (Boopathi & Ki, 2014).



**Figure 5: Saxitoxins chemical structures**

## 2.6 Cyanotoxins exposure and risk assessment

Exposure of cyanotoxins to human can be through several routes such as ingestion of contaminated food, water and dietary supplements, inhalation of contaminated dusts/vapour hemodialysis, and dermal exposure (Buratti *et al.*, 2017). Nature of exposure can determine to what extent the effects could be to human health and animals. Human diseases due to cyanotoxin intoxication vary based on related exposure, each scenario of exposure may affect the amount of internal dose of cyanotoxins in human. Based on the above explanation, the possible human exposure can be grouped in different cyanotoxins sources and exposure scenarios as follows.

### 2.6.1 Drinking water

Ingestion of contaminated water that contain cyanobacteria bloom or their metabolites have been associated with human illness and death (Ballot *et al.*, 2004). Direct use of cyanobacteria or cyanotoxins contaminated water from water sources or poor treated piped water may result in health problems. In developing countries several people use untreated surface water for drinking; this can expose them to released toxins from cyanobacteria. Consumption of untreated water in area with cyanotoxins infestation may lead to acute or chronic illnesses to human depend on cell-bound volume or cyanotoxins concentration of contaminated drinking water (Chorus *et al.*, 2000). In this regards, the World Health Organization (WHO) has, therefore proposed a provisional guideline for the suitable limit of MC-LR concentration in drinking water of 1.0 µg/L (Supply & Programme, 2014). On other hands, National Centre for Environmental Assessment (USA) has suggested the limit for

MC-LR need to be as lower as 0.1 µg/L (Oehrle *et al.*, 2010). Tolerable daily intake (TDI) value of 0.04 µg MC-LR/kg bw/day provided by WHO (WHO 2004), for an adult with 60 kg could be exposed orally up to 2.4 µg per day based on this TDI. In this bases the calculation of provisional guideline value (GV) was made, whereby it was assumed the daily water consumption of 2 L for an adult and allocation factor (AF) of 80% that contribute to total toxin intake of MC-LR comes from drinking water gives the limits of 1.0 µg/L as its show on the equation below:

$$GV = \frac{TDI \times \text{Bodyweight} \times AF}{\text{Daily consumption (C)}}$$

$$GV = \frac{0.04 \mu\text{g/kg} \times 60\text{kg} \times 0.8}{2\text{L}}$$

$$GV=1 \mu\text{g/L for MC-LR}$$

The provisional limit for CYN is 0.8 µg/L, which is considered as the same for MC-LR. The bases for this limit formulated from the following assumption and calculation. Tolerable daily intake (TDI) value of 0.03 µg/kg bw/day provided, an adult with 60 kg could be exposed orally up to 1.8 µg per day. In this base the calculation of provisional guideline value (GV) for CYN was made, whereby it was assumed the daily water consumption of 2 L for an adult and allocation factor (AF) of 90% (0.9) that contribute to total toxin intake of CYN comes from drinking water gives the limits of 0.81 approximately to 1 µg/L as its show in the equation below.

$$GV = \frac{TDI \times \text{Bodyweight} \times AF}{\text{Daily consumption (C)}}$$

$$GV = \frac{0.03 \mu\text{g/kg} \times 60\text{kg} \times 0.9}{2\text{L}}$$

$$GV= 1\mu\text{g/L for CYN}$$

Many countries have not yet set the limit for most of the cyanotoxins and cyanobacteria cells density. This may be due to same challenges including lack of trained personnel, the cost of taxonomic pigment extraction, and expensive equipment involving high-end technology for toxin detection (McQuaid *et al.*, 2011).

### **2.6.2 Recreation**

Exposure through recreation can be in various ways such as professional activities (i.e., fishing), sport, swimming or any other domestic activities using cyanobacteria infested or cyanotoxins contaminated water. Exposure sources in recreation are through inhalation, direct contact and ingestion. In most cases, these types of exposures are associated with acute, or sub-chronic related illnesses depend on the amount and types of toxin in contaminated water. Unique report from England where 50% of exposed 50 soldiers become sick after coming in contact with the massive bloom of *Microcystis* spp. during swimming, whereby two soldiers presented with severe pneumonia. Finally, it was concluded that swimming skills and amount of water ingest are related to a degree in illness (Turner *et al.*, 1990). World Health Organization proposed guidelines for different cell densities of cyanobacteria bloom levels and related health effects, such as for recreational waters cell densities of 20 000 cells/mL is associated with risk of acute adverse health outcomes, and at an advanced cell density of 100 000 cells/mL, excess risk for chronic disease may occur. Harmful Algal Blooms (HABs) scum formation in bathing areas is associated with the risk of potentially severe health illnesses such as throat, skin and eye irritation, stomach upset, headache, nausea, diarrhea, fever, and vomiting (Kibria, 2016). In many developing countries where there is no such control of limits for the number of cyanobacteria cell density in recreation water, the magnitude of the illnesses must be underestimated and go unrecognized.

### **2.6.3 Hemodialysis**

Water used for hemodialysis therapy can present a significant risk to the patient. Surface water used for hemodialysis must be free from any form of cyanobacteria infestation because this route of exposure presents the toxins direct to the bloodstream. Major fatal incident reported by Jochimsen *et al.* (1998) from Caruaru (Brazil) whereby a total of 131 dialysis patients exposed to MC contaminated water used in the process of hemodialysis treatment, of which 56 died and 44 presented symptoms related to MC intoxication. Hemodialysis exposures are quite a few events to be reported but have a significant impact on exposed group hence there is a need to develop a mandatory regulation of the quality of water used for this type of treatment. Awareness of the potential risk of cyanotoxins to be present when surface water is used for hemodialysis ought to be recognized by health sectors and necessary precaution must be taken to minimize or to avoid the risk.

#### 2.6.4 Food and dietary supplement

The threat of cyanotoxins exposure through food and dietary supplementary is based on consumption of fish and other edible aquatic organisms, agriculture products and supplement that bioaccumulates cyanotoxins for the same time. Bioaccumulation of cyanotoxins in this context refers to the process whereby toxins concentration in tissues of aquatics organism exceeds the surrounding water body due to uptake by all exposure route. This process can occur in a wide range of aquatic organism used for human consumption when exposed to cyanotoxins contamination. Aquatic food chain and bioaccumulation enables toxicity enhancement of single or multiple cyanotoxins that ultimately reach humans in higher concentration (Ibelings & Chorus, 2007). A study conducted by Negri *et al.* (1995) reported bioaccumulation of paralytic shellfish poisoning (PSP) from cyanotoxin whereby MCs were detected in freshwater shrimps (*Palaemon modestus*, *Macrobrachium nipponensis*), and red swamp crayfish (*Procambarus clarkii*) in China (Chen & Xie, 2005).

Furthermore, saxitoxins and cylindrospermopsin were also reported in pearl oysters and bivalve hemolymphs, viscera and gonads of the freshwater snail (Drobac *et al.*, 2013). However, most of the aquatic organisms are consumed after boiling, and it should be noted that the boiling process can't destroy cyanotoxins (Dietrich & Hoeger, 2005). Fishes being on the top of the aquatic food chain are probably the most exposed to cyanotoxins, which may accumulate in the various part includes; liver, kidneys, gills, guts and muscles (Magalhaes *et al.*, 2003). Cyanotoxin, especially MCs, can be taken in the liver, disrupting regular cellular activity by inhibiting protein phosphatases. This process impair fish embryogenesis since protein phosphatases are responsible for regulating these critical developmental processes (Malbrouck & Kestemont, 2006).

Cyanotoxins could also be transmitted to plants from surface irrigating waters, whereby plant like lettuce and cabbage that require spray irrigation can potentially be contaminated with cyanobacteria bloom and cyanotoxins (McElhiney *et al.*, 2001; Liu *et al.*, 2016). However, it can be anticipated that the usual washing and rinsing procedure before eating could readily remove cyanobacteria cells. Concerning absorption of dissolved cyanotoxins present in irrigation water by the plant tissues, extracts from rice seedlings exposed to water with MC-LR contamination can be observed in reaped rice (Chen *et al.*, 2012). Climate change brings into attention that there will be water scarcity for irrigation hence, it will be difficult recommended not to use bloom and scum infested waters for irrigation, especially in hot



climate areas. In China they used bloom scums as organic fertilizer in agriculture, this can allow cyanotoxins to leach and contaminate groundwater that can be transferred to drinking water (Chen *et al.*, 2006).

Dietary supplements from cyanobacteria known as Blue- Breen Algae Supplements (BGAS) made from *Aphanizomenon flos-aquae* and *Spirulina* spp, which are grown in artificial ponds or collected directly from the natural environment. Recently dietary supplements from cyanobacteria extract have become popular in developed countries and in the past years this practice was common in China and the same areas in Africa (Dietrich & Hoeger, 2005). BGAS are not drugs rather supplements; therefore, there is neither prescription nor indication for a specific daily dosage. It's becoming difficult to correctly assess the actual exposure and effect of individual nutritional programs. BGAS have become popular due to presumed human health benefits, including support in losing weight during hypocaloric diets, increasing energy and elevated mood for people suffering depression (Dietrich & Hoeger, 2005). Also, the supplements are used for children as an alternative, natural therapy to treat attention deficit hyperactivity disorders. The study conducted on several commercialized dietary supplements revealed presence of ATX-A, microcystins-LR and LA on BGAS (Vichi *et al.*, 2012; Roy-Lachapelle *et al.*, 2017).

## **2.7 Health effect in human and animals**

Exposure to cyanobacteria and cyanotoxins through various routes can be of a significant threat to human and animal, and this causes human health effects and animal death (Codd *et al.*, 1994). The first report of cyanobacterial blooms effect in animals was brought into attention in 1878 in South Australia, whereby the report of animal death after ingestion of cyanobacterial bloom was documented (Francis, 1878). To date numbers of animal's death incidence have been reported that includes birds, dogs, domestic livestock and poultry (Wood, 2016). Evidence that cyanotoxins can cause human illness have been reported in the USA, Canada and Zimbabwe (Codd *et al.*, 1999). All reported incidence involved several thousand cases of human, with liver cancer being the most common outcome. Several acute symptoms have been reported in humans such as stomach upset, vomiting, skin irritation, nausea, diarrhoea, fever, throat irritation, headache, mouth blisters, muscle and joint aches, eye irritation, and allergic reactions (Kibria, 2016). Chronic health effects include the possible human carcinogen for liver and colorectal malignancies (Table -2).



The International Agency for Research on Cancer (IARC) has categorized MC-LR as one of the possible human carcinogens in group 2B with substantial evidence supporting the fact that it can exhibit tumour promotion mechanism (IARC, 2010). Acute cases of microcystin poisoning can lead to humans and animal death (Cheung *et al.*, 2013). Upon ingestion, microcystin is transported to the liver by organic anion transport proteins where they exert their toxicity via inhibition of protein phosphatases 1 and 2A (Runnegar *et al.*, 1995). Inhibition of protein phosphatases leads to excessive phosphorylation of structural filaments, subsequent cytoskeletal degradation and breakdown of hepatic ultrastructure (Sahin *et al.*, 1995). Retraction of hepatocytes from neighboring cells and sinusoidal capillaries causes blood to become pooled in the liver tissues. This ultimately results in local tissue damage, organ failure and haemorrhagic shock (Sahin *et al.*, 1995). A study by Nishiwaki-Matsushima *et al.* (1992) pinpointed this evidence of liver tumour promotion by microcystin-LR. All studies showed a positive association between the risk for hepatocellular carcinoma and MC contaminated water source. A study conducted in China by Chen *et al.* (2009) is the only report that has linked daily exposure of MCs and subsequent health effect. All 35-selected study subject was positive for MCs, of which MCs (-RR, YR and LR) were detected in their serum with a mean and median concentration of 0.389 ng and 0.227 ng respectively.

Furthermore, the study reported positive relationships between serum MC concentration and biochemical liver indices such as ALP, AST, ALT and LDH of serum test (Chen *et al.*, 2009). This is the evidence that MCs target cells of the liver and induce lesions hence causing elevation of liver enzymes, which can be detected in biochemical indices. The same finding was reported in rats/mice treated with MCs whereby increased serum activity was observed after inductive hepatocellular damage, this provided fundamental knowledge of MCs toxicity to mammals (Weng *et al.*, 2007; Billam *et al.*, 2008).

Acute intoxications with microcystins (MCs) is well documented; however, chronic exposure to these toxins is much less understood and importantly long-term exposure, while it has been linked to hepatocellular and colorectal cancer. This requires more definitive and non-invasive tests for reliable epidemiological studies (Meneely & Elliott, 2013). Microcystins also have been linked to possible primary liver and colorectal cancer (Ueno *et al.*, 1996). Microcystins also can cause substantial health hazards and have been implicated in the deaths of birds, aquatic biota, livestock and wildlife (de Figueiredo *et al.*, 2004). Recent experimental in pigs published in nature scientific report reviles that MC-LR could accumulate in the kidney and

large intestine after exposure through drinking contaminated water (Greer *et al.*, 2018). The animal model was used to assess the human risk since pigs and human have similar digestive system hence it helps to understand uptake and accumulation of MC-LR. More health effect, modes of action based on the different type of toxins are detailed in Table 2.

**Table 2: Cyanotoxins from different cyanobacteria genera and their health effects**

Cyanotoxin	Producing Cyanobacteria	Toxic Mechanism	Health Effect	Reference
1 Microcystin (MC)	<i>Microcystis</i> spp, <i>anabaena</i> , <i>Merismopedia</i> , <i>Limnothrix</i> <i>redekei</i> , <i>Phormidium formosum</i> , <i>Hapalosiphon hibernicus</i> , <i>Planktothrix</i> spp, <i>Nostoc</i> , <i>Synechocystis</i> , <i>Cyanobium</i> <i>bacillare</i>	Hepatotoxic, inhibits eukaryotic protein phosphatases (PP1 and PP2A)	Liver inflammation, and hemorrhage and liver failure leading to death, pneumonia, dermatitis, oxidative damage (DNA), Genomic instability, Apoptosis, Cytoskeleton alterations and cellular proliferation	(Carmichael <i>et al.</i> , 2003; Ballot <i>et al.</i> , 2004)
2 Nodularin (NOD)	<i>Nodularia</i>	Hepatotoxic, inhibits eukaryotic protein phosphatases (PP1 and PP2A and3) tumor promoter	Gastrointestinal, liver inflammation, and hemorrhage and liver failure leading to death, pneumonia, dermatitis	(Rinehart <i>et al.</i> , 1988)
3 Cylindrospermopsin (CYN)	<i>Cylindrospermopsis raciborskii</i> , <i>Anabaena</i> spp, <i>Umezakia</i> <i>natans</i> , <i>Aphanizomenon</i> <i>ovalisporum</i> , <i>Aphanizomenon</i> <i>flos-aquae</i> , <i>Rhaphidiopsis</i> <i>curvata</i> , <i>Planktothrix</i>	Hepatotoxic, cytotoxic, neurotoxic; inhibition of glutathione synthesis, protein synthesis and cytochrome P450	An irreversible inhibitor of protein and glutathione cytochrome P-450, overexpression of DNA damage repair protein, Apoptosis, morphological alteration. Gastrointestinal, liver inflammation and hemorrhage, pneumonia, dermatitis	(Li <i>et al.</i> , 2001; Schembri <i>et</i> <i>al.</i> , 2001; Spooof <i>et al.</i> , 2006; Fastner <i>et al.</i> , 2007)

	<b>Cyanotoxin</b>	<b>Producing Cyanobacteria</b>	<b>Toxic Mechanism</b>	<b>Health Effect</b>	<b>Reference</b>
4	Anatoxin-a	<i>Anabaena spp, Microcystis, Aphanizomenon, Cylindrospermum, Raphidiopsis mediterranea, Planktothrix spp</i>	Neurotoxic, mimics the neurotransmitter acetylcholine	Depolarizing neuromuscular blocking, tingling, burning, numbness, drowsiness, incoherent speech, respiratory paralysis leading to death	(Namikoshi <i>et al.</i> , 2003; Wood <i>et al.</i> , 2007)
4	Saxitoxin (STX)	<i>Cylindrospermopsis, Anabeana spp, Aphanizomenon, Lyngbya</i>	Neuromuscular system (Membrane ion channel block)	Blocking neuronal communication by binding to the voltage-gated Na <sup>+</sup> channel, respiratory paralysis leading to death	(Humpage <i>et al.</i> , 1994)
5	LPS endotoxins	All cyanobacteria	Skin and mucosa (irritation, topic effects)	Skin irritation	(McElhiney & Lawton, 2005)
6	Beta-Methylamino-L-alanine (BMAA)	<i>Microcystis, anabaena, Nostoc, Planktothrix</i>	Motor system disorder, glutamate agonist, increasing the intracellular concentration of calcium in neurons and cause hyperexcitation	Increasing the intracellular concentration of calcium in neurons that cause hyperexcitation and motor system disorder	(Lobner <i>et al.</i> , 2007)

## **2.8 Strategic for control of cyanotoxin contamination in water bodies**

The use of freshwater for human drinking and other domestic activities is inevitable, cyanobacteria and cyanotoxins in freshwater must be controlled to minimize the adverse health effect on human and animals. There is economical implication on the cost related to prevention, control and mitigation of cyanobacteria in water bodies. For instance, the USA used about 2.2-4.6 dollars per annum for mitigation activities related to cyanobacteria and cyanotoxins (Dodds *et al.*, 2008). There is a need for the development of new technologies which are economical and sustainable viable to mitigate cyanotoxins hence prevent the public health (Srivastava *et al.*, 2013). The developed approaches must be environmentally friendly, which does not impair aquatic ecosystems. There are several mitigation strategies which can be used to control cyanobacteria includes, biological, physical and chemical means as explained below:

### **2.8.1 Biological approaches**

Biological means of cyanobacteria control is of great advantage compared to chemical and physical ways. Natural approaches such as regulation of availability or nutrient uptake and alteration of the normal physiology of photosynthetic pigment and direct feeding of cyanobacterial biomass by some aquatic organisms is a hopeful way of natural ways ecological restoration (Xu *et al.*, 2007; Zhu *et al.*, 2014). Aquatic species like *Radix swinhoei* snail can feed on cyanobacteria cells and live well without loss infertility and disturbance of aquatic ecosystem (He *et al.*, 2012). Natural manipulation of nutrient such as nitrogen and phosphorus by increasing aquatic plants that compete for nutrient and light with harmful cyanobacteria are assumed to be a limiting factor for algal growth (Wang *et al.*, 2012). More investigations are essential on some aquatic plants that can release natural chemical with an allelopathic effect which prevent the growth of harmful cyanobacteria. Moreover, rival interaction with the naturally occurring compound in water bodies must be studied if they can overpower cyanotoxins production. There are some microorganisms like *Bacillus* spp, *Sphingomonas* spp and *Stenotrophomonas acidaminiphila* can biodegrade cyanotoxin such as MC- (LR, RR, LY, LF) natural (Xuan *et al.*, 2017).

### **2.8.2 Physical approaches**

Bloom control by physical approaches involves mechanical elimination techniques or short wavelength radiation treatment to control the incidence of harmful cyanobacteria. Artificial circulation by crating physical water flows and reduction residence can potentially eliminate

freshwater algal blooms of a reservoir even in nutrient-rich environments (Huisman *et al.*, 2004; Hudnell, 2010). Besides, a solar-powered circulation (SPC) has been designed to generate a long-distances flow of the epilimnion (>200 m) to suppress harmful cyanobacteria in freshwater (Hudnell, 2010). Information attained from a case study of nutrient-enriched water bodies revealed the role of SPC in the decrease of cyanobacterial highest concentration by about 82 and 95% (Hudnell, 2010). Applications of rapid wavelength ultraviolet radiation on the water that is infested by cyanobacteria and cyanotoxins, can quickly cause degradation of the cyanotoxins concentration in water treatment procedures (Beattie *et al.*, 1998). Simulated waterfalls or fountains may also be adequate to control the cyanobacterial blooms in smaller water bodies, hence reduce the risk of HABs exposures (Nally, 2011). Furthermore, cavitation treatment can fragment gas vesicles of cyanobacterial cells and filament, which eliminates up to 99% cyanobacteria growing in water bodies (Jančula *et al.*, 2014).

### **2.8.3 Chemical approaches**

Harmful cyanobacteria and their toxins can be controlled to a certain extent using some chemicals such as inhibitors or flocculants and algicides. Nevertheless, the use of these chemicals can certainly recontaminate water bodies again and impair water quality (Van Hullebusch *et al.*, 2002). A research conducted by Dai *et al.* (2012) has shown to eliminate of MC-LR by a low-cytotoxic microgel- Fe (III) compound up to 99%. Pre-oxidation with chlorine dioxide, followed by flocculation and settling, was found advantageous in eliminating cyanobacterial cells and MCs toxins in contaminated water sources (Bogialli *et al.*, 2012). The use of aluminium salts can be used as algicides for nuisance algae and cyanobacteria control management in a water treatment center (Lelkova *et al.*, 2008). Application of slaked lime calcite (CaCO<sub>3</sub>) in infested water has also been reported to eliminate the algal cells and filament, including cyanotoxins (Prepas *et al.*, 2001). Aluminium compounds can be used to reduce the nutrients from manufacturing industries and domestic wastewaters (De Julio *et al.*, 2010). Likewise, other metals such as iron and copper can be used to control harmful cyanobacteria and reduce toxins in the water. The salt of copper (CuSO<sub>4</sub>.5H<sub>2</sub>O) is extensively used as an algicide in reduction of cell densities in water (Lee *et al.*, 2002). In most cases chlorination is as a treatment choice in several developing countries for many water treatment plant whereby chlorination with 2 mg/L can breakdown cyanotoxins bonds and make water safe for human consumption (Lelkova *et al.*, 2008; Bogialli *et al.*, 2012).

## **2.9 Legislation maximum tolerable limits for cyanotoxins**

Cyanotoxins guidance value varies in each type of toxin and is country-specific most of the countries are yet to develop the limits for food, drinking and recreational water instead, they use WHO provisional guideline (Table 2). Microcystin -LR is the most referred cyanotoxins in terms of limit setting due to available data on animal's studies that are used as a health-based reference value. A provisional TDI of 0.04  $\mu\text{g}/\text{kg}$  bw/day for MC-LR was given by WHO (2008). This is used for toxin exposure assessment for most countries. Moreover, other countries have developed their guidelines for varies limit in water for recreational activities, drinking water and aquatic food. Guidance value also differs in many ways based on their research experience and expert recommendation, e.g. USA has set a limit for infant, school-age and adult this is different from other countries but also the restrictions vary between states. Table 3 below highlights the provisional limit for countries, which set up guideline.

**Table 3: Recommended limit of cyanotoxins exposure to human for drinking water**

<b>Countries/ organization</b>	<b>Source of exposure</b>	<b>Limit</b>	<b>Reference</b>
WHO	Recreation water	Provisional given value >2000 µg/L (Extreme high) 20-2000 µg/L (High) <10 µg/L (Low)	WHO (2003)
	Drinking water	1 µg/L	WHO (2008)
<b>Europe</b>			
Italy Hungary France	Recreation	>25 µg/L No Swimming <4 µg/L Acceptable limit 25 µg/L Not acceptable	Ibelings and Chorus (2007)
Argentina, South Africa Uruguay Brazil Singapore	Drinking water	1 µg/L WHO provisional given value	Chorus (2012)
New Zealand	Recreation	>12 µg/L	Ibelings <i>et al.</i> (2016)
US-EPA* (USA)	Drinking	0.7 µg/L (Infants and Pre-school age for 10 days only) 3 µg/L Adults	US-EPA (2015)
Australia	Fish Prawns Molluscs or Mussels	18 µg/kg 24 µg/kg 39 µg/kg	Mulvenna <i>et al.</i> (2012)
Canada	Food	0.02 µg/kg bw/d	Testai <i>et al.</i> (2016)
	Recreation	<20 µg/L	Ibelings <i>et al.</i> (2016)
	Drinking water	1.5 µg/L	Chorus (2012)

US-EPA\* standard limit for cyanotoxins (MC-LR) in food, drinking water and recreations varies in the different state it's not uniform to all USA.



## 2.10 Cyanotoxins detection method

There are different methods used to detect cyanotoxins, Table 4 summaries range of biological toxin and their detection method.

**Table 4: Detection method for different cyanotoxins**

Biological toxin	Toxin	Detection method	Reference
Hepatotoxic	Microcystin	ELISA, HPLC,	Mekebri <i>et al.</i> (2009)
		PPIA, LC-MS/MS	Neffling <i>et al.</i> (2010) Miles <i>et al.</i> (2012)
	Cylindrospermopsin	MS/MS, ELISA, HPLC	Velichko and Pinevich (2019)
Hepatotoxic	Nodularin	ELISA, PPIA, MS/MS, HPLC	Lundgren <i>et al.</i> (2012)
Neurotoxic	Anatoxin-a/homoanatoxin-a	ELISA, MS/MS,	He <i>et al.</i> (2012)
		HPLC-DAD	Salmaso <i>et al.</i> (2017)
Neurotoxic	Anatoxin-a (S)	AEIA, MS/MS	Paerl and Otten (2013)
Neurotoxic	Saxitoxin	ELISA, HPLC, MS/MS	Loftin <i>et al.</i> (2016)
Cytotoxic, gastroenteritis and dermatotoxic	Lyngbyatoxin	HPLC, LC- MS/MS	Paerl & Otten (2013)
Neurotoxic	Beta-Methylamino-L-alanine (BMAA)	HPLC, ELISA, MS/MS	Esterhuizen-Londt <i>et al.</i> (2011)

HPLC-High performance liquid chromatography, MS/MS mass spectrometry mass spectrometry, AEIA acetylcholine esterase inhibition assay, ELISA enzyme-linked immunosorbent assay.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Study location

##### 3.1.1 Site selection

The study was carried out in Ukerewe District in Mwanza region, Tanzania (Island in the LV). The Island was selected based on the nature of water sources, which is mainly Lake Victoria. Most of Island residents usually draw their water for drinking and domestic purposes from the lake rather than treated pipe water and borehole (wells). There were 23 selected sampling sites for water quality assessment and cyanotoxins occurrences, there were randomly selected from the most used Lake shores list for recreation activities and collection of domestic water. Furthermore, treated piped water and wells water samples (low risk of exposure) were collected for risk of exposure assessment with comparison to Lake water samples (higher risk of exposure) see Table 5 and Fig. 6:

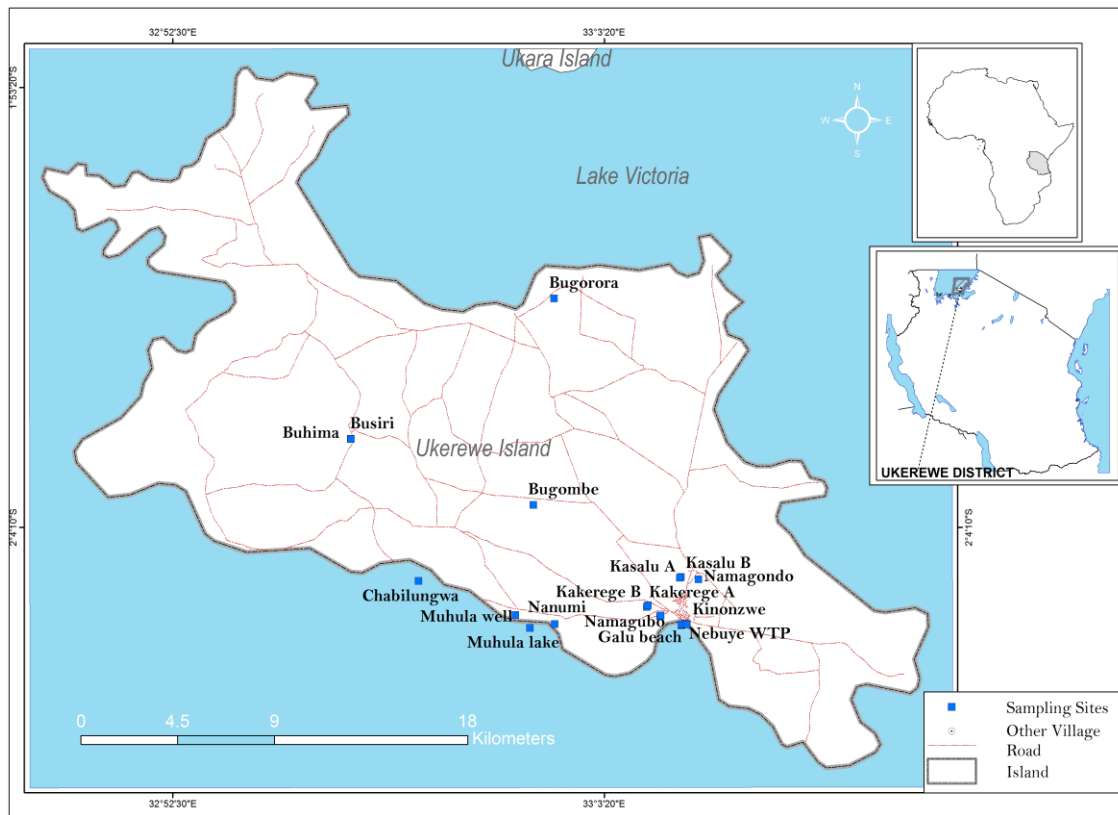
**Table 5: Water sample collection sites**

Lakeshore	Wells	Treated pipe water
1. Bugolora	1. Busiri	1. Household 1 (pipe water)
2. Chabilungo	2. Buhima	2. Household 2 (pipe water)
3. Galu beach	3. Kakerege A	3. Treatment Center (TC)-Treated
4. Water st. Agency	4. Kakerege B	
5. Muhula lake	5. Kasalu A	
6. Nanumi	6. Kasalu B	
7. Treatment Center (TC)-Untreated	7. Muhula well	
8. Namagubo Female	8. Nakatunguru	
9. Managubo Male	9. Kenonzo	
	10. Namagondo	
	11. Pius Msekwa	

##### 3.1.2 Study sites

Ukerewe district comprises 27 islands in Lake Victoria, in northern Tanzania between latitudes 10° 45' and 20° 15' S and longitudes 320° 45' and 330° 45' E (Fig. 6). Lake Victoria is the world's second-largest freshwater body, measured by surface area, and the largest in

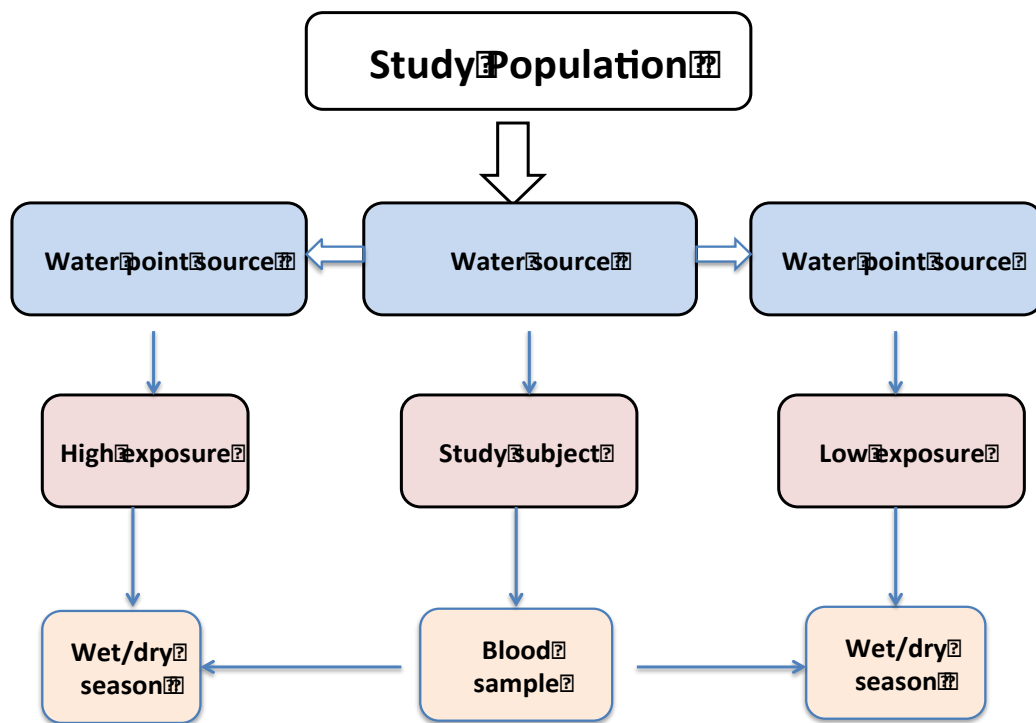
the developing world, with a surface area of 68 800 km<sup>2</sup> and a catchment area covering of 284 000 km<sup>2</sup> (Fig. 6).



**Figure 6: Ukerewe District and sampling sites**

### 3.2 Study design and population

This was a cross section study, whereby study subjects were selected from the same population in different seasons. Selected study subjects were grouped based on their water sources, and random sampling was used to select study subject. Simple random sampling was used for selection of study subject was involved in both phases (I in Feb, 2018 and II in Dec, 2018), whereby the cross-section study design was used to collect information and blood sample in the same study sample in deferent time interval of ten month-long (from Feb, – Dec, 2018).



**Figure 7: Sampling procedure for water and blood sample collection**

### 3.3 Sample size calculation

Simple random sampling was used for the selection of study subject from Ukerewe population, the sample size was calculated with consideration of design effect and response rate adjustment. The obtained sample size was used as a basis to collect data in two groups (high/low risk) of population-based on drinking water source (Fig. 7). The sample size was calculated based on the formula developed by Kothari (2004).

$$N = \frac{Deff}{r} \times \frac{Z^2 P(1-P)}{e^2} \times \frac{N}{N + e^2 \frac{P(1-P)}{e^2}}$$

P =	0.5
R =	0.83
Deff=	0.95
Z=	1.95
E=	0.05
N=	350,146 (Total population)

$$n = 0.95/0.95^2 \times 1.95^2 \times 0.5 \times (0.50/0.05^2) \times 350,146 / 350,146 + 1.96^2 \times (0.05 \times 0.05) / 0.05^2$$

n= 432

During dry season = 432

During wet season = 432 (same population same study subject)

High risk group were study subject that used water from the as their main source of water of which was regarded to have cyanotoxins while low risk was selected among those used wells and treated as the main source of water. Inclusion and exclusion criteria: Information was collected from study population in Ukerewe District, permanent resident only residing at the Island for a list more than five years and above 18 years old. This study did not involve a person below 18 years old and non-resident of Ukerewe Districts.

### **3.4 Data collection and sampling**

#### **3.4.1 Water sampling**

Water samples were collected in three different ways and purposes: The first the sampling focused on water quality parameter assessment whereby water samples were collected for six months consecutively from November 2017 to April 2018. The second water sampling focused on cyanotoxin assessment, whereby water was collected in phases (phase I in February, 2018 and phase II in December, 2018) for three weeks consecutive in each phase. The third samplings were for cyanobacteria species identification and cyanobacteria cells density assessment which was done in December 2018.

##### **(i) Water sampling for water quality parameters assessment**

Water samples were collected from 23 selected sites of Lake Victoria's shores, shallow (<5 m deep) and deep wells (>6 m), a spring, and household water pipes (Fig. 6). One-liter water samples were collected from each site for six months, consecutively from November 2017 to April 2018. The samples were collected into bottles and preserved as per the standard methods for examination of water (APHA, 2012). They were stored in a cool box with ice cubes and transported to Nelson Mandela Africa Institution of Science and Technology in Arusha for analysis.

A multiparameter meter (HI 9829, HANNA Woonsocket, RI, USA) was used on-site to determine temperature, pH, redox potential, DO, EC and TDS, and PC and total chl were measured *in situ* with an Aquafleur handheld field fluorometer model 8000-01 (Turner Designs, San Jose, CA, USA). Before use, the fluorimeter was calibrated according to the

manufacturer's instructions; total chl and PC were both quantified using the intact cells without filtration or extraction. WHO water quality guidelines (Brient *et al.*, 2008) were used to interpret the PC concentration on the basis that a concentration of 30 µg/L is equivalent to WHO alert level 1 (20 000 cyanobacterial cells/mL), and less than 30 µg/L means that the number of cyanobacterial cells/mL is below that level. In the laboratory, the inorganic nutrients analyzed include Nitrate nitrogen, nitrite nitrogen, phosphate and reactive phosphorus that were measured by spectrophotometer (HACH, DR2800).

### **(ii) Water sample for cyanotoxins assessment**

A total of 138 water samples were collected from sites along Lake Victoria's shores (n=54), wells (n=66), and treated pipe water (n=18) from 23 selected site. A volume of one-liter water-samples was collected at an interval of one week for three consecutive weeks from each site in two phases (dry – 69 samples and rainy season-69 samples), the samples were collected and preserved as per the standard methods for the examination of water (APHA, 2012). Samples were stored in cool boxes with ice cubes and transported to Nelson Mandela Africa Institution of Science and Technology (NM-AIST) in Arusha where they were stored at – 20<sup>0</sup>C then shipped to Queens University of Belfast (QUB) for further analysis while frozen in dry ice containers.

### **(iii) Water sample for cyanobacteria identification**

Samples for cyanobacteria species identification were collected from selected sites of the Lake Victoria shores, where water is fetched for drinking, domestic purposes and used for recreation activities at (depth ~ 0.5 m) at each of the established sampling. Water samples were collected at eight selected location along the Lake Victoria shore, namely Galu beach, Barazani, Namagondo, Water Agency Street, Bugolora, Kahama, Nebuye and Lugenzi. At each sampling site 20 L of water was concentrated using a 13 µm phytoplankton net to get the desirable sampling volume of 20 mL of water. The concentratedwater samples were preserved with Lugols solution (0.7%) in 20 mL vials.

### **3.4.2 Blood samples collection for liver function and toxins analysis**

Simple random sampling was used to select human study subject whereby each person in the study population had equal chance to be chosen. Blood samples were collected to selected study subject in both high and low risk area as regards to water sampling sites. A semi-structured questionnaire was administered to obtain key information about sources of

drinking water, fish-eating behaviour and other risky activities that increase exposure to cyanotoxin contaminated water. A 5 mL volume of whole blood from all selected study subjects was collected by a trained nurse in red top vacutainer tube and all blood samples were stored at the district hospital. A total of 732 vacutainers of whole blood were collected from elected study subject in ten wards namely Nansio, Kakerege, Bukongo, Mahande, Murutungura, Muhula, Chabilubgwa, Nakatunguru, Igala and Namagondo at Ukerewe district in two phases. The blood samples were centrifuged at 2 500 RPM for 10 min, and then the serum samples were separated and collected in aliquots of 1 mL at Ukerewe district hospital. Serum samples were transported to National Health Laboratory Quality Assurance and Training Centre (NHL-QATC) Dar es Salaam and were stored at -20<sup>0</sup>C refrigerator. Samples were screened for HIV, HCV and HBV and Liver function test then negative samples were transported to QUB for further toxins analysis. The remaining samples after serological test in two phases were as follows; Phase I was in dry season – February 2018 (n=374) and phase II in rainy season - December 2018 (n=264). Samples were stored at -20<sup>0</sup>C then shipped to Queens University Belfast (QUB) on dry ice for analysis. At QUB.

### **3.5 Sample analysis**

Samples analysis were done in different methods based on the outlined objectives below. For each objective a detailed method is stipulated hereunder:

#### **3.5.1 Cyanotoxins assessment of different surface waters**

##### **(i) Materials for cyanotoxin assessment**

Standards for microcystins (-LA, -LF, -LR, -LY, -LW, -RR, -YR, -WR, dm MC-RR and dm MC-LR) and nodularin were purchased from Enzo Life Science (UK). Anatoxin-a was purchased from the National Research Council, Canada and cylindrospermopsin were obtained from n'Tox, France. Water was supplied from an in-house 18 MΩ Millipore water system, Millipore Ltd. (Hertfordshire, UK). Acetonitrile, Methanol and formic acid were purchased from Sigma Aldrich (Dorset, UK).

##### **(ii) Analytical Standard preparation**

Anatoxin-a was provided reconstituted at a concentration of 4.96 µg/mL, whereas the other toxins were in powder form (100 µg). Cylindrospermopsin was reconstituted in water whereas microcystin and nodularin standards were reconstituted in pure methanol (100 µL) to give stock standards of 1 mg/mL. Working standards of 10 µg/mL were further prepared by

diluting the stock standards of 1 mg/mL 1:100 (v/v) with 80% aqueous methanol (v/v) and water for cylindrospermopsin. The multi-toxin stock standard containing all thirteen standards was then prepared at a concentration of 500 ng/mL by dilution of the 10 µg/ mL working standards 1:20 (v/v) and in the case of anatoxin-a by 1:9.92 (v/v) with 80% aqueous methanol (v/v).

### **(iii) Extraction method**

Sample extraction was conducted after lyophilisation of a 100 mL aliquot of water sample from 23 selected site. Samples were then extracted by resuspension in 5 mL of 75% aqueous methanol (v/v). Samples were briefly vortexed for 1 minute, then transferred to a 15 mL falcon tube, whereby they were vortex mixed for a further 30 minutes at room temperature before was centrifuged at 4500 rpm for 15 minutes. The supernatant was collected and evaporated to dryness under a gentle stream of nitrogen using a turbovap, at 50 °C, then the residue was dissolved in in 200 µl of 80% aqueous methanol (v/v) and transferred to a micro vial for analysis. Quantification of any toxins present was attained by the use of a seven-point extraction solvent calibration curve with a range of 5 ng/mL to 1000 ng/mL based on an initial sample size of 100 mL. This was achieved by spiking with the MTS standard at 5, 50 or 500 ng/mL. Calibrants used were prepared based on the method above in section 2.5.

### **(iv) Water Sample Preparation and extraction**

A total of 138 water samples were collected from 23 field sites as shown of Fig. 6 at Ukerewe district. Samples collection was divided into two phases; Phase I was in dry season – February 2018 (n=69) and phase II in rainy season - December 2018 (n=69), whereby 54 (39%) samples were from the Lake, wells 66 (48%) and treated piped water 18 (13%). At Queens University of Belfast (QUB) samples were separated in 100 mL aliquot from 1L collection bottles from the field then stored at -20<sup>0</sup>C before freeze-drying. Sample extraction was as per section (iii).

### **(v) Analysis on TQ-MS**

Analysis of the thirteen toxins was performed using liquid chromatography mass spectrometry (UPLC-MS/MS) machine from Waters, (Manchester, UK). The system was operated in electrospray positive mode (ESI+) with the capillary voltage set at 1 kV, source and desolvation temperatures at 150 °C and 400 °C respectively, and desolvation gas flow at 700 L/hr, optimised to give the best sensitivity across all analytes. Detection and



quantification were achieved using targeted analysis via Multiple Reaction Monitoring (MRM) (Table 6) involving fragmentation of specific precursor ions (parent) using argon as the collision gas, to at least two product ions (daughters), with the cone voltages and collision energies for each analyte optimised manually. The separation was achieved using a CORTECS UPLC T3 column, 100 mm x 2.1 mm i.d., 1.6  $\mu\text{m}$  particle size, 120Å pore size (Waters, UK) with the column maintained at 45°C. The mobile phases A and B consisted of water containing 0.1% formic acid (v/v) and acetonitrile respectively. The flow rate of mobile phase was set at 0.45 mL/min with the acetonitrile held at 2% for 1min, followed by an increase to 70% over 9 min, washed for 1 min at 90% before returning to 2% for a 1min re-equilibration before the next injection. L.M- resolution ranges from 1-2.85 and H.M resolution ranges from 1-14.8. The injection volume was set at 2  $\mu\text{L}$ .

**Table 6: Optimised MRM/SRM transitions for the 13 freshwater cyanotoxins**

Analyte	Precursor Ion(m/z)	Cone (V)	Base Fragment Ion (Q)(m/z)	Collision Energy (eV)	Qualifier fragment (q)	Collision Energy (eV)	Retention time (min)
ATX-A*	166.10	25	149.0	15	131.05	15	1.79
CYN	416.20	35	194.1	40	336.2	20	1.09
dmMC-RR	512.95	35	135.2	40	107.2	50	5.43
MC-RR	519.95	35	135.0	30	127.1	40	5.51
NOD	825.50	65	135.1	65	70.0	75	5.89
MC-LA	910.50	32	135.1	65	213.1	60	7.57
dmMC-LR	981.55	50	135.1	70	107.1	70	6.33
MC-LF	986.50	35	135.1	70	213.1	55	8.59
MC-LR	995.60	55	135.0	75	107.05	80	6.29
MC-LY	1002.5	35	135.15	70	163.1	60	7.72
MC-LW	1025.6	25	135.1	65	107.1	65	8.40
MC-YR	1045.5	60	135.2	70	107.1	75	6.14
MC-WR	1068.55	60	135.1	75	107.1	75	6.53

Optimised MRM/SRM transitions for the 13 freshwater toxins (cyanotoxins) including; quantifier ion ( $Q$ ) and qualifying ion ( $q$ ). \*ATX-A has a second qualifier fragment ( $q1$ ) which is the diagnostic ion and is used to prevent misidentification;  $q1 = 166.1 > 42.95$  with a collision energy of 20 eV (not shown in table).

### 3.5.2 Cyanobacteria species identification and health risks assessment

#### (i) Cyanobacteria identification

Phytoplankton identification to the genus level was done on a light microscope following morphological descriptions given by Anagnostidis and Komárek (1988) and Komárek and Kling (1991). The counting of phytoplankton classification were done by using inverted

microscope at 400 x magnification, morphological species identification criteria were according to freshwater phytoplankton keys (Whitton *et al.*, 2002). Ten fields were randomly selected during counting and observation of the species from the sedimentation chambers. Different species were counted by numbers of filaments and cells depending on the nature of the species. Phytoplankton abundance was calculated using the formula by (Greenberg, 1992):

$$Abundance = \frac{C \times A_t \times v}{A_f \times F \times V \times V_i}$$

Where:

C = number of organisms counted,

A<sub>t</sub> = total area of bottom of settling chamber (mm<sup>2</sup>),

V = volume of concentrated sample (20 ml),

A<sub>f</sub> = area of field (mm<sup>2</sup>),

F = number of fields counted,

V = volume of sample observed (2 ml) and

V<sub>i</sub> = volume of the sedimented sample.

### 3.5.3 Cyanotoxins detection method validation and biochemical indices analysis

#### (i) Cyanotoxins detection in serum

Cyanotoxins detection method was developed for the purpose of validation its performance and then the method was test in the collected human serum samples using the following protocol

#### **Materials**

Standards for MCs (-LA, -LF, -LR, -LY, -LW, -RR, -YR, -WR, dm MC-RR and dm MC-LR) and NOD were purchased from Enzo Life Science (UK). Cylindrospermopsin was obtained from n<sup>o</sup>Tox, France. Water was supplied from an in-house 18 MΩ Millipore water system, Millipore Ltd. (Hertfordshire, UK). Methanol, acetonitrile, trifluoroacetic acid, formic acid, human serum (from male AB clotted whole blood) and ENVI-Carb SPE cartridges (250 mg, 3 mL) were purchased from Sigma Aldrich (Dorset, UK). OASIS PRiME HLB SPE cartridges (60 mg, 3 mL) were from Waters (Dublin, Ireland).

### ***Analytical Standard preparation***

Cyanotoxins was reconstituted in water whereas the MC and NOD standards were reconstituted in pure methanol (100 µL) to give stock standards of 1 mg/mL. Working standards of 10 µg/mL were further prepared by diluting the stock standards of 1 mg/mL 1:100 (v/v) with 80% aqueous methanol (v/v) and water for cylindrospermopsin. The multi-toxin stock standard containing all twelve standards was then prepared at a concentration of 500 ng/mL by dilution of the 10 µg/mL working standards 1:20 (v/v).

### ***Extraction of cyanotoxins from serum for method validation***

Toxin extraction and enrichment were developed using 250 µL aliquots of human serum (from male AB clotted whole blood). Samples (250 µL of serum) were extracted by addition of 1.5 mL of 75% aqueous methanol (v/v), vortex mixed briefly and sonicated at room temperature for 30 min to extract any potential toxin(s) present, followed by centrifugation at 16 000 g for 15 min to remove cell debris/matrix and protein. The supernatant was removed and diluted 5-fold with water to achieve a 15% methanol content for loading onto SPE cartridges; OASIS PRiME HLB and ENVI-Carb. Before loading samples, SPE cartridges were conditioned with 3 mL of MeOH, followed by 3 mL of water, then 3 mL of 15% aqueous methanol (v/v). Samples were loaded onto the Oasis PRiME HLB cartridges, the flow-through collected then passed through the ENVI cartridges. Cartridges were cleaned with water and 20% aqueous methanol (v/v), then briefly dried before elution into clean glass tubes.

The analytes were eluted with 2 x 1.5 mL of 80% aqueous methanol (v/v) containing 0.1% TFA for OASIS PRiME and 2 x 1.5 mL methanol/dichloromethane (4:1 v/v) comprising 5% formic acid (v/v) for the ENVI. The subsequent eluents were collective, dried under a stream of nitrogen before reconstitution in 75 µL of 80% aqueous methanol (v/v) and transported to a microvial for analysis.

### ***Cyanotoxins extraction in serum samples for method validation***

Sera samples of 250 µL were extracted as detailed by Gree (2018). Quantification of any toxins present was attained by the use of seven-point extracted matrix matched calibration curve with a range of 0.01 ng/mL to 2.5 ng/mL. This was achieved by spiking with the multi-toxins standard.

### ***Analysis by UPLC-MS/MS of in serum samples***

Analysis of the twelve toxins in sera samples was performed using a liquid chromatography mass spectrometry (UPLC-MS/MS), Xevo TQ-S (triple quadrupole MS/MS) from Waters (Manchester-UK). The system was operated in electrospray positive mode (ESI+) with the capillary voltage set at 1 kV, source and desolvation temperatures at 150 °C and 400 °C respectively, and desolvation gas flow at 700 L/hr, optimised to give the best sensitivity across all analytes. Detection and quantification were achieved using targeted analysis via Multiple Reaction Monitoring (MRM) involving fragmentation of specific precursor ions (parent) using argon as the collision gas, to at least two product ions (daughters), with the cone voltages and collision energies for each analyte optimised manually. The separation was achieved using an ACQUITY UPLC Cortecs T3 column, 2.1 x 100 mm i.d., 1.6 µm particle size, 120 Å pore size (Waters, Ireland) with the column maintained at 45°C. The mobile phases A and B consisted of water containing 0.1% formic acid (v/v) and acetonitrile respectively. The flow rate of mobile phases was set at 0.45 mL/min with the acetonitrile held at 2% for 1 min, followed by an increase to 70% over 9 min, washed for 1 min at 90% before returning to 2% for a 1min re-equilibration before the next injection. The injection volume was set at 2 µL.

### **(ii) Serology and liver function test**

Liver function was performed to detect levels of biochemical indices as an indicator of liver damage using the following protocol and materials

#### ***Materials***

Rapid HIV test 1 (SD Bioline) and HIV test 2 (uni-gold) were from Standard diagnostics, INC. (Republic of Korea). Rapid immunochromatographic test for detection of Hepatitis B surface Antigen in serum (HBsAg WB) and HCV were from Standard Diagnostics, INC. (Republic of Korea).

The following list of reagent from Roche (Germany) used for liver function test on a Cobas: Integra 400 plus automated chemistry analyzer

Cobas ISE Deproteinizer (6x21ml)

Cobas (Activator Roche Diagnosis kit)

Cobas C.f.a.s (Roche Diagnosis kit, C.f.a.s (12x3ml))

Cobas Precicontrol (PeciControl CC Multi 1 (4x5ml))

Cobas cleaner solution (1L)  
Cobas ALP (Alkaline Phos)  
Cobas BIL-D (Roche Diagnosis kit)  
Cobas BIL-T (T-Bilirubin total G.3)  
Cobas TP (Roche Diagnosis kit)  
Cobas ALB (Roche Diagnosis kit)  
Cobas ALTL (GPT) - (Roche Diagnosis kit)  
Cobas ASTL (GOT) (Roche Diagnosis kit)

### ***Sample preparation***

Serum samples were separated for liver function test and serological test. Blood sample used was free from haemolysis, lipemia and icterus. A volume of 250  $\mu$ L of serum used for liver function test and 100  $\mu$ L for serological test.

### ***Serology test for for HCV, HBV and HIV***

Tests for HIV, HBV and HCV were done using rapid immunochromatographic HIV test (SD Bioline), HBsAg and HCV respectively.

### ***Analysis on Cobas Integra 400 plus***

Analysis of liver function test was performed using Cobas Integra 400 automated chemistry Analyzer plus from Roche diagnostic Basel (Switzerland). Also, transaminases (ALT & AST) were determined on Cobas Integra 400 plus automated clinical chemistry analyzer and the method was according to the IFCC, but without pyridoxal – 5- phosphate. The enzyme ALT / AST were catalyzed by the reaction between L- alanine/ aspartate respectively and 2-oxoglutarate. The pyruvate/oxaloacetate formed react with NADH, in the presence of dehydrogenase to form NAD. The rate of the Nicotinamide Adenine Dinucleotide (NADH) oxidation is directly proportional to the catalytic ALT/AST activities and determines by measuring the decrease in absorbance at 340 nm. The absorbance of test is directly proportional to the concentration of ALT/AST presence in the specimen.

Method calibration was done according to each test requirement, controls calibrator for automated systems (Cfas) from Roche commercial control was used for quality control. Controls were run before processing patient sample for each day and/or after every 30 samples have been run test for the field-collected sample. A volume of 250  $\mu$ L was used for

all required test. An electronic database was created with patient ID, date of sample collection and demographic information as a requirement of automated result from Cobas Integra 400 plus. The instrument was set to run the test in order of arrangement and results was automatically produced after the validation then printed. Interpretation of results was based on the normal ranges of biochemical liver indices whereby below and above the normal range were considered as abnormal range results hence used as proxy indicator for liver damage due to toxin exposure.

The list of the range used as a reference for the liver function test result are shown in Table 6. All results that were out of range consider having liver problem.

**Table 7: Liver biochemical indices normal reference range**

<b>Index</b>	<b>Gender</b>	<b>Normal range</b>
1 ALP	Men	40-129 U/L
	Female	35-104 U/L
2 Alb		25-55 g/L
3 ALT	Men	4-41 U/L
	Female	2-33 U/L
4 AST	Men	2-40 U/L
	Female	2-32 U/L
5 Total Protein		66-87 g/L
6 T.BIL		0-21 $\mu\text{mol/L}$
7 D.BIL		0-3.4 $\mu\text{mol/L}$

### **3.6 Statistical analysis**

#### **3.6.1 Water quality parameters**

Data were collected and prepared using Microsoft Excel (MS) spreadsheet and analyzed using Open Source software, R statistical package version 3.5.0 (R. Core Team, 2018). Generalized linear mixed models (GLMMs) with a Gaussian distribution were used to model variations in the amount of PC for different environmental variables. The mixed model was used to account for pseudo-replication during sampling. The amount of PC was included in the model as a response variable, while different variables of interest were included as fixed factors. In the univariate analysis, means and their 95% confidence intervals were reported in tables while in the multivariate analysis adjusted means with their 95% confidence intervals were reported. The outcomes were considered significant when the p-value < 0.05. All graphs were generated using R statistical software with a ggplot2 (Grammar for Graphic plot) package (Wickham, 2016)

### **3.6.2 Health effect due to cyanobacteria exposure**

Data was collected through open data kit (ODK) then exported to and cleaned using Microsoft Excel (MS). The analysis was done by Epi Info Version 7.2.1 statistics software. The outcome of interest was the observed health effects such as stomach upset, vomiting, diarrhea, and skin, eye and throat irritation. Each outcome of interest was independently tested in Univariate analysis using two by two table and Chi-square - Mantel-Haenszel (2-tailed p-value) was used to determine the level of significance. For the response with less than five in the two by two table, Fisher exact test was used to determine level of statistical significance. All statistically significant outcomes variables in the univariate analysis were further put together in the multivariate analysis.

Multivariate analysis was done by a backward method where the outcome with the weakest association was removed in the model successively until the best-fit model was comprehended. In the univariate analysis crude odds ratio (cOR) and their 95% confidence intervals (CI=95%) were reported, while in the multivariate analysis the adjusted odds ratio (AOR) with their (CI=95%) were reported. The results were measured as significant when the P-value (<0.05.)

### **3.6.3 Cyanotoxin associated illnesses assessment**

Health effects were self-reported by the study subjects, based on the following definition: Having diarrhea was considered when an individual reported having three or more loose stools in the duration of 24-hour. Vomiting; ejection of abdominal contents through the mouth, stomach upset; syndrome of digestive system functions characterized by uneasiness. All the three symptoms together (vomiting, stomach and upset diarrhea,) were regarded as gastrointestinal illness (GI) that interfere with regular activities. Skin irritation was defined as a condition of swelling, itching or reaction of the skin. Throat irritation, defined as pain, itchiness of the throat that can lead to cough and runny nose as general upper respiratory illness. Eye irritation was whichever eye infection, eye itching, or/and watery eyes (Collier *et al.*, 2015)

### **3.7 Ethical consideration**

The permission to carry out this study was sought from the Tanzania National Institute for Medical Research (NIMR) with reference number NIMR/HQR.8a/Vol. 1X/2436. Consent was obtained from each of the nominated study subjects before their involvement in the data

collection. Local authorities (Region, District, Village) were informed on purpose and importance of the study before study implementation and permission was granted to conduct the survey in their areas.

Participants who agreed willingly to participate in the study signed the consent form, which abides the rules and regulations of research in human from NIMR. Confidentiality of the study participants was strictly observed and the findings of this research will be given to the Ministries responsible for human health, water authority, Regional and District Officers where this research was conducted. Material and data transfer agreement between QUB and NM-AIST was sought from NIMR.



**CHAPTER FOUR**  
**RESULTS AND DISCUSSION**

**4.1 General results on humans**

**4.1.1 Questionnaires results**

A total of 432-study subjects were selected to participate in this study. The mean age of the participants was 42 years old with a range from 18 to 86 (SD=15) of which 186 (43%) were aged from 18 to 39 years and 246 (57%) were above 40 years. There were more male study subjects 234 (54.2%) than female 198 (45.8%). Almost 80% of the study subjects completed primary school, more than 50% were farmers and 90 (20%) were fisherman (Table 7). The mean weight was 60 kg (SD=11).

**Table 8: Demographic characteristics of study subjects (N=432)**

<b>Characteristics</b>	<b>Frequency</b>	<b>Percentage (%)</b>
<b>Sex</b>		
Male	234	54
Female	198	46
<b>Age group</b>		
18-39	186	43
40+	246	56
<b>Level of education</b>		
Never attended school	43	9.9
Primary school	340	79
Secondary school	44	10.1
Tertiary education	5	1
<b>Occupation</b>		
Fisherman	90	21
Farmer	301	70
Employed	17	4
Unemployed	24	5

**4.1.2 Serological results for HCV, HBV and HIV**

The total of 732 blood samples were collected in both phases and screened for HCV, HBV and HIV. In phase one 432 samples of blood were collected then screening, results indicated that 58 were positive for screening test of which 37 were positive for HBV, 3 for HVC and 18 for HIV. In phase two, a total of 300 blood samples were collected, screen results show

that 36 were positive whereby one, 11 and 24 were positive for HIV, HCV and HBV respectively. The positive samples were removed from the list of serum stock and the total of 638 negative samples remained, whereby in phase I were 374 and phase II were 264. The negative samples from HIV, HBV and HCV were tested for presence of cyanotoxins in both phases.

## **4.2 Results on surface water**

### **4.2.1 Cyanotoxins contaminations in surface water**

Table 5 and 6 shows the the cyanotoxin positive water samples from different collection sites during phase I and II. A total of 138 water samples were collected from the Lakeshores 54 (39%), wells 66 (48%) and treated piped water 18 (13%) in both phases. Results indicate that cyanotoxins were detected in eight (89%) out of nine-selected sites from the Lakeshore waters. Several toxins such as CYN, microcystin congeners; (-RR, -LR and -YR) and NOD, were identified in phase I. In the phase II cyanotoxins were identified in 4 (44.4%) sites only, whereby CYN and MC (-RR and -LY) were detected.

CYN was detected in 8 (89%) of Lakeshore collection sites with the concentration range from 3.6 to 10.8 ng/L. NOD was detected in one site with the concentration of 10.4 ng/L. The study shows the presence of microcystin congeners -RR, -LR and -YR with the concentrations range from 2.8 to 8.6 ng/L for MC- RR, 10.2 to 11.8 ng/L for MC-LR and for MC-YR from 11.8 to 13 ng/L in phase I. Furthermore in phase one, MC-RR was detected in 5 (55.6%) of the Lakeshore samples collection site while MC-LR in 2 (22.2%) and MC-YR in 4 (44.4%) (Table 8). There were no cyanotoxins detected in water samples from the wells and piped water.

**Table 9: Positive sample showing toxins profile and their detection levels in phase I**

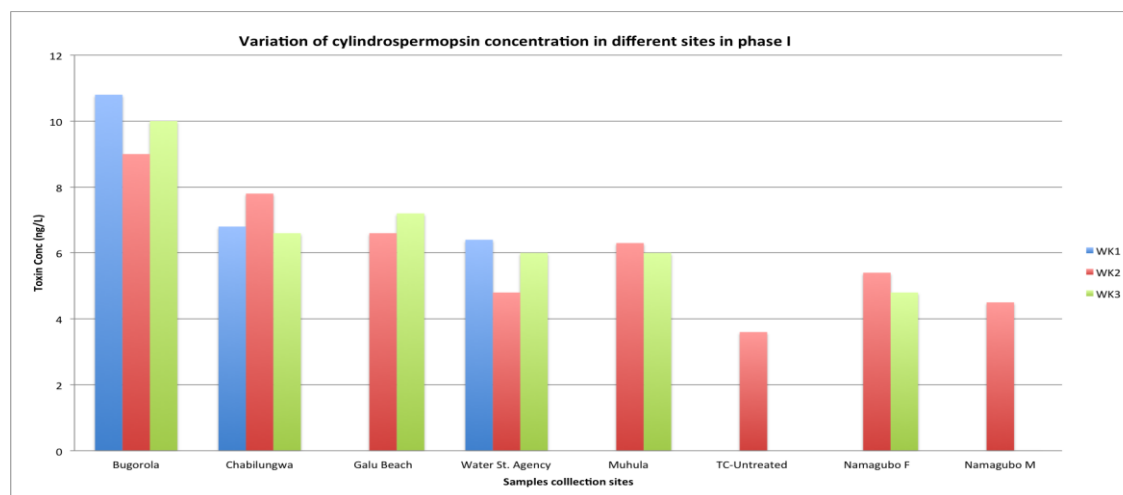
Sampling location	Cyanotoxin concentrations														
	CYN (ng/L)			MC-RR (ng/L)			MC-LR (ng/L)			MC-YR (ng/L)			NOD (ng/L)		
	WK1	WK2	WK3	WK 1	WK 2	WK3	WK1	WK2	WK3	WK1	WK2	WK3	WK1	WK2	WK3
Bugorola	10.8	9	10	8.6	8	5.4									10.4
Chabilugwa	6.8	7.8	6.6												
Galu Beach		6.6	7.2	3.5											
Water Agency Street	6.4	4.8	6	2.8	2.8										12.8
Muhula		6.3	6			2.8			11.8						11.8
Nanumi															
TC-Untreated		3.6													13
Namagubo Female		5.4	4.8		2.9									11.8	
Namagubo Male		4.5							10.2						

Phase II results indicate that CYN was detected in the water sample from the Lake with the concentration range from 4 –12.2 ng/L in 3 (44%) collection sites. Microcystin-LR was detected in one site with the concentration of 9.6 ng/L while MC-RR was present in two sites with the concentration range from 3.8 to 4.2 ng/L (Table 9). No cyanotoxins were detected in water samples from the wells and treated pipe water in phase II.

**Table 10: Positive sample showing toxins profile and their detection in phase II**

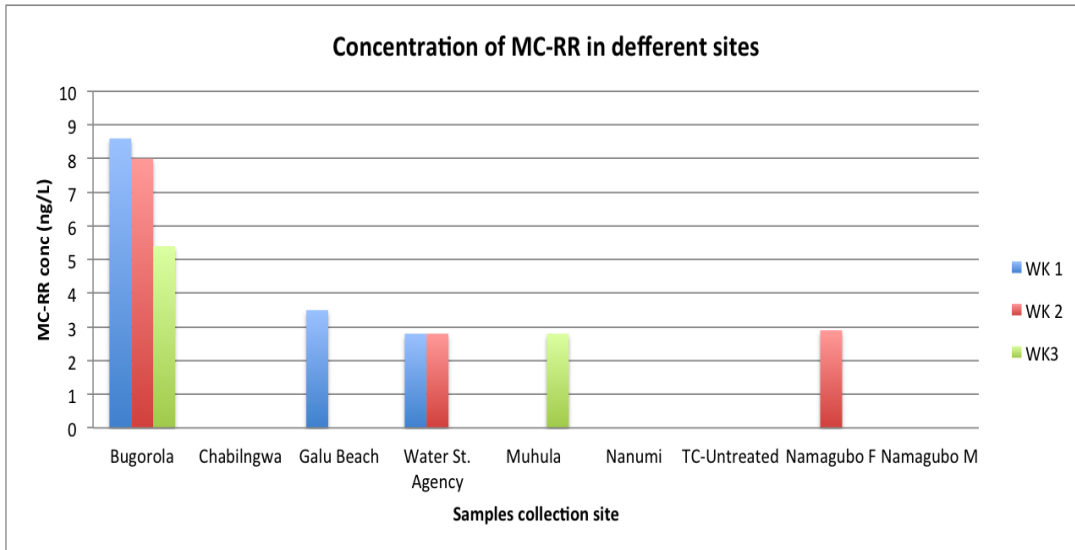
Name	CYN (ng/L)			MC-RR (ng/L)			MC-LR (ng/L)		
	WK1	WK2	WK3	WK 1	WK 2	WK3	WK1	WK2	WK3
Bugorola	12.2			4.2					
Chabilungwa	4								
Water Agency Street							9.6		
Muhula Lake	7.8			3.8					

Concentrations of cyanotoxins in phase I were very high as compared to phase II in general except for CYN where there is a slight increase of maximum levels of concentrations of 10.8 ng/L in phase I to 12.2 ng/L in phase II. Microcystin congeners -RR, -LR and -YR, CYN and NOD were detected in phase I as compared microcystin congeners -RR, -LR and CYN detected in phase II.



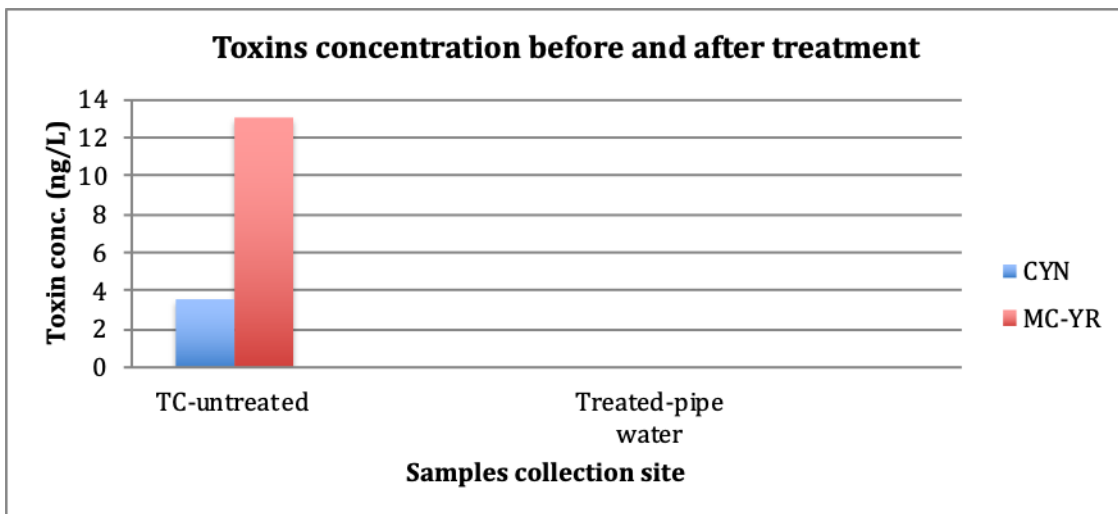
**Figure 8: Cylindrospermopsin concentrations for three weeks period in phase I**

Cylindrospermopsin was the most abundant cyanotoxin observed in 89% of all the collection sites, and in week two it was detected in all eight sites while in week three it was detected in six sites (Fig. 8).



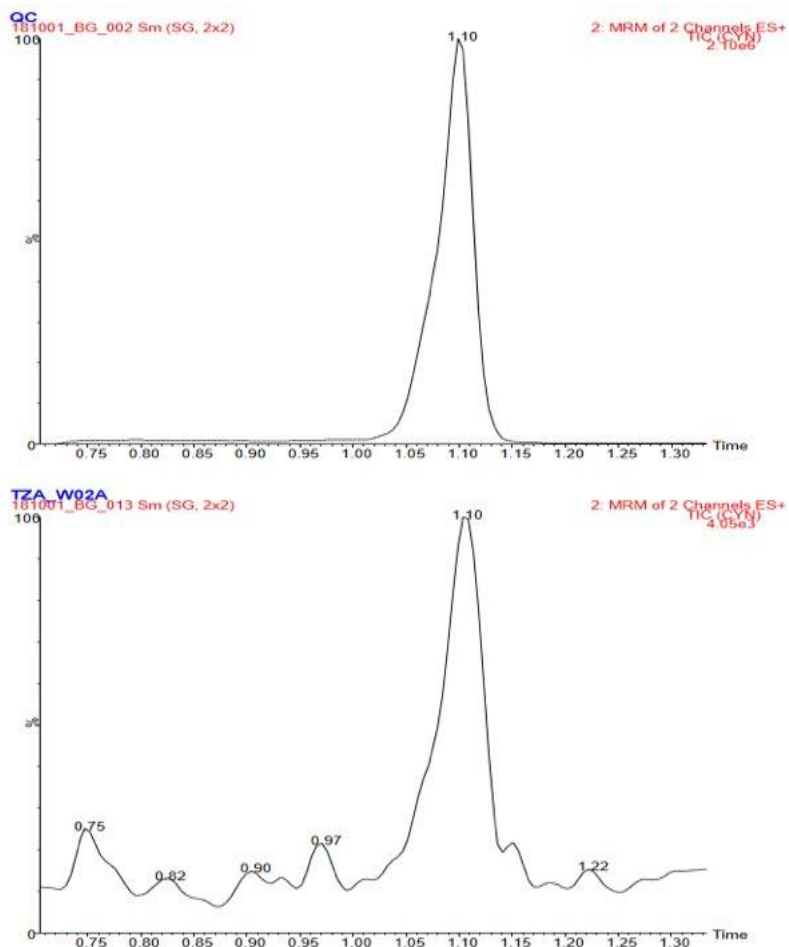
**Figure 9: Microcystin-RR concentrations in samples collected for three weeks in phase I**

Samples from the lake indicated the presence MC-RR in 5 (55.6%) of the collection sites, and this makes it the second most abundant MC toxin in the collection sites (Fig. 9). Among the sites that MC-RR was detected, only one site exhibited the toxin in the entire period of three weeks.



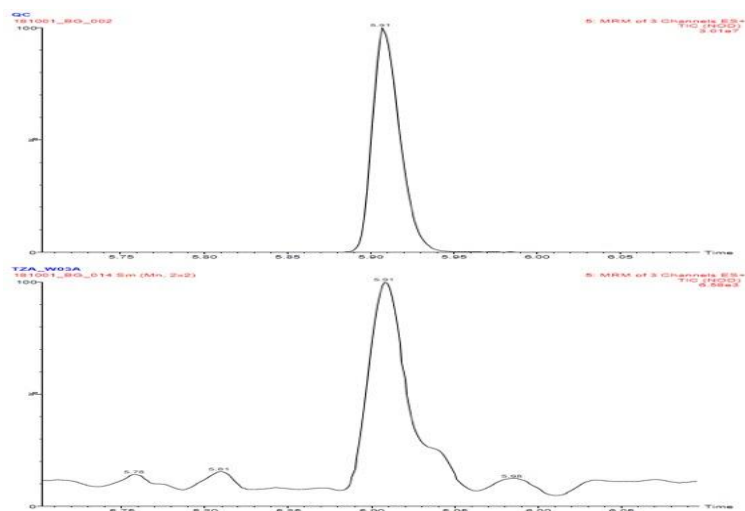
**Figure 10: Cyanotoxins at the treatment center (TC) in phase I before and after treatment**

Water samples collected at the catchment area of the treatment plant in phase I were found with CYN and MC-RR toxin with the concentration of 3.6 ng/L and 13 ng/L of MC-YR, respectively. No toxin was detected after treatment in phase I however in phase II no toxins were detected before and after treatment (Fig. 10).



**Figure 11: Chromatograms of the cyanotoxin CYN**

The chromatogram on top shows the CYN standard, with the bottom chromatogram indicating a sample positive for CYN (TZA\_W02A is water sample of week two from Bugolora site).



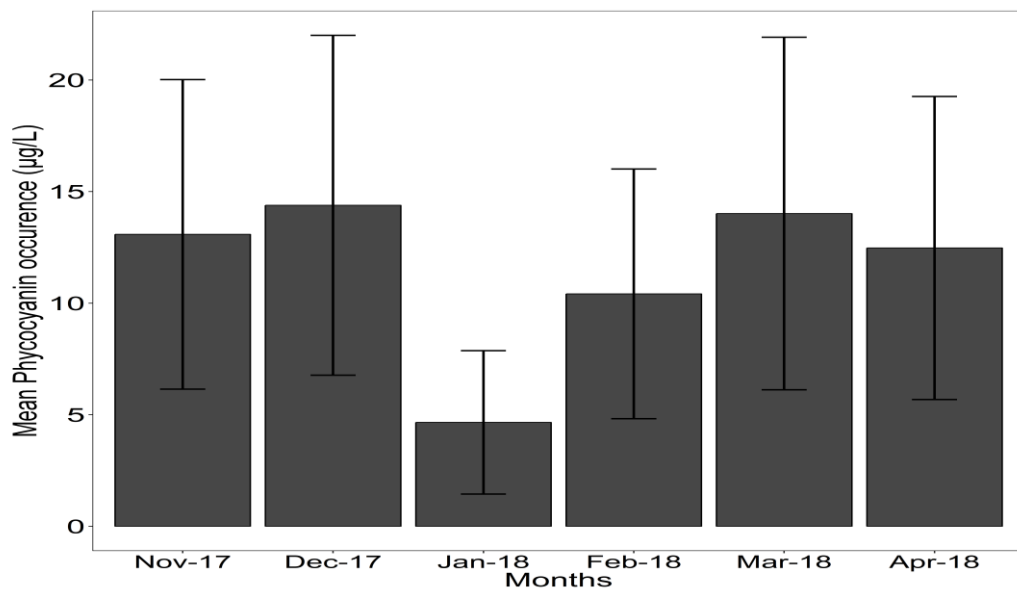
**Figure 12: Chromatograms of the cyanotoxin NOD**

The chromatogram on top shows the NOD standard, with the bottom chromatogram indicating a sample positive for NOD (TZA W03A is a water sample of week 3 from Bugolora site).

### 4.3 Results on water quality assessment

#### 4.3.1 Water quality parameters as a proxy indicator for cyanotoxins existence

A total of 138 samples was collected from water sources, which were divided into four main categories – lake shores 54 (39%), deep wells 18 (13%), natural springs 12 (9%), shallow wells 36 (26%) and piped water 18 (13%).



**Figure 13: Phycocyanin concentration means from November 2017 to April 2018**

The mean PC concentrations found in December 2017 and March 2018 were higher than in other months (Fig. 13). The concentration was lowest in January 2018. Other studies conducted in Lake Victoria show that algal blooms vary slightly but can occur throughout the year (Okello *et al.*, 2010).

#### 4.3.2 Water quality parameters from selected sampling site as shown below (11-14)

Water quality parameters were measured from different selected sampling sites to look at their level of concentration for six consecutive months as the data presented in sites specific in Table 11 – 14:

**Table 11: Water quality parameters from selected sites on Lake Victoria's shore**

Sample collection site		Temp (°C)	Redox	pH	DO (mg/l)	EC	TDS	PC	Total chl	NO <sub>3</sub> -N	NO <sub>2</sub> -N	PO <sub>4</sub> <sup>3-</sup>	P
						(µS/cm)	(mg/l)	(µg/L)	(mg/l)	(mg/l)	(mg/l)	mg/l	(mg/l)
Bugorola	Min	24	55	7	6	248	161	6	26	16	18	0.25	0.01
	Max	29.6	298	9	7	641	416	24	138	30.8	43	0.55	0.26
	SD	2	82.1	0.6	0.4	143.4	92.8	6.1	49.4	6	9.3	0.1	0.1
Namagobo-Male	Min	25	88	7	6	161	155	5	27	11	11.9	0.29	0.09
	Max	29	297	9	8	372	249	40.2	160	30.9	51.8	0.81	0.38
	SD	1.5	76.5	0.8	0.7	74.2	44	13.1	58.1	7.1	14.5	0.2	0.1
Namagobo-Female	Min	25	83	7	5.55	244	158	6	36	21.2	9.7	0.16	0.05
	Max	28	251	8	8	387	285	33	176	33	33.9	0.96	0.55
	SD	1	74	0.5	0.9	49.4	42.6	9.6	63.8	5.4	9.1	0.3	0.2
Galu beach	Min	24.9	97	7	6	198	128	5	33	17	7	0.17	0.05
	Max	29	260	9	8	412	267	44	129	52	37	0.74	0.41
	SD	1.5	66.8	0.8	0.8	76.3	49.5	13.2	39.6	14.2	10.4	0.2	0.1
Water agency-Street	Min	25	95	8	6.37	274	178	11	18	24	24.3	0.24	0.08
	Max	29.5	387	9	8	398	413	48	201.3	60.9	64	2.04	1.06
	SD	2	122.8	0.4	0.6	46.6	97.9	13.5	70.5	12.7	14.9	0.7	0.4
Chabilugwa	Min	25	81	8	6.4	200	97	14	23	24.6	16.6	0.14	0.05
	Max	28	245	9	7	391	286	39	156	65.3	73	16.17	0.79
	SD	1	62.6	0.4	0.2	75.1	77	9.6	52.5	15.9	18.9	6.4	0.3
Muhula- Lake	Min	24.5	80	7.6	5	170	89	25	29	27	17.8	0.14	0.04
	Max	29	286	8	7	298	166	49	195	51	75	18.15	0.65
	SD	1.6	84.9	0.2	1.1	50	28	9.5	65.7	9.7	20	7.1	0.2
Nanumi	Min	25.2	99	7	5	158	48	28	69	29.3	27.3	0.17	0.06
	Max	28	271	8	7	301	183	58.4	213	72.9	84	22.14	0.96
	SD	0.9	66.1	0.4	1.1	59.7	49.9	12	65.3	17.8	20.3	9.1	0.4



Sample collection site		Temp ( <sup>0</sup> C)	Redox	pH	DO (mg/l)	EC (μS/cm)	TDS (mg/l)	PC (μg/L)	Total chl (mg/l)	NO <sub>3</sub> -N (mg/l)	NO <sub>2</sub> -N (mg/l)	PO <sub>4</sub> <sup>3-</sup> mg/l	P (mg/l)
Nebuye Intake	Min	25	32.5	7	6	239	108	3	53	15	18	0.94	0.62
	Max	29	191	9	7	440	271	32	159	53.9	59	2.32	1.9
	SD	1.4	66.7	0.8	0.4	90.8	63.2	6	39.6	12.9	15.1	0.6	0.4

**Table 12: Water quality parameters from selected deep wells**

Sample collection site		Water quality parameter											
		Temp ( <sup>0</sup> C)	Redox	pH	DO (mg/l)	EC (μS/cm)	TDS (mg/l)	PC (μg/l)	Total chl (mg/l)	NO <sub>3</sub> -N (mg/l)	NO <sub>2</sub> -N (mg/l)	PO <sub>4</sub> <sup>3-</sup> mg/l	P (mg/l)
Bogombe	Min	25	33	5	6	185	119	0.1	0.36	1.4	11.6	0.19	0.06
	Max	28	232	7	8	440	286	1.21	23	5.7	25	0.59	1
	SD	1	74.3	0.8	0.7	101.6	68	0.4	8.7	1.9	5	0.2	0.5
Mahula well	Min	25	78	5	5	73	52	0	0.06	0.3	1.6	0.19	0.03
	Max	28	251	8	8	284	185	0.7	27	10.4	34.2	1.25	0.44
	SD	1	61.9	1.2	1	85.3	46	0.2	10	4.2	13.4	0.4	0.1
Nakatunguru	Min	25	84	6	5	879	490	0.01	0.02	9	25.8	0.83	0.76
	Max	28	237	7	7	3733	2426	0.5	6.3	35.6	98	4.25	1.5
	SD	1.3	55.1	0.5	0.7	1004.1	670.1	0.2	2.5	9.5	27.2	1.3	0.3

**Table 13: Water quality parameters from selected shallow wells**

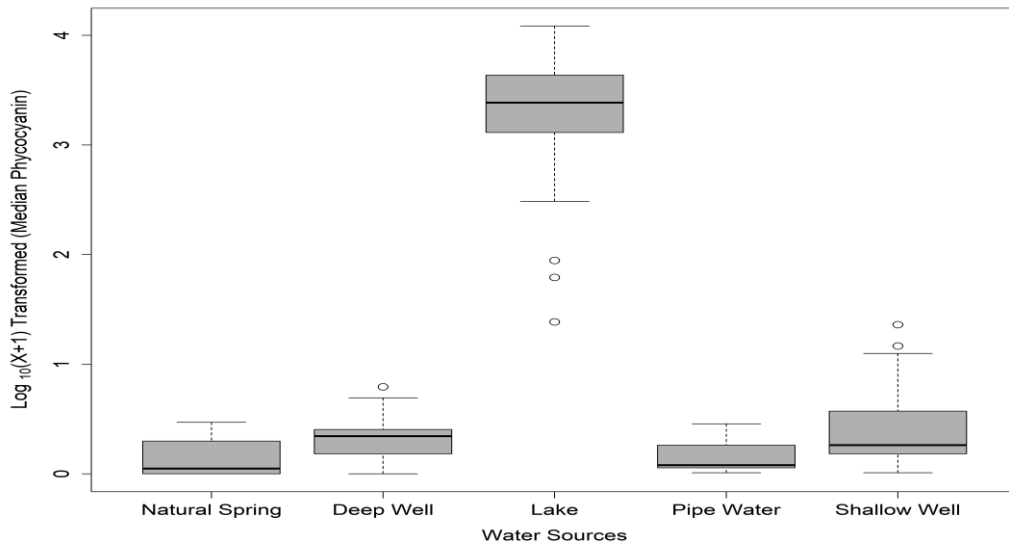
		<b>Water quality parameter</b>											
<b>Sample collection site</b>		<b>Temp (°C)</b>	<b>Redox</b>	<b>pH</b>	<b>Do (mg/l)</b>	<b>EC (µS/cm)</b>	<b>TDS (mg/l)</b>	<b>PC (µg/l)</b>	<b>Total chl (mg/l)</b>	<b>NO<sub>3</sub>-N (mg/l)</b>	<b>NO<sub>2</sub>-N (mg/l)</b>	<b>PO<sub>4</sub><sup>3-</sup> (mg/l)</b>	<b>P (mg/l)</b>
Namagondo	Min	23	44	5	3	60	78	0.3	8	0.9	24.1	0.21	0.07
	Max	27	257	7	8	226	187	2.9	39	8.4	52	0.42	0.9
	SD	1.4	83.4	0.6	1.7	53.9	37.9	1.2	13	3.1	10.2	0.1	0.3
Kakerege A	Min	25	75	6	6	321	178	0.11	1.84	4.3	6.1	0.17	0.06
	Max	28	256	8	7	1092	710	0.65	15	39	97.2	1.8	0.41
	SD	1.2	62.5	0.8	0.5	312.8	193.7	0.2	5.3	11.7	39.5	0.6	0.1
Kakerege B	Min	25	76	6	5	166	143	0.1	2.5	8.4	22.3	0.31	0.1
	Max	27	324	7	7	1125	732	0.9	13	16.7	56	1.9	0.49
	SD	1	82.2	0.5	0.8	402.2	205.2	0.3	4.6	3	15.2	0.6	0.1
Kinonzwe	Min	25	111	6	6	701	100	0.2	16	2	3.1	0.14	0.04
	Max	27	210	7.2	8	786	510	2	47	5	11	1.3	0.42
	SD	0.8	42.4	0.6	0.8	32.1	171.2	0.7	12.1	1.1	3	0.4	0.1
Kasalu A	Min	25	78	6	6	31	147	0.01	8	1.3	2.8	0.12	0.04
	Max	28	239	7	8	914	594	0.9	41	6.1	24	0.71	0.3
	SD	1.3	78.4	0.5	0.8	342.9	166.5	0.4	11.3	1.8	8.4	0.2	0.1
Kasalu B	Min	25	66	5	5	294	105	0.1	4	0.5	3	0.16	0.05
	Max	27	226	8	8	951	497	0.5	35.6	7	12.3	0.76	0.13
	SD	1	75.6	1	1	325.3	134.3	0.2	10.7	2.3	3.6	0.2	0.08

**Table 14: Water quality parameters from springs**

		Water quality parameter											
Sample collection site		Temp (°C)	Redox	pH	Do (mg/l)	EC (µS/cm)	TDS (mg/l)	PC (µg/l)	Total chl (mg/l)	NO <sub>3</sub> -N (mg/l)	NO <sub>2</sub> -N (mg/l)	PO <sub>4</sub> <sup>3-</sup> mg/l	P (mg/l)
Buhima	Min	25	37	5	5	117	76	0	0.01	1	9.3	0.14	0
	Max	27	256	7	8	269	174	0.5	34	5.2	21	0.48	0.81
	SD	0.8	78.6	1	1.2	59.5	37.3	0.2	14.2	1.5	4	0.1	0.3
Busiri	Min	26	42	5	6	88	57	0	0.03	1.4	12.3	0.12	0.02
	Max	27	277	7	8	288	187	0.6	7	11	38.3	0.82	0.27
	SD	0.5	82.5	0.9	0.7	73.4	44.2	0.3	3.1	3.7	11.9	0.3	0.1

**Table 15: Water quality parameters from selected piped water supplies**

		Water quality parameter											
Sample collection site		Temp (°C)	Redox	pH	Do (mg/l)	EC (µS/cm)	TDS (mg/l)	PC (µg/l)	Total chl (mg/l)	NO <sub>3</sub> -N (mg/l)	NO <sub>2</sub> -N (mg/l)	PO <sub>4</sub> <sup>3-</sup> mg/l	P (mg/l)
Nebuye WTP	Min	26	131	6	6.1	112	163	0.03	6.52	0.3	3.1	0.14	0.04
	Max	27	266	7	8	395	272	0.58	15	11	21.1	0.8	0.5
	SD	0.4	49.5	0.4	0.7	99.4	50.3	0.2	3.4	4.8	6.8	0.3	0.2
Household 1	Min	25	92	6	6	266	167	0.01	1	0.7	3.01	0.12	0.04
	Max	27	665	7	8	483	314	0.33	12	9.4	12.2	0.56	0.18
	SD	0.8	212.5	0.5	0.7	90.8	59.2	0.1	4.2	3.2	3.8	0.2	0.1
Household 2	Min	26	112	6	6	265	168	0.01	0.95	0.7	3	0.13	0.04
	Max	27	171	7.4	8	407	266	0.3	12	8.8	11.2	0.4	0.15
	SD	0.7	21.3	0.6	0.6	69.3	44.4	0.1	4.3	3	3.9	0.1	0



**Figure 14: Phycocyanin distribution by water source**

**Table 16: Analysis for different water sources associated with the presence of PC**

Water Sources	Univariate		
	Comparison Factors ( $\mu\text{g/L}$ )	95% CI	P-value
Spring	Ref	-	-
Deep Well	0.24	-9.67, 10.17	0.962
Lake	28.61	20.11, 37.11	<b>&lt;0.001</b>
Piped supply	-0.02	-9.94, 9.91	0.998
Shallow Well	0.4	-8.47, 9.28	0.93

**(ii) Phycocyanin association with water quality parameters**

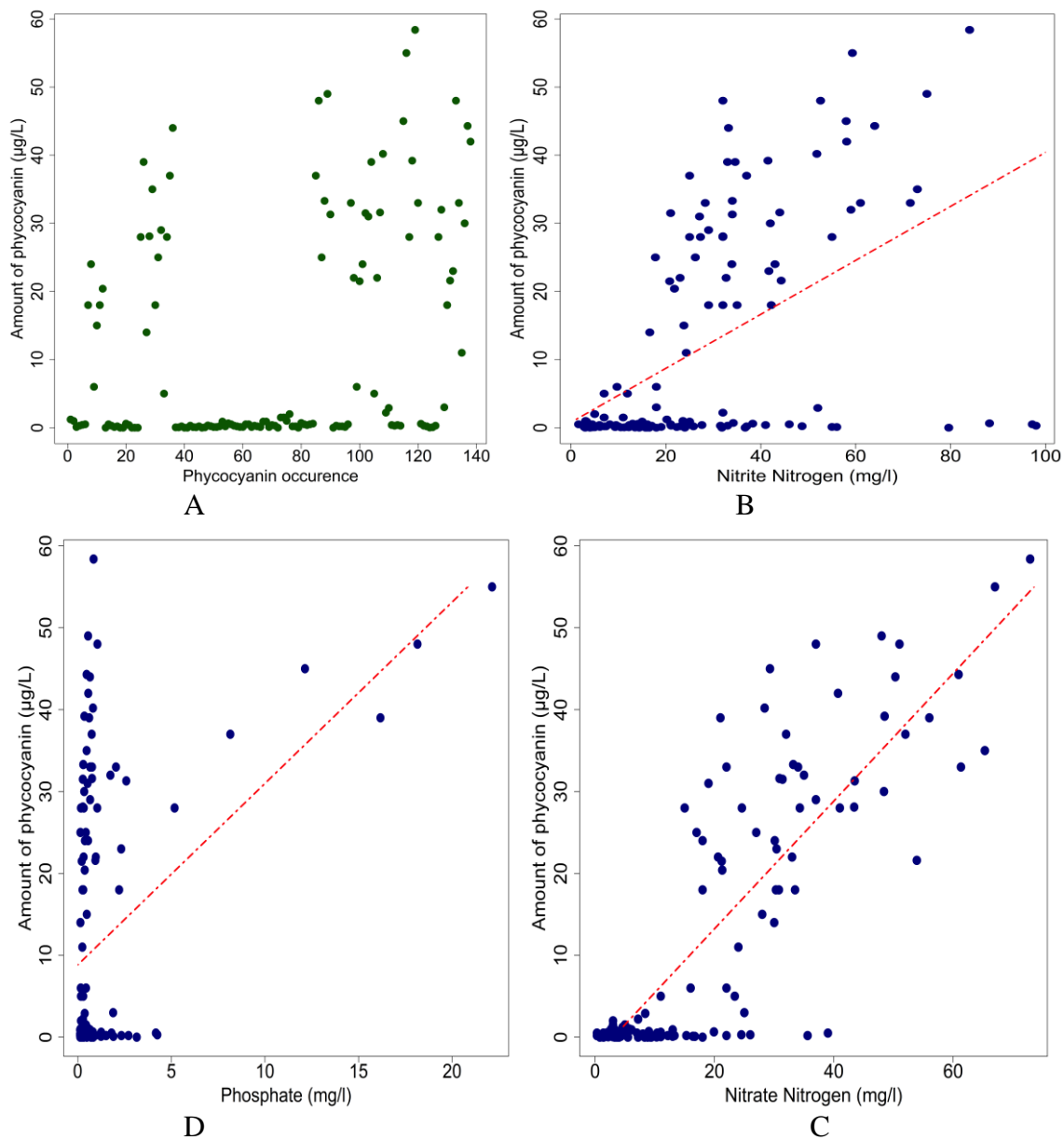
**Table 17: Analyses of water quality parameters and their association with PC**

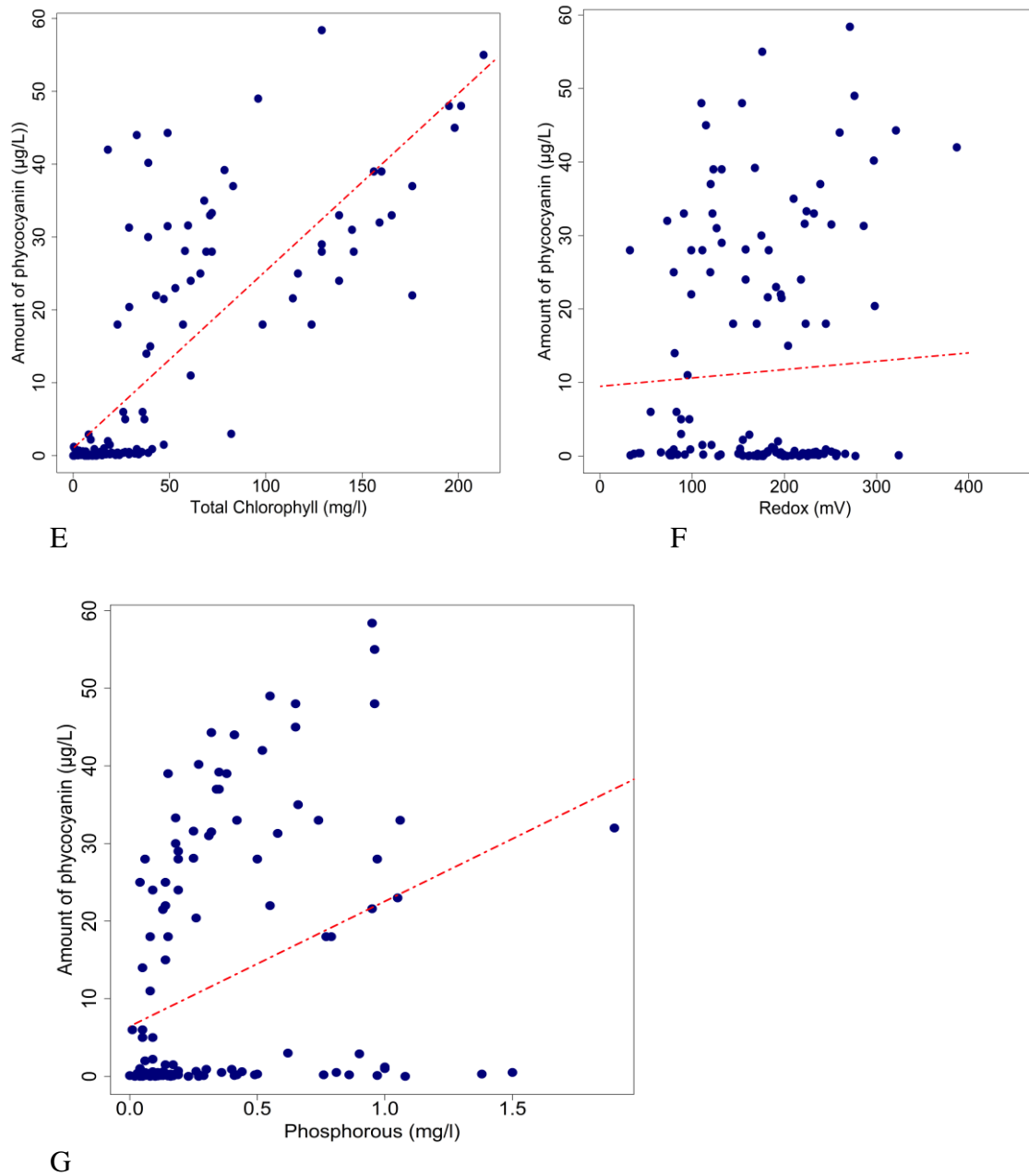
Variable	Univariate			Multivariate		
	Comparison factor ( $\mu\text{g/L}$ )	95% CI	P-value	Comparison factor ( $\mu\text{g/L}$ )	95% CI	P-value
Temperature	-3.04	-3.95, -2.13	<b>&lt;0.001*</b>	-1.26	-2.21, -0.32	<b>&lt;0.05*</b>
Redox	1.6	0.43, 2.77	<b>&lt;0.01*</b>	1.33	0.42, 2.23	<b>&lt;0.05*</b>
pH	-0.62	-2.44, 1.19	0.507			
DO	-0.19	-1.45, 1.08	0.773			
EC	-0.16	-2.07, 1.74	0.867			
TDS	-0.17	-2.01, 1.66	0.856			
Total Chl	5.67	4.12, 7.23	<b>&lt;0.001*</b>	4.6	2.98, 6.23	<b>&lt;0.001*</b>
Nitrate ( $\text{NO}_3\text{-N}$ )	9.55	7.62, 11.48	<b>&lt;0.001*</b>	5.06	3.12, 6.96	<b>&lt;0.001*</b>
Nitrite ( $\text{NO}_2\text{-N}$ )	4.74	3.21, 6.26	<b>&lt;0.001*</b>	0.89	-0.51, 2.29	0.217
Phosphate ( $\text{PO}_4^{3-}$ )	2.57	1.28, 3.87	<b>&lt;0.001*</b>	0.07	-1.01, 1.15	0.898
Phosphorus (P)	4.38	2.76, 5.99	<b>&lt;0.001*</b>	0.31	-0.97, 1.60	0.633

\*Refers to statistically significance variable where  $P < 0.05$

The univariate relationship between water quality parameters and PC indicates statistically significant associations with temperature, redox potential, total chlorophyll, nitrate nitrogen, nitrite nitrogen, phosphate and reactive phosphorus, for all of which  $P < 0.001$  (Table 16).

All water quality parameters reported as statistically significant in the univariate analysis were subjected to the multivariate model. Redox potential, Temperature, total chl and nitrite nitrogen all correlated with PC with  $P < 0.05$  (Table 17).





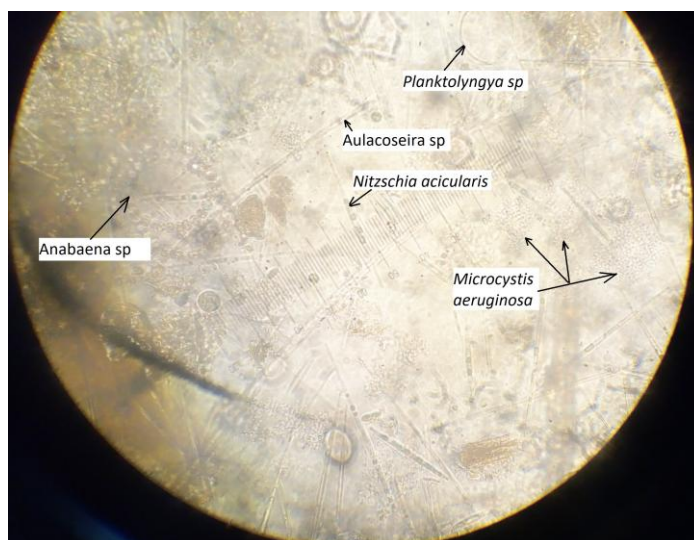
**Figure 15: Predictions of PC and its association with water quality parameters (B-G)**

#### 4.4 Harmful Algal Bloom and associated health risks

Result of cyanobacteria identification in different selected samples collection site are present based on their concentration in cell density in each site in the Table below:

**Table 18: List of Phytoplankton species found at Ukerewe area in Lake Victoria**

List of Phytoplankton species found at seven selected Lake Victoria lake shores										
Taxa		Selected Sites Along Lake Victoria Shores								
Chlorophyceae (Green algae)		Unit	Lugenzi	Water Agency St.	Namagobo	Barazani	Bugolora	Nebuye	Kahama	Galu Beach
1	<i>Ankistrodesmus sp</i>	cells/ml	90.25	150.9	75	33.85	22	10	21	80
2	<i>Coelastrum microporum</i>	cells/ml	0	12.10	33.85	0	33.85	4	2	13
3	<i>Pediastrum sp</i>	cells/ml	0	0	0	33.85	0	0	0	0
4	<i>Scenedesmus spp</i>	cells/ml	0	0	0	135.41	270.83	1 218.72	1 624.96	3 656
<b>Cyanophyceae (Cyanobacteria)</b>										
5	<i>Anabaena spp</i>	cells/ml	677 068	4 814 702	142 316.75	174,054	112 310	67 321	73 292	13 310
6	<i>Chroococcus dispersus</i>	cells/ml	32	42.00	812.48	12	812.48	31	11	54
7	<i>Merismopedia spp</i>	cells/ml	2 843.69	201 113	2 843.69	1 421	2 447.60	4 361.00	32 562.00	189 815
8	<i>Microcystis aeruginosa</i>	cells/ml	90 361.63	3 032 031	148 139.02	111 239	136 104	92 302	104 032.00	154 212
9	<i>Microcystis flos aquae</i>	cells/ml	14 387.70	443 507	10,156.02	5 078	8 463.34	100 950	100 239.86	203.12
10	<i>Microcystis sp</i>	cells/ml	110	21 202	302	13 541	6 770.68	321	2 761.00	5 211
11	<i>Planktolyngbya circumcreta</i>	filament/ml	118.49	0.00	67.71	50.78	78.98	0	0	0
12	<i>Planktolyngbya spp</i>	filament/ml	50.78	0	169.27	50.78	0	0	0	0
<b>Bacillariophyceae (Diatoms)</b>										
13	<i>Aulacoseira spp</i>	cells/ml	1 692.50	1 402 6	1 777.30	4,824	761.7	0	0	3 046.80
14	<i>Cyclotella sp</i>	cells/ml	84.63	308.13	67.71	50.78	67.71	101.56	203.12	541.62
15	<i>Fragilria spp</i>	cells/ml	33.85	0	50.78	67.71	33.85	0	101.56	372.32
16	<i>Navicula sp</i>	cells/ml	0	0	67.71	0	67.71	101.56	101.56	101.56
17	<i>Nitzschia acicularis</i>	cells/ml	1 263.85	0.00	1 218.72	220	327.23	0	0	203.12
18	<i>Synedra cunningtonii</i>	cells/ml	440.09	210	293.37	84.63	67.71	0	0	0
<b>Dinophyceae</b>										
19	<i>Glenodinium sp</i>	cells/ml	0	0	0	203	33.85	0	0	0



**Figure 16: Image of different cyanobacteria species under light microscopy**

During this study, while collecting the samples, water colour was observed to be greenish with bloom on the surface, which was a clear indication that cyanobacteria member dominated the areas, four main groups of phytoplankton namely cyanobacteria, dinophyceae, diatoms and chlorophyceae (Table 18). Results indicate that Cyanobacteria (Cyanophyceae) were flourishing in all eight sample collection sites; they formed more than 97% of all phytoplankton in the Lake Victoria. The most common members were *Microcystis*, *Anabaena* and *Merismopedia* Fig. 16. The concentration of *Microcystis aeruginosa* class ranged from 90 361 to 3 032 031 cells/mL, on selected eight sample collection sites 5 (62.5%) had above 100 000 cells/mL and 3 (37.5%) had less than 50 000 cells/mL. *Anabaena spp.* cells abundance with the maximum concentration levels of 4 814 702 cells/mL of which 7 (87.5%) sites had above 100 000 cells/mL and 1 (12.5%) less than 100 000 cells/mL. *Merismopedia spp* cells concentration maximum levels were 201 113 cells/mL where only 2 (25%) sites had cells above 100 000 cells/mL (WHO) safety limit and 6 (75%) sites had less than 100 000 cells/mL (Table 18). Treated pipe and well water were not tested for availability of cyanobacteria.

#### **4.4.1 A reported health effect from the study population**

The study was conducted in December 2018, and a total of 432-study subject participated in this study. Of the studied population 134 (31%) stated using LV water as their primary source of water, while 229 (53%) used well water and 69 (16%) used treated supplied pipe water as their primary source of water for drinking and domestic usages. Reported health effects among study subject were; 78 (18.06%) did not report any health effect while 71 (16.44%) reported one health effect, 81 (18.75%) reported two health effects, 124 (28.70%) reported 13



illnesses and 4 (0.98%) specified all six-health results. The study observed that there is a mixture of water uses and different exposure whereby a person can use treated distributed pipe water from Ukerewe water authorities for cooking or drinking but use LV water for bathing and other water activities and the vice versa is true. More than 50% of the study subject reported skin irritation, stomach upset and eye irritation followed by diarrhoea 32% while vomiting 9% and throat irritation 10% was minimum reported in total.

**Table 19: Reported health effect from various water sources**

Health Effect	Water source	Response		Univariate cOR (95% CI)	P- value	Multivariate AOR (95% CI)	P- value
		Yes	No				
Vomiting	Lake	27	107	6.1 (2.8-13.58)	<b>&lt;0.001</b>	2.8 (1.18-6.4)	<b>0.01</b>
	Wells	9	220				
Diarrohea	Lake	48	86	1.5 (0.95-2.38)	0.08		
	Wells	62	167				
Skin Irritation	Lake	80	54	1.21 (0.78- 1.86)	0.3		
	Wells	126	103				
Eye irritation	Lake	69	65	0.86 (0.56-1.33)	0.5		
	Wells	126	103				
Throat irritation	Lake	23	111	6.57 (2.74-15.79)	<b>&lt;0.001</b>	4.3 (1.5-11.76)	<b>0.004</b>
	Wells	7	222				
Stomach upset	Lake	119	15	8.14 (4.49-14.78)	<b>&lt;0.001</b>	7.7 (4.2-14.4)	<b>&lt;0.001</b>
	Wells	113	116				
Vomiting	Lake	27	107	17 (2.28-127.2)	<b>&lt;0.001</b>		
	Pipe	1	68				
Diarrohea	Lake	48	86	1.19 (0.64-2.2)	0.5		
	Pipe	22	47				
Skin Irritation	Lake	80	54	0.65 (0.35-1.2)	0.16		
	Pipe	48	21				
Eye irritation	Lake	69	65	0.46 (0.24-0.85)	<b>0.01</b>	0.2 (0.07-0.57)	<b>0.002</b>
	Pipe	48	21				
Throat irritation	Lake	23	111	Undefined	<b>0.005</b>		
	Pipe	0	69				
Stomach upset	Lake	119	15	45.2 (19.72-111.81)	<b>&lt;0.001</b>	58.96 (21-162.73)	<b>&lt; 0.001</b>
	Pipe	10	59				

In univariate analysis, individuals who used the lake as their source of drinking water were six times more likely to have vomiting compared to those individuals who used wells as their primary source of drinking water, this was statistically significant  $P < 0.001$ . The risk of getting throat irritation when using the lake as a source of drinking water was six times higher than when using wells as the main source of drinking water  $OR = 6.57$  (95%  $CI = 2.74-15.79$ ),  $P < 0.001$ .

Those who had used the LV as their primary water source were revealed to be associated with more stomach upsets ( $OR = 8.4$ ,  $P < 0.001$ ) compared to those using wells as their significant consumption source water (Table 19). The odds of vomiting when an individual consumed lake water was observed to be seventeen times higher compared to when an individual used treated supplied piped water (95%  $CI = 2.28-127.2$ )  $P < 0.001$ . Bathing using treated supplied pipe water was protective against eye irritation as compared with contaminated LV water source with cyanobacteria  $OR = 0.46$ , 95%  $CI = 0.24-0.85$  this statistically significant  $P = 0.01$ . Reporting GI when a person drinks cyanobacteria contaminated LV water was 45.2 folds higher than when they used treated supplied pipe water for drinking,  $P < 0.001$ .

**Table 20: Reported health effect based on bloom availability**

Health Effect	Variable	Response		Univariate cOR (95% CI)	P-value	Multivariate AOR (95%CI)	P-value
		Yes	No				
Vomiting	Bloom	29	220	3.95 (1.49-10.44)	<b>0.003</b>		
	No bloom	5	150				
Diarrohea	Bloom	85	164	2.25 (1.39-3.6)	< <b>0.001</b>	2.4 (1.5-4)	< <b>0.001</b>
	No bloom	29	126				
Skin Irritation	Bloom	159	90	1.5 (1-2.3)	<b>0.04</b>		
	No bloom	81	74				
Eye irritation	Bloom	140	109	1.2 (0.8-1.8)	0.36		
	No bloom	80	75				
Throat irritation	Bloom	24	225	4 (1.37-11.8)	<b>0.006</b>		
	No bloom	4	151				
Stomach upset	Bloom	171	78	3.4 (2.28-5.28)	< <b>0.001</b>	3.39 (2.2-5.2)	< <b>0.001</b>
	No bloom	60	95				

The odds of vomiting when consuming water with visible bloom was almost four times higher compared to drinking water without visible bloom,  $P < 0.05$  (Table 20). The odds of getting diarrhoea when drinking water with visible bloom is two times higher as compared with consuming water source without cyanobacteria bloom (95% CI=1.39-3.6),  $P < 0.001$ .

The likelihoods of getting skin irritation among those individuals using water with visible bloom was almost two times higher as related to those who used water for bathing without visible bloom, 95% CI=1.0-2.3,  $P < 0.05$ . Study subjects who reported drinking water with visible bloom were four times more associated with throat irritation than those who consumed water with no visible bloom OR=4,  $P < 0.05$ . The study showed that the probabilities of getting stomach upset when drinking water with visible bloom was three folds higher than those who drank water with no visible bloom this was statistically significant OR=3.4,  $P < 0.001$ .

**Table 21: Reported health effect based on occupation**

Health Effect	Variable	Response		Univariate cOR (95% CI)	P-value	Multivariate AOR (95% CI)	P-value
		Yes	No				
Vomiting	Fisherman	13	77	2 (1-4.5)	<b>0.02</b>	2.2(1.05-4.4)	<b>0.03</b>
	Non-fisherman	24	318				
Diarrhoea	Fisherman	40	50	2 (1.2-3.3)	<b>0.003</b>	2 (1.2-3.2)	<b>0.004</b>
	Non-fisherman	97	245				
Skin Irritation	Fisherman	63	27	1.8 (1-2.9)	<b>0.02</b>		
	Non-fisherman	194	148				
Eye irritation	Fisherman	53	37	1.1 (0.7-1.8)	0.5		
	Non-fisherman	190	152				
Throat irritation	Fisherman	11	69	2.4 (1.1-5.1)	<b>0.02</b>		
	Non-fisherman	19	323				
Stomach upset	Fisherman	53	37	1.1 (0.7-1.9)	0.53		
	Non-fisherman	189	153				

The study subject occupation was compared between fishers and those with non-fishing activities such as employed in areas other than fishing activities. Individuals who were in the fishing activities were more likely to have reported symptoms of vomiting as compared with their counterparts who were related to other jobs rather than fishing activities OR=2, 95%

CI=1-4.5, P< 0.05. This study reveals that being a fisherman was two times more likely to have reported symptoms of diarrhoea as compared with other non-fishing occupations with 95% CI=1.2-3.3, P=0.003. The odds of getting skin irritation among fishers were almost two folds higher (95% CI=1-2.9, P=0.02) as related to persons with other non-fishing occupations (Table 21).

Study subjects with a fishing occupation were more than two times related to getting throat irritation as compared to those with other jobs (95% 1.1-5.1, P< 0.05).

**Table 22: Reported health effect based on the amount of water consumption**

Health Effect	Variable	Response		Univariate cOR (95%CI)	P-value	Multivariate AOR (95% CI)	P-value
		Yes	No				
Vomiting	Less than 1lt	19	204	1 (0.5-2)	0.88		
	> 1lt	17	192				
Diarrhoea	Less than 1lt	79	144	1.6 (1.06-2.44)	<b>0.023</b>		
	> 1lt	53	156				
Throat irritation	Less than 1lt	35	188	4 (2-7.7)	<b>&lt; 0.001</b>	3.3 (1.6-7)	<b>0.001</b>
	> 1lt	10	199				
Stomach upset	Less than 1lt	121	102	0.86 (0.58-1.26)	0.44		
	> 1lt	121	88				

Diarrhoea and throat irritation were reported to be mainly associated with the consumption of more than one liter of lake water OR=2, 95% CI=1-2.4, P=0.02 and OR=4, 95% CI=2.11-8.9, P<0.001 respectively as shows in the table above.

#### 4.5 Validation of cyanotoxins detection method and biochemical liver indices

Liver biochemical indices were measure with reference to nomal rangers for each specific index, the result differ in each phases as its shows in Table 23:

**Table 23: Liver biochemical indices test result**

Index	Normal range	No. Out of range (%)	
		Phase I	Phase II
ALP	35-129 U/L	119 (28)	1 (0.3)
Alb	25-55g/L	31 (7)	1 (0.3)
ALT	2-41 U/L	7 (1.5)	2 (0.7)
AST	2-40 U/L	32 (7.4)	20 (6.6)
Total Protein	66-87g/L	80 (18.5)	15 (5)
T.BIL	0-21 µmol/L	1	4 (1.2)
D.BIL	0-3.4 µmol/L	0	26 (8.6)

Results indicate that there were more liver biochemical indices elevations observed in phase I as compared to phase II. Liver biochemical indices such as ALP, ALb, Total protein and AST were highly detected whereby in phase II direct DB, AST and total TP were mostly recorded. The observed liver function result corresponds well with the variation of toxins, as reported previous that more toxins were detected in phase I as compared to phase II (Table 23).

**Table 24: The potential risk that associate with elevation of liver biochemical indices**

<b>Variables</b>	<b>OR</b>	<b>95% CI</b>	<b>P-Value</b>
<b>Occupation</b>			
Fisherman	5	1.01-32.6	0.04
Farmers	0.9	0.1- 5	0.99
Employed	0.38	0.2-5.6	0.49
<b>Water sampling location</b>			
Chabilugwa	14	3 – 66.9	0.001
Bugorola	25	4 - 141	0.000
Muhula lake	5	1.1- 23.9	0.02
Namagubo	9	2 - 58	0.013
Water St. Agency	5.2	1.6 - 17	0.005
Galu beach	9.3	1.5 - 56	0.015

The fishermen are five times more likely to have their liver biochemical indices evaluated than other occupation  $P < 0.04$  (Table 24). Liver biochemical indices elevation were strongly related with the concentration of toxins level observed at different collection point whereby the odds of were 25, 14, 9 for Bugorola, Chabiligwa and Galu beach respectively  $P < 0.05$ . The higher observed toxins concentration at samples collection points, the higher the liver biochemical indices elevation.

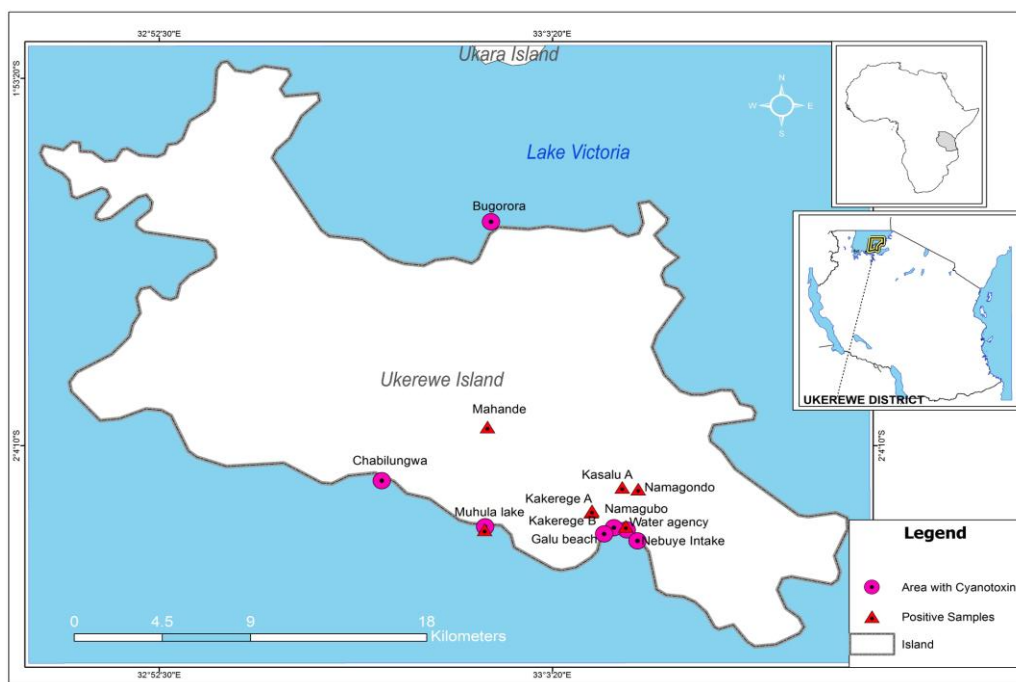
**Table 25: Level of cyanotoxins detected in human serum and liver biochemistry index**

ID	CYN	MC-LR	dm MC-LR	MC-RR	NOD	Liver biochemistry index
20	< LOD	< LOD	< LOD	< LOD	Trace <0.2 ng/mL	ALP
22	< LOD	< LOD	< LOD	Trace <0.2 ng/mL	< LOD	
23	< LOD	Trace <0.2 ng/mL	< LOD	< LOD	< LOD	
25	< LOD	0.11 ng/mL	< LOD	< LOD	< LOD	
26	< LOD	0.10 ng/mL	0.05 ng/mL	< LOD	< LOD	ALP
27	< LOD	< LOD	< LOD	< LOD	< LOD	ALP
28	< LOD	< LOD	< LOD	< LOD	< LOD	
31	< LOD	0.11 ng/mL	< LOD	< LOD	< LOD	
32	Trace	0.07 ng/mL	< LOD	< LOD	< LOD	ALP
40	Trace	0.08 ng/mL	< LOD	< LOD	< LOD	ALP, Alb
11	Trace 0.02 ng/mL	< LOD	< LOD	< LOD	< LOD	
11	Trace 0.03 ng/mL	< LOD	< LOD	< LOD	< LOD	
11	Trace	< LOD	< LOD	< LOD	< LOD	
11	Trace 0.09 ng/mL	< LOD	< LOD	< LOD	< LOD	D-Bill
11	Trace 0.08 ng/mL	< LOD	< LOD	< LOD	< LOD	D-Bill
11	Trace	< LOD	< LOD	< LOD	< LOD	
12	Trace 0.02 ng/mL	< LOD	< LOD	< LOD	< LOD	
12	Trace 0.03 ng/mL	< LOD	< LOD	< LOD	< LOD	
14	Trace 0.12 ng/mL	< LOD	< LOD	< LOD	< LOD	
14	Trace 0.07 ng/mL	< LOD	< LOD	< LOD	< LOD	ALP, AST
14	Trace 0.08 ng/mL	< LOD	< LOD	< LOD	< LOD	
15	Trace 0.03 ng/mL	< LOD	< LOD	< LOD	< LOD	ALP
15	Trace 0.14 ng/mL	< LOD	< LOD	< LOD	< LOD	ALP
15	Trace 0.01 ng/mL	< LOD	< LOD	< LOD	< LOD	ALP, TP
16	Trace 0.15 ng/mL	< LOD	< LOD	< LOD	< LOD	ALB, TP
17	Trace 0.06 ng/mL	< LOD	< LOD	< LOD	< LOD	ALP, Alb, TP, ALT, AST

Cyanotoxins detection in human serum shows the presence of CYN, NOD and MCs congener (-LR, -RR and dmMC-LR) toxins, which have potential to damage liver cells (hepatotoxins). The concentration of CYN detected range from 0.02 to 0.15 ng/mL. The concentration of MC-LR range from 0.2 to 0.11 ng/mL, MC-RR < 0.02 ng/mL and dmMC-LR <0.05. Concentration of NOD detected was < 0.05 ng/mL (Table 25). Cyanotoxins detected in human serum and liver biochemistry indices elevation, indicate that there is an association between the two with correlation coefficient of 0.33. Liver biochemical indices elevations

depend on variation of unit increase of toxins concentration or number of cyanotoxins detected in the serum. Therefore, coefficient of determination of liver biochemical indices is 0.78.

The distribution of cyanotoxins in water samples in the district indicated that its mainly around the Lake shores, whereas detections of cyanotoxins in human serum shows that it's distributed even in the area where no toxin detected in water samples (Fig. 17). There are possibilities that the exposure of cyanotoxins to human at Ukerewe is more than water source, it may be fish and other aquatic organism are the source of exposure of toxins to human.



**Figure 17: Distribution of cyanotoxins in water samples and from human serum**

Analytical performance of the developed cyanotoxins detection method was assessed using MS/MS machine whereby the results of thirteen cyanotoxins are detailed on the (Table 25-36) below. Signal 1 (+intense) is a signal from the parent compound and signal 2 is the daughter ion, the split facilitates the machine to detect more accurately the compound of interest. The linearity of the coefficient of determination was measured in relation to 1 as reference for perfect liner equation, whereby  $R^2 = 0.99$  was referred to as acceptable coefficient of determination.



Repeatability, signal relative standard diversion (rsd%) was referred to be acceptable when is less than 15%. Relative Retention Time (rsd) referred as the time where the compound of interest elutes from the colum. Ratio (rsd) is the proportion between the intensity of signal one and two whereby the lower the concentration the lower the intensity, the higher the picks and vice versa.

Trueness: Added concentration ppb (ng/mL) is the added concentration of toxin to be detected by the method. Observed concentration ppd is the observed concentration of toxin detected by the method and shift is the change or different of observed and added concentration of the toxin. Limit of quantification (LOQ) is referred to as minimum amount of analyte in a sample that can be quantified with the acceptable precision and accuracy understated operation condition of the method. Limit of qualification is determined by the analysis of sample with known concentration of analytes and establishing the minimum level at which the analyte can be reliably quantified. Limit of Detection (LOD) is the lowest amount of the analytes in a sample that can be detected but not necessarily quantified. The following table are the results of cyanotoxins detection method validation (Table 26-37).

**Table 26: Validation report cylindrospermopsin toxin (CYN)**

<b>Analytical Parameters</b>	<b>Validation Parameters</b>	<b>Signal 1 (+ intense)</b>	<b>Signal 2 (- intense)</b>
<b>Linearity</b>	Coefficient of determination (R <sup>2</sup> )	0.9987	0.9980
	Slope (a)	1156.0000	587.5000
	Intercept (b)	8.5500	5.6500
<b>Repeatability</b>	Signal (rsd %)	7.5%	10.0%
	RRT (rsd %)		2.0%
	Ratio (rsd %)		4.4%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>2.11</b>	2.05
	Shift (%)	5.6%	2.7%
<b>Limits</b>	CC $\alpha$	<b>0.02</b>	<b>0.04</b>
	CC $\beta$	<b>0.03</b>	<b>0.04</b>

**Table 27: Validation report MC-RR toxin**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination (R <sup>2</sup> )	0.9970	0.9928
	Slope (a)	9458.0000	3165.0000
	Intercept (b)	17.2000	6.3000
<b>Repeatability</b>	Signal (rsd %)	3.1%	4.6%
	RRT (rsd %)		1.6%
	Ratio (rsd %)		3.4%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>2.02</b>	2.06
	Shift (%)	0.9%	2.9%
<b>Limits</b>	CC $\alpha$	<b>0.004</b>	<b>0.005</b>
	CC $\beta$	<b>0.005</b>	<b>0.005</b>

**Table 28: Validation report dm MC-RR**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination (R <sup>2</sup> )	0.9980	0.9950
	Slope (a)	7000.9000	2348.4000
	Intercept (b)	12.1500	7.6000
<b>Repeatability</b>	Signal (rsd %)	4.9%	5.5%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		3.2%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>1.88</b>	1.73
	Shift (%)	-5.8%	-13.6%
<b>Limits</b>	CC $\alpha$	<b>0.004</b>	<b>0.011</b>
	CC $\beta$	<b>0.005</b>	<b>0.012</b>

**Table 29: Validation report NOD toxin**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination (R <sup>2</sup> )	1.0000	0.9997
	Slope (a)	18892.0000	8068.0000
	Intercept (b)	8.2000	7.3500
<b>Repeatability</b>	Signal (rsd %)	8.5%	8.0%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		2.2%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>1.84</b>	1.77
	Shift (%)	-8.0%	-11.5%
<b>Limits</b>	CC $\alpha$	<b>0.001</b>	<b>0.003</b>
	CC $\beta$	<b>0.001</b>	<b>0.003</b>

**Table 30: Validation report MC-LA**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination (R <sup>2</sup> )	0.9990	0.9981
	Slope (a)	2598.0000	1049.0000
	Intercept (b)	17.4000	21.3000
<b>Repeatability</b>	Signal (rsd %)	10.7%	9.4%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		5.4%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>1.87</b>	1.82
	Shift (%)	-6.6%	-8.9%
<b>Limits</b>	CC $\alpha$	<b>0.02</b>	<b>0.07</b>
	CC $\beta$	<b>0.02</b>	<b>0.08</b>

**Table 31: Validation report of dm MC-LR toxins**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination (R <sup>2</sup> )	0.9991	0.9984
	Slope (a)	2239.8000	843.0000
	Intercept (b)	3.7500	3.0500
<b>Repeatability</b>	Signal (rsd %)	6.7%	7.1%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		4.6%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>1.89</b>	1.91
	Shift (%)	-5.3%	-4.6%
<b>Limits</b>	CC $\alpha$	<b>0.01</b>	<b>0.01</b>
	CC $\beta$	<b>0.01</b>	<b>0.02</b>

**Table 32: Validation report of MC-LF toxins**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination (R <sup>2</sup> )	0.9999	0.9996
	Slope (a)	1465.8000	842.0000
	Intercept (b)	18.1500	26.4500
<b>Repeatability</b>	Signal (rsd %)	5.5%	9.5%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		5.8%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>1.90</b>	1.74
	Shift (%)	-4.8%	-12.8%
<b>Limits</b>	CC $\alpha$	<b>0.02</b>	<b>0.05</b>
	CC $\beta$	<b>0.03</b>	<b>0.07</b>

**Table 33: Validation report of MC-LR toxin**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination ( $R^2$ )	0.9993	0.9982
	Slope (a)	1687.7000	752.7300
	Intercept (b)	8.2000	6.9000
<b>Repeatability</b>	Signal (rsd %)	5.4%	9.4%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		5.4%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>1.88</b>	1.83
	Shift (%)	-6.2%	-8.6%
<b>Limits</b>	CC $\alpha$	<b>0.01</b>	<b>0.03</b>
	CC $\beta$	<b>0.01</b>	<b>0.03</b>

**Table 34: Validation report of MC-LY toxin**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination ( $R^2$ )	0.9999	0.9991
	Slope (a)	1810.9000	681.9300
	Intercept (b)	8.2500	8.8000
<b>Repeatability</b>	Signal (rsd %)	10.4%	12.2%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		4.4%
<b>Trueness</b>	Added conc. (ppb)		2.00
	Observed conc. (ppb)	1.88	1.83
	Shift (%)	-6.0%	-8.3%
<b>Limits</b>	CC $\alpha$	0.01	0.05
	CC $\beta$	0.02	0.06

**Table 35: Validation report MC-LW toxin**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination ( $R^2$ )	0.9994	0.9990
	Slope (a)	532.0000	270.0000
	Intercept (b)	7.3500	5.4500
<b>Repeatability</b>	Signal (rsd %)	11.4%	13.1%
	RRT (rsd %)	0.1%	
	Ratio (rsd %)	6.3%	
<b>Trueness</b>	Added conc. (ppb)	<b>2.00</b>	
	Observed conc. (ppb)	<b>2.28</b>	2.27
	Shift (%)	14.1%	13.6%
<b>Limits</b>	CC $\alpha$	<b>0.05</b>	<b>0.08</b>
	CC $\beta$	<b>0.06</b>	<b>0.11</b>

**Table 36: Validation report of MC-YR toxin**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination ( $R^2$ )	0.9999	0.9997
	Slope (a)	4926.4000	1877.7000
	Intercept (b)	24.1500	24.6500
<b>Repeatability</b>	Signal (rsd %)	5.7%	8.1%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		5.4%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>1.75</b>	1.75
	Shift (%)	-12.6%	-12.5%
<b>Limits</b>	CC $\alpha$	<b>0.01</b>	<b>0.02</b>
	CC $\beta$	<b>0.01</b>	<b>0.03</b>

**Table 37: Validation report of MC-WR toxin**

Analytical Parameters	Validation Parameters	Signal 1 (+ intense)	Signal 2 (- intense)
<b>Linearity</b>	Coefficient of determination ( $R^2$ )	0.9996	0.9996
	Slope (a)	838.7500	333.9500
	Intercept (b)	3.7000	5.3500
<b>Repeatability</b>	Signal (rsd %)	23.5%	24.3%
	RRT (rsd %)		0.1%
	Ratio (rsd %)		9.3%
<b>Trueness</b>	Added conc. (ppb)		<b>2.00</b>
	Observed conc. (ppb)	<b>1.89</b>	1.96
	Shift (%)	-5.7%	-2.0%
<b>Limits</b>	CC $\alpha$	<b>0.02</b>	<b>0.06</b>
	CC $\beta$	<b>0.03</b>	<b>0.11</b>

## 4.6 Discussion

### 4.6.1 Cyanotoxins in water

This study presents for the first time the detection of CYN and NOD toxins in Lake Victoria (Fig. 11 and 12). In this study, 13 cyanotoxins were analysed and of these, CYN was the most abundantly detected in 8 (89%) and 3 (33%) of the collection sites in phase I (dry season) and phase II (wet season), respectively. Originally it was thought that CYN was predominantly found in toxic blooms of subtropical, tropical or arid zone freshwater bodies; however, the hepatotoxin has been increasingly identified in temperate European waters such as Germany and France (Fastner *et al.*, 2003) and Ireland (Greer *et al.*, 2016). Nodularin have similar mechanisms of action as MC, as demonstrated by Yoshizawa *et al.* (1990). The hepatotoxins have been reported to be potent protein phosphatase 1 and 2A inhibitors which have been shown to have long term cumulative toxic effect for potential tumour formation (Rastogi *et*

*al.*, 2015). However, comprehensive studies have been carried out on MC in comparison to NOD. The toxicity and carcinogenic potential of NOD in humans has not been well characterized and therefore, the findings of this investigation emphasize the need for further work in the detection and effects of NOD on human health.

This study further found Microcystins in seven (78%) of the sites, and the main variants identified included MC-LR, MC-RR and MC-YR, which was slightly higher than the results reported in cyanotoxin surveillance in European countries by Greer *et al.* (2016). The occurrence of MC-LR, MC-RR and MC-YR toxins has been related with the presence of dominant cyanobacteria species *Anabaena* and *Microcystis* that produce microcystins. This was also reported in a study conducted in Lake Victoria in the Mwanza and Musoma regions in Tanzania by Mbonde *et al.* (2015). Furthermore, the occurrence of these toxigenic cyanobacteria has been documented in studies carried out in Kenya and Uganda on the Lake Victoria shores (Okello *et al.*, 2010; Sitoki *et al.*, 2012).

It has been shown that MC-RR and MC-LR are the most occurring types of MC toxins in Lake Victoria (Okello *et al.*, 2010; Mbonde *et al.*, 2015), which was the case in this study. However, the variation of MC detection and concentration in different location of the lakeshores may be due to the life cycle of different cyanobacteria species as well as bloom concentration. Microcystin toxins concentration have been shown to exhibit seasonal variation, whereby it has been observed to be higher during the dry season compared to the rainy season. In this study it was found the concentrations of microcystin congeners -RR, -LR and -YR ranged from 2.8 to 13 ng/L in phase I, considered as the dry season, while in phase II (rainy season), only MC-RR and MC-LR were observed with a concentration ranging from 3.8 to 9.6 ng/L.

The concentrations of cyanotoxins detected in this study were below the WHO provisional acceptable limit of 1.0 µg/L for MC-LR in drinking water (Organization & others, 2008). In this study, however, it was found to be far lower concentrations of MCs in Lake Victoria waters compared to studies conducted in other locations along Lake Victoria (Sekadende *et al.*, 2005; Okello *et al.*, 2010; Mbonde *et al.*, 2015). This could be due to seasonal variation, influenced by the nutrients load of the water body, as well as the levels of eutrophication during different sampling periods and selected sites. This seasonal variation of cyanotoxins has been observed in studies done elsewhere in Uganda along the Lake Victoria shores. (Okello *et al.* , 2010).

The cyanotoxins detected included the cyclic peptides MC and NOD, as well as tricyclic alkaloid CYN, which are all hepatotoxic. Microcystin and NOD work by causing inhibition of protein phosphatase type 1 and 2A (PP1 and PP2A) in the liver cells (Runnegar *et al.*, 1991). International Agency for Research on Cancer (IARC) characterized MC-LR as Group 2B carcinogen with substantial evidence supporting the fact that it can exhibit tumor promotion mechanism (IARC, 2010).

The existence of multiple toxins in Lake Victoria freshwaters may be compounded with multiple exposures to the human population from recreational activities, fishing and consumption of contaminated aquatic organisms and water (Rastogi *et al.*, 2015). Existence of multiple toxins in the Lake increases the consequence of toxicity enhancement and therefore an increased possibility of bioaccumulation. The risk of an increase in toxin production in Africa is expected to be higher due to the rise in temperature (Liu *et al.*, 2011). In the developing countries little has been done to develop strategies for cyanotoxin prevention and control in food and water supplies. This raises concerns that multiple toxins exist and which have been detected, therefore increasing the risk to human health (Rastogi *et al.*, 2015).

Microcystins are reported to have cumulative effects by Fitzgeorge *et al.* (1994), which may be explained by the irreversible covalent binding of the toxin to the protein phosphatases and subsequent substantial damage to the cell structure (Greer *et al.*, 2018). The cumulative effects of toxins may cause sub-acute liver cell injury, which is likely to go unnoticed up to a level closer to severe acute toxicity. In most cases, the lack of apparent symptoms at moderate exposure to these toxins is likely to continue, as individuals are unaware of the repeated exposure they are subjected to. The repetitive and multiple toxin exposures, even at relatively low doses may cause cumulative liver damage, which in the extensive-term may lead to chronic liver diseases such as cancer (Chorus *et al.*, 2000; Chen *et al.*, 2009).

Samples from treated piped water in different distribution sites in Ukerewe district did not show the presence of cyanotoxins, whereas in the catchment (Lake Victoria) area of this water before treatment both CYN and MC-RR were detected. Water treatment at the district involves the application of Aluminium Sulphate (5 mg/L) followed by flocculation then chlorination (2 mg/L). Chemical water treatment approaches have proven to be the most effective means of treating cyanobacterial blooms and cyanotoxins (Bogialli *et al.*, 2012). Lelkova *et al.* (2008), observed similar findings where aluminium sulphate was found to be

effective in the treatment algae and cyanobacteria. Based on these finding it is therefore crucial for the Tanzanian water authority to acknowledge the occurrence/existence of cyanotoxins and its potential health risk to the population that consumes water from Lake Victoria freshwaters and mitigation measures to control cyanobacteria blooms.

#### **4.6.2 Water quality parameters**

##### **(i) Temperature and pH**

The temperature ranged from 25 to 29.6 °C in the lake water and from 24 to 28 °C in the shallow wells, the temperature recorded from the lake exceeding that in 2004 (Kishe, 2004). Temperature has been reported to have a direct relationship with algal blooms and toxin production (Davis *et al.*, 2009). The temperature increase is thought to be a factor contributing to the global rise in algal bloom globally – continental Africa is heating up faster than the rest of the world (Liu *et al.*, 2011). The pH recorded was between 7 and 9 in the lake water samples, and 5 and 8 from the deep wells (Table 11-14). Springwater had the lowest pH range (5 to 7) and piped the narrowest (6 to 7). The pH range of the lake water is that most favoured for PC and cyanobacterial production. Other studies have also reported that this pH range contributes to increased cyanobacterial bloom (Ndlela *et al.*, 2016; Dalu & Wasserman, 2018).

##### **(ii) Electro conductivity, total dissolved solid and dissovolved oxygen**

Electro conductivity varied significantly between the sampling sites, the highest range was in the deep wells, from 73 to 3 733  $\mu\text{S}/\text{cm}$  , followed by shallow well water ranging from 31 to 1 125  $\mu\text{S}/\text{cm}$  (Table 8). The narrowest range recorded was in spring water, ranging from 88 to 288  $\mu\text{S}/\text{cm}$ .

The TDS concentration varied substantially in all sources, from 52 to 2 426 mg/l, 78 to 732 mg/l, and 48 to 416 mg/l in water from deep and shallow wells, and lake water, respectively. The DO concentration in the lake water ranged from 5.5 to 8 mg/l and from the shallow wells from 3 to 8 mg/l. Dissolved oxygen is an important water quality parameter reflecting the physical and biological processes prevailing in the water (Trivedy & Goel, 1984). Waters with low DO concentrations can be aesthetically displeasing in colour, taste and/or odour, as well as resulting in the microbial reduction of nitrate to nitrite WHO (2006).



### **(iii) Total chlorophyll and Phycocyanin pigment**

Total chlorophyll reported high concentrations in the lake and shallow well samples, with ranges of 18 to 213 mg/l and 4 to 47 mg/l, respectively. Other water sources generally reported much lower concentrations. Phycocyanin reported the to have highest levels in lake water samples with a range of 5 to 58.4 µg/L (Fig. 14) as compared to shallow well waters with a range of 0.01 to 2.9 µg/L. PC concentrations in other sources were very low, ranging from 0 to 1.21 µg/L, 0 to 0.6 µg/L and 0.01 to 0.58 µg/L in deep well, spring and piped waters respectively. The maximum PC concentration in a lake water source was 58.4 µg/L, which exceeds WHO's "alert level 1" (Brient *et al.*, 2008). Univariate analysis for the different water source types associated with PC indicated that lake water could contain concentrations of almost 30 µg/L ( $P < 0.001$ ) – as shown in Table 16. The lake environment favours cyanobacterial growth leading to PC and chlorophyll production due to the inflow of effluents from human habitats. Because of the high PC concentrations in the lake, it is crucial to institute control measures to help lake water users.

### **(iv) Nitrate, Nitrite and Phosphate**

The nitrate ( $\text{NO}_3\text{-N}$ ) concentration varied from different water sources with a range of 11 to 72.9 mg/l in the lake, 0.3 to 35.6 mg/l in deep wells and 0.9 to 39 mg/l in shallow wells. The nitrite concentration also varied – ranges of 1.6 to 97.2 mg/l in deep wells, 2.8 to 97.2 mg/l in shallow wells, and 7 to 84 mg/l in lake waters.

Phosphate ( $\text{PO}_4^{3-}$ ) was found at high concentrations in lake water, ranging from 0.14 to 22.14 mg/l. Spring waters reported lower levels ranging from 0.12 to 0.82 mg/l. It is thought that the higher phosphate concentrations in the lake might be related to the elevated pH, which could promote desorption of sedimentary inorganic phosphorus (Gao *et al.*, 2012).

### **4.6.3 The predictive and association between PC and water quality parameters**

The statistical model developed in this study shows that some water quality parameters are associated with the presence of PC. Those with univariate association include temperature, redox potential, total chl,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{PO}_4^{3-}$  and P, with  $p < 0.001$ . The same finding was reported for the same parameters in a study conducted by Marion *et al.* (2012). The multivariate model indicates that; temperature, redox potential, total chl and  $\text{NO}_3\text{-N}$  are all statistically significant, with  $p < 0.001$  (Table 17). The associations were extra enumerated concerning the extent that the parameters contribute to increases in PC.

Nitrate contributes highly to PC occurrence, with a unit increase (1 mg-N/l) causing an increase in PC concentration of 9.55 µg/L (P<0.001), while a unit increase of P (1 mg/l) can increase PC concentration by 4.38 µg/L (P<0.001). Other parameters such as total chl, nitrite, PO<sub>4</sub><sup>3-</sup> and redox potential all also have positive correlations with PC concentration (P<0.001) as presented in Fig. 15. It was shown that, in essence, the nitrate and phosphorus loads determine the rate and magnitude of cyanobacterial growth (PC concentration). The higher the loads the greater the potential for algal growth (Wetzel, 2001). The associations observed can be used as water quality surveillance indicators that can be invoked easily and cheaply using simple detection methods.

#### **4.6.4 Harmful Algal Bloom identification and associated health risks**

Based on WHO guideline cyanobacteria cell concentration at 20 000 cells/mL is associated with the danger of short-term adverse health outcomes and at 100 000 cells/mL, risk for long-term illness exists; possibly severe health consequence. Detected levels of cyanobacteria's scum concentration are very high and utilization of water from the LV may lead to severe health problem. The predominance of cyanobacteria in the plankton of Lake Victoria has been related to eutrophication of the lake that may be stimulated by extreme nutrients inflow such as nitrogen and phosphorus nutrient (Hecky, 1993). Studies conducted in LV have indicated that occurrence of *Anabaena* and *Microcystis* species in higher abundances in Lake Victoria has threatened the water quality due to the ability of these species to produce toxins (Sekadende *et al.*, 2005; Mbonde *et al.*, 2015). The leading class of cyanobacteria observed like *Anabaena* and *Microcystis* when are consumed by an aquatic organism and human can result to health and ecological problems (Chorus & Bartram, 1999; Semyalo *et al.*, 2010).

##### **(i) *Microcystis*, *Anabaena* and *Merismopedia* and their health effect**

The observed dominant cyanobacteria of genera *Anabaena*, *Merismopedia* and *Microcystis*, (Fig. 17) can produce variants of Microcystin toxin that can lead to hepatotoxicity, inhibits eukaryotic protein phosphatases in human. These toxins have potential health effects such as gastrointestinal illness, liver inflammation, and hemorrhage and liver failure leading to death, pneumonia, and dermatitis (Chorus, 2000; Boopathi & Ki, 2014). *Anabaena* spp can also produce cylindrospermopsin toxins that are hepatotoxic, cytotoxic, neurotoxic, which will increase inhibition of glutathione synthesis, protein synthesis and cytochrome P450 in human. Cylindrospermopsin toxin has the following health effect; pneumonia, liver inflammation, and hemorrhage, gastrointestinal and dermatitis (Carmichael, 2001). Anatoxin-

a toxins are one of the cyanobacteria toxins, which are produced by *Anabaena* spp, which lead to neurotoxicity and imitation of the neurotransmitter acetylcholine. Anatoxin-a toxins can cause number of health effects include: tingling, burning, numbness, drowsiness, incoherent speech and respiratory paralysis leading to death. The observed dominant *Anabaena ssp* in the lake can potentially produce Saxitoxin, which causes neurotoxicity and blockage of voltage-gated Na<sup>+</sup> channels in humans and other vertebrates. The toxin can cause numbness, burning, tingling, drowsiness, incoherent speech and respiratory paralysis leading to death (Boopathi & Ki, 2014). Apart from the long-lasting health effect, the dominant species can potentially cause acute symptoms such as stomach upset, vomiting, skin irritation, nausea, diarrhoea, fever, throat irritation, headache, mouth blisters, muscle and joint aches, eye irritation and allergic reactions (Kibria, 2016).

#### **(ii) Health effect reported from different water sources utilization**

Assessment of water use among study subjects indicates that 31% use Lake water as the primary source of water, 53% use well water and 16% used treated supplied pipe water as the main water source for drinking. Study participants identified vomit as one of the health effects occurs after consumption water from the lake source, which is infested with cyanobacteria; the risk of getting vomit is 6.1 folds higher compared to those drinks water from the boreholes sources  $P < 0.001$ . The risk of vomiting when consuming bloom contaminated water is 17 folds compared to treated supplied pipe water  $P < 0.001$ - (Table 19). The same result that cyanobacteria infested water can cause vomiting was reported in Australia (Pilotto *et al.*, 1997) and UK (Turner, 1990). Vomiting was still statistically significant in multivariate analysis  $P < 0.05$  (Table 19). The likelihoods of getting throat irritation when drinking cyanobacteria contaminated water from the lake source is 6.57 higher than drinking from the boreholes (well) source  $P < 0.001$ , this association was also strong in multivariate analysis  $P < 0.05$  (Table 19). Throat irritation is one of the documented health effects after ingestion of cyanobacteria infested water in most recreation activity or water-related exposure (Kibria, 2016).

The gastrointestinal illness was significantly higher among study subject used water from the Lake as compared to pipe and well water users. Having stomach upset when drinking cyanobacteria contaminated water from the lake source is 45.2 and 8.4 folds higher than drinking treated supplied pipe and well water respectively  $P < 0.001$ . This finding was also reported by Hilborn *et al.* (2014) and Collier *et al.* (2015). The association between stomach

upset and infested LV water was perceived to be strong in multivariate analysis  $P < 0.001$ . Bathing using treated supplied pipe water is protective against eye irritation as compared to contaminated lake water source  $OR = 0.46$ ,  $P < 0.05$ . The association was the same on multivariate analysis  $P < 0.05$ . The Acute and short-term health effects can be preventable with adequate treatment that significantly reduce cyanobacteria cell number up to 99% hence make the water safe for human use (Dietrich & Hoeger, 2005; Funari & Testai, 2008). World Health Organization reported that swimmers in water containing cyanobacteria might suffer from allergic reaction such as eye irritation (WHO, 2014).

### **(iii) Bloom availability on the Lake water source**

The evidence of an epidemiological link to the physical and visible bloom for most HAB-related illness is critical, documented domination of *Anabaena spp* and *Microcystis spp* in the Lake Victoria, which forms scums of blooms is associated with several health effects. The scum of cyanobacteria may be visible at some time during the day and disappear. Therefore, it was essential to associate the illnesses risk with the visible algal scum in the water. The study reveals the odds of vomiting when consumed water with visible bloom is almost four times compared to drinking water without visible bloom,  $P < 0.05$ . The likelihoods of GI were higher when consuming water with visible bloom, for diarrhoea and stomach upset the chances were 2 and 3.4 respectively  $P < 0.001$  as compared with water source with no visible bloom. The same association was reported on multivariate analysis that diarrhoea and stomach upset were still statistically significant link with bloom availability  $P < 0.001$ . The same finding was reported by Stone and Bress (2007) and Collier *et al.* (2015). The probabilities of reported skin and throat irritation among water users with visible bloom is almost two and four times higher as linked with those used water without visible bloom respectively,  $P < 0.05$  (Table 20). Harmful Algal Bloom-related Illness Surveillance System (HABISS) of USA reported an association of bloom availability in water with the related infection for the year 2009-2010 (Hilborn *et al.*, 2014).

### **(iv) Occupation risk and amount of water consumption**

Occupation of the study subject was compared between fisherman and other non-fishing activities, which include (employed, non-employed and farmers). Fishing is reported to be one of the risk activities of contracting health effect related to cyanobacteria bloom and cyanotoxins. Gastrontatinal illnesses (GI) was strongly related to fishing occupation whereby vomiting and diarrhoea were reported among fisherman as compare to non-fisherman,  $OR = 2$ ,

$P < 0.05$ , vomiting and diarrhoea show strong association in multivariate also  $P < 0.05$ . Fishing occupation is strongly linked exposure factor of getting throat irritation 2.4 higher as compare to non-fisherman,  $P < 0.05$  (Table 21). The higher risk to fisherman was also reported in china (Chen *et al.*, 2009). Amount of water consumption was reported to be one of the factors that may contribute to reported health risk, whereby the use of more than one liter is more risk than less than one liter. Diarrhoea and throat irritation was reported to be associated with the amount of water consumption were the likelihoods was 2 and 4 time more than these reported to consume less than one liter respectively  $P < 0.05$ , throat irritation was a strong link with the amount of water intake in multivariate analysis  $P < 0.05$  (Table 21). The association of the amount of water ingested and the degree of infections was also described in the UK were military soldiers become sick after ingestion of bloom water contain *Microcystis spp* (Turner *et al.*, 1990) and in the USA (Wade *et al.*, 2008; Collier *et al.*, 2015).

#### **4.7 Cyanotoxin in human serum and liver damage**

Detection and quantification of cyanotoxins from human serum are of the challenges due to handling of samples and extraction procedures. Water safety and risk of exposure assessment after human intoxications is of concern to human health. Therefore, the development of cyanotoxins detection method from human tissues is critical to estimate the exposure risk. The challenges for proper detection of cyanotoxins in human tissues occurs during; sample collection, handling, storage, and extraction. These challenges may be attributed by chemical properties of different cyanotoxins and their behaviour related to chemical and biological systems. Cyanotoxins, especially MC congeners exhibit different biochemical properties based on the position of amino acids such as leucine, arginine, tryptophan, alanine, tyrosine and phenylalanine. The variation of amino acids position makes the MC congeners to have two main characters of hydrophobicity and surface adhesion capacity (Heussner *et al.*, 2014).

Based on the reported linearity, the detection method proved to be usefully for detection of thirteen cyanotoxins as results show the coefficient of determination ( $R^2$ ) =0.99, which is close to 1 as a reference for perfect coefficient. The method is capable of detecting almost similar amount of added concentration in the sample, this is demonstrated by the small percentage or shift between the added and observed concentration by the method. For all thirteen cyanotoxins detected by this method the signal relative standard diversions (rsd%) were less than 15%, this signifies that the method is perfect to be used (Table 26-37).

This is the first study to report the presence of cyanotoxins in the human serum in Tanzania and Africa. The study reports for the first the detected of CYN, dmMC-LR and NOD from human serum after exposure from contaminated water. Presence of cyanotoxins such as MCs (-LR, -RR) in human serum was also reported in Caruaru from haemodialysis patients, which were exposures in MC-LR contaminated water used for dialysis (Azevedo *et al.*, 2002). The first and only study that report the presence of cyanotoxins after exposure from drinking contaminated water in China documented detected of MCs (-LR, -YR) from the fisherman after exposure of 5 to 10 years (Chen *et al.*, 2009). The maximum (0.15 ng/mL) concentration of cyanotoxins detected in this study is small as compared to other two studies with concentration of 31.4 ng/mL and 1.83 ng/mL in Brazil and China respectively (Azevedo *et al.*, 2002), this difference may be attributed to the amount of toxins present in water and detection method used.

The study reveals the fishermen are more likely to have their liver biochemistry indices evaluated than other occupation. Liver biochemical indices elevation was strongly related to the concentration of toxins level observed at different collection point. These findings relate with study conducted in China (Chen *et al.*, 2009) whereby ALT, AST and ALP were reported to have positive relationship with cyanotoxins. Furthermore, the study demonstrates liver damage is relatively proportion with the increase in cyanotoxins concentration or existence of multiple toxins in serum hence toxicity enhancement has potential in level damage. In this study the Pearson correlation test shows the moderate relationship between liver biochemistry indices such as ALP, Alb, TP, ALT and AST. The moderate relationship is due to low concentration of toxins observed in sample. Studies conducted on mice treated with MC toxins indicated there were increase serum activities of enzymes that indicate hepatocellular damage due to toxins exposure. Concentration of cyanotoxin detected in human serum and liver biochemistry indices elevation, shows an association between the two with correlation coefficient of 0.33 for MC-LR while for combined cyanotoxins of MC-LR, CYN and NOD is 0.78. This provides knowledge of cyanotoxin toxicity of mammals so as human (Billam *et al.*, 2008). Human liver damage and elevation of liver biochemical indices such as ALT, AST, and ALT can occur even in a relatively low dose of prolonged MC-LR exposure was confirmed by Chen *et al.* (2009) in China.

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

This is the first study to report CYN and NOD cyanotoxins in the freshwater of Lake Victoria. Existence of multiple toxins in Lake Victoria poses a higher risk to humans when water is consumed without treatment. Multiple and repeated exposures to humans may enhance levels of toxicity and synergistic effects. There is a need for development of structured surveillance systems for detection of toxins in freshwaters used for human consumption and recreation purposes. Long-term studies should be conducted to enhance our understanding of the effects that attribute to the increase of cyanotoxins and emerging toxins such as CYN and NOD.

This is the pioneering study to detect CYN, dmMC-LR and NOD in human serum globally and it is the second report documenting the existence of MCs cyanotoxins in human serum from population study in world. A validated method for cyanotoxins detection by MS/MS can be used to detect and quantify the existence of cyanotoxins in human serum. This is a groundbreaking study in Tanzania and Africa at large indicating that cyanotoxins is ignored public health problem that requires special attention. The observed cyanotoxins in Lake Victoria freshwater were also detected in human serum from general study population and reveal that the problem could be bigger to the highest risk group. Existences of multiple toxins, which are particularly hepatotoxins, enhance toxicity level hence accelerate liver damage. More studies are required providing evidence that cyanotoxins may attribute the increased observed cancer in Lake zone.

The PC proxy indicator is a surveillance tool that enables anticipation of water body contamination by cyanobacteria. The concentrations of parameters like redox potential, total chlorophyll, nitrate, nitrite, phosphate and reactive phosphorus all have positive correlations with PC concentration and can be measured and monitored easily, to enable prediction of increasing PC. This will address the challenges of lack of advanced technological equipment in district-level government bodies in most developing countries for identifying, monitoring and managing cyanobacterial blooms. The predictive model developed in this study has quantified the water parameters that affect PC concentrations based on a case study in Ukerewe District. To validate this approach, more long-term studies are needed on several water bodies, which will also enable it to be used more efficiently.

The concentration of cyanobacteria blooms found in this study goes beyond WHO acceptable. Consequently, water uses from the lake source was found to be associated with acute health outcomes. The result indicates there are potential health risks associated by using lake water without any treatment for human consumption. It is therefore advised to continue to monitor the water quality at Ukerewe area to understand its spatial and temporal dynamism of phytoplankton. The long-term study of Phytoplankton helps to understand the nature of nutrients or pollution entering the water body because phytoplankton's are the good and cheaper indicator of environmental change as compared to chemical indicators. The documented illness associated with cyanobacteria infested water can be used as a baseline to improve case detection at the district and contribute to the development of evidence-based prevention strategies to mitigate adverse health outcome that may result to long-term exposure to HABs.

## **5.2 Recommendations**

- (i) Awareness creation to community, stakeholders, public health researchers and governments on the health risks associated with existence of Cyanobacteria and Cyanotoxins in the Lake
- (ii) Reducing cyanotoxins exposure to human through provisional of safe and clean water will reduce the health risk of exposure and decreases illnesses
- (iii) Proper water quality management must be instituted in the area where have been identified with a high risk of infestation of cyanobacteria. Cyanotoxin monitoring should be part of the water quality parameters monitored by water authorities
- (iv) Establishment of a health surveillance system that will be able to capture and record the illnesses associated with cyanobacteria.
- (v) More research should be conducted in this new research area whereby less is known on the diversity of cyanobacteria along Lake Victoria shores. Moreover, a cohort studies should be conducted to monitor long term effects of prolonged exposure to cyanotoxins
- (vi) Solution for portable water treatment to the community that can not access treated pipe water is critical to be researched on and developed.
- (vii) Recommendation of further research on the following:
  - Synergistic or antagonistic effects of cyanotoxins
  - Health effect due prolonged exposure to cyanotoxins
  - Diversity of cyanobacteria's and their abundance around the lake



- Development of actual biomarker for detecting toxins in human serum
- To explore more on other sources of exposure of toxins rather than drinking water
- The association between liver damage and exposure toxicity levels
- Mapping of risk areas in relation to seasonal variation on cyanobacteria and cyanotoxins occurrence

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