HEALTH RISK OF TOXIC CYANOBACTERIA IN DRINKING WATER IN THE NYANZA GULF WATER, LAKE VICTORIA KENYA

BY

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DECLARATION

I declare that this thesis is my original work and has not been presented elsewhere for an academic award by any other person in any other university.

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DEDICATION

This thesis is dedicated to the communities around Lake Victoria for whom my desire is that they have a clean source of drinking water.

ABSTRACT

Cyanobacteria are single-celled algae that thrive in warm and nutrient rich water bodies including lakes. They can produce different kinds of toxins called cyanotoxins. Microcystin is the most common and most studied cyanotoxin. Microcystin mostly affects the liver. Epidemiological studies in China and Serbia have shown an association between cyanotoxins and occurrence of Primary Liver Cancer. Cyanobacteria have been reported in Lake Victoria, which is an important source of drinking water for the riparian communities, thus posing a danger to human health. This is as a result of eutrophication in Lake Victoria increasing the levels of cyanobacteria and cyanotoxins in the Nyanza Gulf. However, the health risk from exposure to toxic cyanobacteria in the Nyanza Gulf water, remains unknown. The purpose of this study was to assess the health risk of toxic cyanobacteria to the riparian communities in the Nyanza Gulf. The specific objectives were to determine the concentration of microcystins, identify and quantify microcystins and evaluate the health risk of microcystins in household and Lake Victoria water for Nyanza Gulf residents. In a longitudinal study adopting survey and experimental design, six beaches were studied and 127 samples were collected monthly from both households and beaches over six months. Cyanobacterial levels were determined using an enzyme assay method (PP2A) and microcystin strains identified using High Performance Liquid Chromatography (HPLC). Two-way ANOVA was done to determine statistical significance of Microcystins in levels. The results showed that all beaches were eutrophic resulting in flourishing of cyanobacteria. 84% of water samples contained Microcystins. Concentration of Microcystins was 3.44µg/L which is over the WHO limit of 1µg/L. Microcystin RR (MC-RR) is the most abundant cyanotoxins followed by Microcystin YR (MC-YR) and Microcystin LR (MC-LR) is the least abundant in the Nyanza Gulf. There was significant variation between different beaches and different months (ANOVA: $F=12.09$, $p<0.0005$) and no variation between beaches and water treatment (ANOVA: $F=0.97$, $p=0.47$). The health risk factor of cyanotoxins in drinking water is 3.86. There is a health risk posed by cyanotoxins to the residents of the Nyanza gulf who use the lake water for drinking since is over the WHO limit. This information provides an insight into the quality of Lake Victoria water for drinking. The study recommends regular monitoring of cyanobacterial cells, development of cyanobacteria removal methods as well as sensitizing the riparian communities on the health risk of cyanotoxins in drinking water.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMNS

ANOVA	:-	Analysis of Variance
BMU	:-	Beach Management Unit
CHl	:-	Chlorophyll
HPLC	:-	High Performance Liquid Chromatography
LVFO	:-	Lake Victoria Fisheries Organization
LC_{50}	:-	Lethal Concentration 50
MC	:-	Microcystin
MC-LR	:-	Microcystin LR
MC-RR	:-	Microcystin RR
MC-YR	:-	Microcystin YR
MC-LA	:-	Microcystin LA
mcy	:-	gene of the MC synthetase gene cluster
NGOs	:-	Non-governmental Organisations
PCR	:-	Polymerase Chain Reaction
PLC	:-	Primary Liver Cancer
PP2A	:-	Protein phosphatase 2
TDI	:-	Tolerable Daily Intake
TSI	:-	Trophic State Index
WHO	:-	World Health Organization
IARC	:-	International Agency for Research on Cancer
U.S.A	: -	United States of America

DEFINITION OF OPERATIONAL TERMS

Cyanobacteria :- Microscopic bacteria found in freshwater lakes, streams, oceans and are capable of photosynthesis

Cyanotoxin :- Toxins produced and contained in the cyanobacterial cells

Eutrophication : - The enrichment of an ecosystem with chemical nutrients typically compounds containing nitrogen, phosphorus or both leading to excessive plant and algal growth

Health risk :- Something that could cause harm to people's health

Household head :- The individual in one family setting who is responsible for making decisions

Microcystin :- A class of cyanotoxins

Nyanza Gulf :- The north-eastern part of Lake Victoria that extends into Western Kenya

The lake :- Lake Victoria which is the focus of this study

Water quality :- The fitness and safety of water to be used for drinking

Water treatment method:- Any process for removing undesirable chemicals, biological contaminants, suspended solids and gases from water so as to produce water fit for drinking

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Cyanobacteria, also known as blue-green algae, is a family of single-celled algae that proliferate in water bodies such as ponds, lakes, reservoirs, and slow-moving streams when the water is warm and nutrients are available (Mur *et al.*, 1999). Rapid, excessive cyanobacteria growth, often referred to as a "bloom", is linked to eutrophication. The increased eutrophication is associated with urbanization, agricultural malpractices and deforestation. Globally, the frequency and intensity of cyanobacterial blooms in fresh water are increasing due to eutrophication caused by climate change and anthropogenic activities (Paerl *et al*., 2011; Paerl, 2012; Taranu *et al*., 2015). Many genera of cyanobacteria are known to produce toxins. These toxins, which are referred to as cyanotoxins make up a large group of chemical compounds that differ in their molecular structure and toxicological properties. They are generally grouped into major classes according to their toxicological targets: cell, liver, nervous system, skin, and tumor promotion. Microcystins are hepatotoxins commonly produced by the cyanobacteria genera *Anabaena*, *Microcystis*, *Oscillatoria*, *Planktothrix*, *Nostoc*, and *Hapalosiphon* (Szlag *et al.*, 2015). Cyanobacteria can cause serious threats to drinking water supplies using surface water as source (Li *et al.*, 2017). Lake Victoria has experienced major deterioration in its water quality mainly due to pollution. Nyanza Gulf is one of the bays of Lake Victoria that is most affected by nutrient enrichment (Gikuma-Njuru *et al*. 2013).It is estimated that only about 20% of the Kenyan rural population has access to safe water but for both Nyanza and Western provinces, it is only 8% (LBDA, 2004).

Lake Victoria and its catchments have undergone eutrophication over the last three decades (Verschuren *et al.*,2002). In 1984 massive fish kills were observed in the Nyanza Gulf of Lake Victoria, Kenya, which coincided with the occurrence of cyanobacteria (Ochumba, 1990). Sitoki, *et al*. (2012) carried out a study in the lake which showed that cyanobacteria are the most abundant phytoplankton. About 54% of the samples contained microcystin and thereby recommended regular monitoring of the cell numbers of toxigenic cyanobacteria in the raw water since it is used as supply of drinking water. Temporal variability in microcystin (MC) concentration has been described along the shoreline of wind-exposed systems worldwide and has been considered as an important variable in estimating health risks through exposure to toxic algal blooms. Maximum concentration of microcystins (MCs) are typically observed along the shore (Humbert & Fastner, 2017). Drinking water sources are unique and need to be investigated to determine the risk and the best management strategy for cyanotoxins risk reduction. Different drinking water treatment technologies are applied in different countries and contexts. Studies investigating cyanotoxins in drinking water and their removal during the water treatment have been even scarcer on the entire African continent (Addico *et al.,* 2017).Although studies have been carried out to measure the cyanotoxins in the Nyanza Gulf, none has been done to determine the concentration of cyanotoxins in both lake water and household water.

Microcystins (MCs) are the most frequently found cyanotoxins which can be produced by various cyanobacterial genera including water bloom- and scum-forming planktonic cyanobacteria such as *Dolichospermum* (formerly *Anabaena*), *Microcystis* or *Planktothrix* (Manganelli *et al*., 2012). To create a comprehensive cyanotoxins contingency plan, familiarity with the physical and chemical properties of the toxin, the nature of the toxin and effective treatment processes is necessary (Westrick *et al.*, 2010). Based on their toxin production, to which human can be exposed via different routes, the World Health Organization (WHO) has

listed cyanobacteria among the emerging health issues (Manganelli *et al.*,2012). WHO has set the Tolerable Daily Intake (TDI) of Microcystin-LR (MC-LR) to be 0.04µg/kg-bw/day. For drinking water, the levels of MC-LR should not exceed 1µg/L.

Cyanobacterial toxins are well recognized as a cause of livestock poisoning, which has been extensively reported in the Americas, Europe, Asia and Australasia (Falconer, 2005). The most well characterized case was the poisoning of renal dialysis patients in a clinic in Caruaru, Brazil, in 1996, where the patients treated in a dialysis clinic during one week suffered severe illness following perfusion, with hepatic failure and death in more than 50 cases. Investigation of the water treatment unit at the clinic found contamination of the filters by two types of cyanobacterial toxin, cylindrospermopsin and microcystins (Jochimsen *et al*., 1998; Carmichael, 2001). Animal experiments have shown that chronic exposure to microcystins affect liver histology and function and may cause liver cancer with long-term exposure (Beasley*et al*.,2000; Grosse*et al.*, 2006). Ueno *et al*. (1996) hypothesized that the high incidence of primary liver cancer (PLC) in south east China is likely related to microcystin contamination in drinking water (Ueno *et al*., 1996). Another study carried out in Brazil identified microcystin in the serum of highly exposed fishermen in addition to indication of liver damage (Chen *et al.*, 2009).The first officially confirmed case of toxic algal poisoning in Kenya is the widely reported death of thousands of marine wildlife along the Kenya Coast at the beginning of 2002 when huge numbers of fish, including manta rays, sharks and tuna were washed ashore (Tomlinson, 2002). Several green and hawksbill turtles were also found dead. It is believed that their death was caused by a bloom of naturally occurring toxic algae triggered by an upwelling of nutrient rich oxygen poor deep ocean water (Agence France-Presse 2002; Wild Net Africa 2002).The symptoms of poisoning by the main toxic cyanobacteria in drinking water reservoirs overlap with a range of other gastrointestinal illnesses, largely caused by infectious disease organisms.

As a result, during an outbreak of enteric disease, the pathogens are investigated first, as the most probable cause, and only after exhaustive exploration are toxins of any type evaluated. Agricultural chemicals and industrial pollutants such as heavy metals are likely to be next suspected, with cyanobacterial toxins ignored until well after the event (Teixeira *etal.*,1993). As such, this study established the concentration of cyanotoxins in both Lake Victoria water and household drinking water for Nyanza Gulf. The microcystin present were identified and quantified based on their chemical properties and the risk they may be potentially posing to users of the Lake Victoria water was determined. This was done against the TDI and the provisional guideline value set by the WHO.

1.2 Statement of the Problem

Lake Victoria is an important source of drinking water to the riparian communities. However, the lake has undergone major dramatic water quality and biological changes in the recent past associated with increased nutrient input, introduction of exotic fish species and climate change leading to cyanobacterial blooms. Cyanobacteria toxins produced by some species of cyanobacteria are potentially harmful to human health.

It is estimated that only about 20% of the Kenyan rural population has access to safe water but for both Nyanza and Western provinces, it is only 8%. In 1998, the World Health Organization released the provisional guideline value of Microcystin LR to be 1 μgL^{-1}. Although studies have shown the MC-LR concentrations in Lake Victoria water, the level in household waters already stored for drinking is not known. The health risk of toxic cyanobacteria in the Nyanza Gulf of Lake Victoria, Kenya also remains unknown. There is no single method of getting rid of cyanotoxins in the water drawn from Lake Victoria for drinking. Previous studies have shown the presence of cyanobacteria in the Nyanza Gulf water but there is no evidence that methods to

curb and eliminate the cyanotoxins from drinking water have been developed and adopted by the riparian communities.

MCs are highly toxic for mammals. Their acute effects are primarily manifested in liver but MCs have been shown to induce gastrointestinal and renal damage or neurological symptoms as well. Chronic exposures to MCs have been linked to tumor promoting and carcinogenic effects which is based on laboratory animal and *in vitro* experiments and supported also by results of epidemiologic studies of human population consuming drinking water contaminated by these cyanotoxins. Concerns regarding the presence of cyanotoxins in drinking water and associated health effects have raised research and public health interest worldwide. Drinking water sources are unique and need to be investigated to determine the risk and the best management strategy for cyanotoxins risk reduction.

1.3 Objectives of the Study

1.3.1 General objective

To assess the health risk of toxic cyanobacteria in drinking water in the Nyanza Gulf of Lake Victoria, Kenya.

1.3.2 Specific objectives

1. To determine the concentration of cyanotoxins in both Lake Victoria water and household drinking water for Nyanza Gulf residents.
2. To identify the cyanotoxins in Lake Victoria and household drinking water for Nyanza Gulf residents.

3. To determine the health risk of cyanotoxins in both Lake Victoria water and household drinking water for Nyanza Gulf residents.

1.4 Research Questions

1. What was the concentration of cyanotoxins in both Lake Victoria water and household drinking water for Nyanza Gulf residents?
2. What cyanotoxins were found in Lake Victoria and household drinking water for Nyanza Gulf residents?
3. What was the health risk of cyanotoxins in both Lake Victoria water and household drinking water for Nyanza Gulf residents?

1.5 Justification and Significance of the Study

Cyanobacteria produce toxins that may present a hazard for drinking water and whose effects range from liver damage, liver cancer and neurotoxicity (Hitzfeld*et al.*,2000). There is a growing concern about the non-lethal, acute and chronic effects of microcystins globally (Hitzfeld*et al.*, 2000). Toxic cyanobacteria *Microcystis* spp. and *Anabaena* spp. were observed in Nyanza Gulf in a study (Sitoki*et al*.,2012) and the concentration of microcystin at several sites in Kisumu Bay of Nyanza Gulf was in excess of the guideline value set by WHO. There is a major gap in the synthesis and dissemination of available information on cyanobacteria and their toxins. A priority area is to develop a further understanding of the human health significance of cyanobacteria and individual cyanotoxins, and the practical means for assessing and controlling exposure to cyanobacteria and to cyanotoxins.

It was therefore important to establish the presence and concentration of cyanotoxins in both Lake Victoria water and household drinking water so as to evaluate the health risk in both Lake

Victoria water and household drinking water for Nyanza Gulf residents. With the knowledge developed from this study and other similar ones, there will be need to develop effective treatment procedures to remove these toxins in water. This information will also form a basis of advising county governments and the local residents on how to treat lake water before household use. These strategies would further lead to a decrease in disease incidences associated with cyanotoxicity.

1.6 Limitations of the Study

Data collection involved travelling to remote villages where members of households were involved in various activities sometimes far away from home. As a result, there were missed opportunities which necessitated planning for return visits by the investigator and research assistants so as to ensure adequate coverage. The research tool was not translated to the local language which might have led to some misinterpretations of some words or questions by the research team. To mitigate this, research assistants were drawn from the locality who would understand the tool. They were also trained on the tool, how to ask questions and when and how to probe.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Introduction

Cyanobacteria are a major group of bacteria that occur throughout the world. They are a morphologically diverse group of photosynthetic prokaryotes that occupy a wide range of niches, from freshwater to hydrothermal vents, from desert rocks to Antarctic lakes. Cyanobacteria can form a wide variety of secondary metabolites. These metabolites show various types of biological or biochemical activities and cyanotoxins have been identified as potent toxins. The cyanotoxins are a diverse group of compounds, both from the chemical and the toxicological points of view. Based on the target of theirtoxins, cyanotoxins arehepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins (Wiegand & Pflugmacher, 2005). Cyanobacteria have been reported in freshwater lakes, basins, rivers, irrigation channels, brackish and sea waters, salty lakes, as pelagic or benthic organisms. Cyanobacteria are expanding geographically and now threaten bodies, including Lake Victoria (Paerl *et al*., 2011).

The growth of aquaculture production systems in recent years together with a lack of control has led to an increase in eutrophication and cyanobacterial blooms (Chen *et al*., 2009). Cyanobacteria blooms have been reported in many fresh water lakes of the world as a severe problem (Pu *et al.*, 2007). Blooms can affect the water as indicated by changes in pH, transparency and biodiversity and produce a variety of toxins including microcystins (Gilroy *et al.*, 2000).

Cyanobacteria are mostly abundant in shallow, warm, nutrient rich or polluted water low in oxygen and can grow to form thick scum that could color the water, creating blooms (Stotts *et al.*, 1993). Most blooms disappear in a few days. Cyanotoxins may be released during the life of

the cyanobacterial cell and in large quantities after the death of the cell by passive leakage of cell content (Fitzgerald, 2001). Cyanobacterial blooms formed by planktonic species or mats of benthic cyanobacteria have severe impacts on ecosystem functioning, e.g., disturbances of relationships among organisms, changes of biodiversity, light conditions or oxygen concentrations. The occurrence of cyanobacterial mass populations can create a significant water quality problem, especially as many cyanobacterial species are capable of synthesizing a wide range of odors, harmful compounds or potent toxins (Sivonen & Jones, 1999).The cyanobacterial toxins produced create human and animal health hazards, which can cause risks of illness and mortality at environmentally relevant concentrations (Codd *et al.*, 2005).

Eutrophication is the enhancement of the natural process of biological production in rivers, lakes and reservoirs, caused by increases in levels of nutrients, usually phosphorus and nitrogen compounds (Falconer *et al.*,1999). Anthropogenic loading of nitrogen (N) and phosphorus (P) to freshwaters and coastal marine systems is a global environmental problem (Smith *et al.*, 2006; Smith, 2003). Eutrophication was recognized as a pollution problem in many western European and North American lakes and reservoirs in the middle of the twentieth century (Rohde, 1969). Since then, it has become more widespread, especially in some regions; it has caused deterioration in the aquatic environment and serious problems for water use, particularly in drinking-water treatment.

Eutrophication because of human activities on surface waters leads to accelerated growth of photoautotrophic organisms including cyanobacteria. Immense proliferations of cyanobacteria in freshwater, brackish and coastal marine ecosystems have become a worldwide environmental problem. More than 40% of lakes and reservoirs in Europe, Asia and America are now eutrophic and offer favorable conditions for cyanobacterial mass development (Falconer *et al.*, 1999).

Approximately 25 to 75% of cyanobacterial blooms are toxic (Bláhová *et al*. 2008; Bláhová, *et al.,* 2007;Chorus, 2001). The cyanobacterial toxins produced create human and animal health hazards, which can cause risks of illness and mortality at environmentally relevant concentrations (Codd *et al*., 2005).

Even though eutrophication has been acknowledged as a growing concern since the 1950s, only recently have cyanobacterial toxins become widely recognized as a human health problem arising because of eutrophication (Falconer *et al*., & Codd, 1999). The significance of such toxins, in relation to other water-health issues, can only be estimated currently.

2.2Cyanotoxins in water

Several cyanobacteria species produce toxins as secondary metabolites, which can impact on ecosystems, animal and human health (Chorus & Bartram, 1999; Funari & Testai, 2008). In the recent years, principal laboratories worldwide have undertaken research in cyanotoxins. Genes used for the synthesis of microcystins which is the most studied group of cyanotoxins have been isolated and characterized from several different cyanobacteria species (Fujii *et al.*, 2002; Rouhiainen *et al.*,2004).

Concern over established acute and the possible chronic effects has increased the pressure to monitor levels of these toxins in drinking water. It is known that microcystins are produced within the cyanobacterial cell and become free in the water column after cell lysis and thus it is therefore important not only to develop a rapid method of analysis but also to provide a means of assessing intracellular levels of toxins in addition to those free in water. Knowledge of the levels of intracellular toxins also permits advance warning of potentially high levels of free toxin (Lawton *et al*.,1994).

Over 90 analogues of microcystin have been identified and found to be toxic. However, they are not being monitored presently. This is because their Tolerable Daily Intake (TDI) cannot be derived because toxicological data is lacking (Nováková *et al.*,2011). All species with microcystin-producing strains also include related strains that lack the ability to produce this toxin. The risk assessment response to the increasing occurrence of cyanotoxins has been seriously constrained due to the limited number of available standards and the limited analytical capability of some laboratories (Fortin *et al.*,2010).Detection and characterization of toxin-producing cyanobacteria require reliable tools. Enzyme-linked immunosorbent assay (ELISA) and high-pressure liquid chromatography (HPLC) is currently the most widely used techniques to evaluate whether toxins are present in water samples.

The most likely route for humans to be exposed to cyanotoxins is via drinking water, medical dialyses, recreational waters or consumption of cyanobacterial health food products (Gilroy *et al.,* 2000).Cyanobacterial toxins are well recognized as a cause of livestock poisoning, which has been extensively reported in the Americas, Europe, Asia and Australasia (Falconer, 2005). However, epidemiological data for human poisoning by cyanobacterial toxins is not well documented. The most well characterized case was the poisoning of renal dialysis patients in a clinic in Caruaru, Brazil, in 1996, where the patients treated in a dialysis clinic during one week suffered severe illness following perfusion, with hepatic failure and death in more than 50 cases. Investigation of the water treatment unit at the clinic found contamination of the filters by two types of cyanobacterial toxin, cylindrospermopsin and microcystins (Carmichael, 2001; Jochimsen *et al.*, 1998). Animal experiments have shown that chronic exposure to microcystins affect liver histology and function and may cause liver cancer with long-term exposure (Beasley *et al.*, 2000; Grosse *et al.,* 2006). Ueno *et al.* (1996) hypothesized that the high incidence of primary liver cancer (PLC) in south east China is likely related to microcystin contamination in

drinking water (Ueno *et al.*,1996). Another study carried out in Brazil identified microcystin in the serum of highly exposed fishermen in addition to indication of liver damage (Chen *et al*., 2009).

In Ghana, about 40% of the population do not have access to treated piped water and thus rely on surface water for drinking. It was reported that cyanotoxins occur very frequently in the raw water in Ghana and often at concentrations higher than the WHO limit (Addico, *et al*. 2017). Cyanobacteria were found to be the dominant phytoplankton species in Murchison Bay of Lake Victoria in Uganda (Haande *et al*., 2011) Cyanotoxin levels in the Nyanza Gulf have been reported to be over the WHO limit of 1µg/L (Kotut *et al.,* 2006: Sitoki *et al*., 2012). Although studies have been done to quantify the cyanotoxins in the Nyanza Gulf, none has been done to quantify the cyanotoxins in the households that rely on the lake water for drinking.

2.3 Identification of Cyanotoxins

Cyanotoxins are naturally occurring low molecular weight compounds and can be characterized by their chemical or toxicological properties. Toxicologically, they fall into three main groups: hepatotoxic cyclic peptides (microcystins and nodularins); alkaloids, both neurotoxic (anatoxin-a, saxitoxin, β-Methylamino-Lalanine and cytotoxic/hepatotoxic (cylindrospermopsin); and irritant lipopolysaccharides (Carmichael, 2001; Sivonen & Jones, 1999).A study done revealed that an average of 59% of blooms contains toxins, and hepatotoxic blooms are more common than the neurotoxic ones (Rantala *et al*., & Sivonen, 2006). Other studies have shown that toxigenic and non-toxigenic strains of cyanobacteria coexist within populations of the same species and that the proportion of toxigenic and non-toxigenic cells in a population can be quite variable (Mbedi *et al.,* 2005; Vezie *et al.,* 1998).

The most common and very much studied of these toxins is microcystin, which is a toxin that targets the liver (Sivonen and Jones 1999; Carmichael 2001). These are a group of toxins produced by different species of freshwater cyanobacteria, specifically *Microcytis*, *Anabaena* and *Oscillatoria* (An & Carmichael, 1994; Trogen *et al.*, 1996). The composition of cyanobacteria communities determine the particular cyanotoxins that will be present, for instance, microcystins are chiefly produced by Microcystis, Planktothrix or Anabaena species (Sivonen, 2009). Microcystins are probably the most prevalent cyanotoxins in the environment and they are present in high amounts in cyanobacterial biomass that is, up to 1% of dry weight (Welker & Von Döhren, 2006). Their natural physiological or ecological function is not well understood despite rigorous research. The chemical structure of microcystins comprise two variable amino acids and an unusual aromatic amino acid, ADDA (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid), containing a substituted phenyldecadienoic acid (Botes *et al.*,1985). Diverse microcystins have different lipophilicities and polarities, which could affect their toxicity. Microcystins are very soluble in water and of the over 90 reported variants, four microcystins (LR, RR, LA, and YR) are of special concern. The main structural difference between the microcystin variants arise from the substitution of single amino acids (Westrick *et al.*,2010).

Microcystin-LR was the first microcystin chemically identified and to date, most work has been conducted using this microcystin. It has been associated with most of the incidents of toxicity involving microcystins in most countries (Fawell *et al.*, 1999).The World Health Organization (WHO) in 1998 derived a value of tolerably daily intake (TDI) for human health risks assessment purposes for microcystin-LR. The TDI of 0.04 μg/kg bw/day (WHO, 1998) was used for calculation of guidance value for the maximal acceptable concentration of microcystin-LR in drinking water, 1 μg/L (WHO, 1998).Commonly occurring MC variants identified in the

examined reservoirs were MC-LR, -LF, -RR and -YR. The highest diversity of MC variants was observed in the Weija Reservoir in Ghana, where also two additional peaks possessing MC-like UV absorption spectrum were identified (Addico *et al*. 2017).

Microcystins are potent inhibitors of protein phosphatases 1 (PPl) and 2A (PP2A) from animals and higher plants (MacKintoshet al., 1990; Matsushima *et al.*, 1990). PP2A is one of the major protein phosphatases in eukaryotic cells which have been shown to be important enzymes in tumor suppression (Cohen, 1989). The action of microcystins in inhibiting such enzymes might suggest that they may in turn act as tumor promoters (Nishiwaki-Matsushima *et al*., 1992).

During the water treatment process, concentrations of both intracellular as well as extracellular toxins might increase or decrease in concentration. In a reservoir being monitored in Ghana, increases in concentration of both intracellular and dissolved MC were observed in some cases after the flocculation/sedimentation steps of water treatment (Addico *et al.* 2017).

Until recently, cyanobacteria identification had been done using the traditional taxonomic methods for phytoplankton analysis, by using morphological characteristics as a basis. These methods have been proven not satisfactory in many occasions (Vasconcelos, 2006). The need for new techniques that could be used for both naturally collected species and laboratory isolates led to the development of molecular techniques for the identification of cyanobacteria species (Neilan *et al.*, 1997; Saker *et al*., 2005). The discovery of the gene sequences responsible for the production of the most common cyanotoxins was a breakthrough in this area (Fujii *et al.*, 2002; Rouhiainen *et al.,* 2004).Toxicity is not species specific, thus making microscope analysis of limited utility for monitoring blooms. So, the utilization of molecular markers that may detect only the toxic populations, independently of their taxonomic identification is extremely useful (Bittencourt-Oliveira, 2003).

2.4 Health risk of cyanotoxins

Public health concern regarding cyanobacteria lies on the ability of many species and strains of these organisms to produce cyanotoxins. Cyanotoxins can fall into two of the four groups of water-related diseases. They may cause waterborne disease when ingested, and water contact disease primarily through recreational exposure. In hospitals and clinics, exposure through intravenous injection has led to human fatalities from cyanotoxins (Falconer *et al.,* 1999).Major routes of exposure to cyanotoxins include oral and dermal routes through drinking water and recreational water use. There is very limited information that suggest that inhalation of aerosols may be an equally important route (Fitzgeorge *et al.,*1994). Adverse health consequences for swimmers exposed to cyanobacterial blooms such as skin irritation due to contact with cyanobacteria in bathing water has been reported (Pilotto *et al.,* 1997). This is due to either direct response to toxins or allergic reactions. Cyanobacterial dietary supplements available in some countries may constitute a major route of oral intake for a small sub-population, if the cyanotoxin levels in the supplements are not controlled (Falconer *et al.,* 1999).

Direct ingestion of contaminated drinking water is a frequent route of cyanotoxin intake. If the water is obtained from a surface water source during cyanobacterial bloom, it is possible that the water had become contaminated with toxins released during cell decomposition (Chorus & Bartram, 1999). Cyanobacterial toxins have been detected in raw and final water in numerous cases throughout the world including Argentina, Australia, Bangladesh, Canada, Czech Republic, China, Finland, France, Germany, Latvia, Poland, Thailand, Turkey, Spain, Switzerland and U.S.A (Dietrich & Hoeger, 2005; Hoeger *et al.*, 2005; Hoeger *et al.,* 2004; Westrick, 2003).

Microcystins require additional attention not only for their ability to cause acute poisoning but also for their ability to initiate cancer through acute doses and potentially promote it through chronic exposure to low microcystin concentrations in drinking water (Lun *et al.*, 2002; Svirčev *et al.*, 2009).Chronic exposures to cyanobacteria and their toxins – *e.g.*, via contaminated drinking water – have been associated with increased occurrence of liver and colorectal cancer (Zhou *et al.*, 2002; Svircev *et al.*, 2009). The International Agency for Research on Cancer (IARC) performed an assessment of the carcinogenesis of MC-LR in 2006 in Lyon, France. They concluded that MC-LR is a possible carcinogen for humans and classified it as group 2B carcinogen (Grosse *et al.*, 2006).Studies carried out have revealed that microcystin toxicity is cumulative and that microcystins are liver carcinogens which could increase cancer risk to humans following continuous, low level exposure (Fitzgeorge *et al.*, 1994; WHO, 2003) A lethal dose of microcystins in vertebrates causes death by liver necrosis within hours or up to a few days. Liver injury is likely to go unnoticed and results in noticeable symptoms only when it is severe (WHO, 2003).

Epidemiological studies of human populations have shown symptoms of poisoning or injury linked to the presence of cyanotoxins in water or other water sources (Kuiper-Goodman *et al.*,1999). High levels of risk to human health are linked to the ingestion of large cyanotoxin quantities from water or the intake of small doses during extended chronic exposure (Svirčev *et al.*,2010). The cyanobacterial toxins provide a risk to human health when the population of toxic cyanobacteria in drinking water sources rises to bloom proportions. The present assessments of Guideline Values for these toxins as chemical, non-carcinogenic contaminants indicate that a safe concentration in drinking water is around 1μg/L, a concentration that has been exceeded in numerous water storages (Falconer & Humpage, 2005).

Microcystins and cylindrospermopsin have caused excess tumors in rodent experiments. Several experiments with mammals (rodents, pigs) showed significant sub-chronic and chronic toxicity of orally administered microcystins (Falconer & Humpage, 2005; Fawell *et al*., 1999),where harmful effects of microcystins such as increased mortality, liver injury such as histopathological changes, chronic inflammation, degeneration of hepatocytes, increased liver enzyme levels, renal damage or slightly higher number of tumors were observed. Microcystinsare considered to be tumor promotion factors. There has been evidence of tumor promotion properties of microcystins from several animal experiments (Dietrich & Hoeger, 2005; Humpage *et al*., 2000).

Animal studies have demonstrated that microcystins are amongst the most potent liver carcinogens yet described. Following the ingestion of microcystin-containing cyanobacteria, acute poisoning leading to death from massive hepatic hemorrhage has been reported in animals. A number of incidents of human illness have been attributed to microcystins in drinking water or at recreational sites (Kuiper-Goodman *et al.*,1999).

Hepatotoxins are potent inhibitors of protein phosphatases types 1 and 2A. These enzymes are fundamental to cell growth and tumor suppression (MacKintosh *et al*., 1990; Matsushima *et al*., 1990; Runnegar *et al*., 1995;Yoshizawa *et al*., 1990). These toxins have been shown to inhibit protein phosphatases 1 and 2A at Nanomolar concentrations by bonding covalently to these enzymes (MacKintosh *et al*., 1995; Runnegar *et al*., 1995). When hepatotoxins are ingested, they are absorbed across the ileum transported to the liver where they are taken up by hepatocytes. Chronic ingestion of sub lethal doses has been further epidemiologically linked to PLC and colorectal cancer in humans (Lun *et al.*, 2002).Acute poisoning by the hepatotoxins causes weakness, anorexia, pallor of the mucous membranes, vomiting, cold extremities and diarrhea

(Carmichael, 1992). Death due to intrahepatic hemorrhage and hypovolemic shock may occur within a few hours. Death may occur later because of hepatic insufficiency.

Epidemiological studies in Serbia have shown that the consumption of drinking water contaminated by microcystins could be connected to PLC (Svirčev *et al.,* 2010; Svirčev *et al.*,2009;Svirčev *et al.,* 2007). In Krusevac, Serbia, a bloom of Aphanizomenon, Anabaena and Microcystis occurred in 2004. Samples of water obtained from the reservoir had 650µg/L of MC while the tap water had a final concentration 2.5µg/L of MC. Epidemiological research has shown a significant association between cyanobacterial blooms in reservoirs used to draw drinking water for supply and the increased incidence of PLC in Central Serbia (Svirčev *et al.,* 2009).The association between an increased risk of PLC and the quantity of surface water was detected in Florida (Fleming *et al.,* 2002). A study conducted in China implied that hepatotoxins from water reservoirs containing cyanobacteria triggered the development of PLC (YU, 1995). These reports only go to show that microcystins could be an important chemical and external factor in the development of PLC.

Primary Liver Cancer is the fifth most common malignancy and the third most common cause of cancer mortality in the world (Li *et al.,* 2011). Apart from the well-known and generally accepted risk factors for PLC such as chronic viral infection, aflatoxins in food, liver cirrhosis and excessive use of alcohol, cyanotoxins have been taken to having potential carcinogenic effect (Svirčev *et al.,* 2009). From the present state of knowledge, it can be concluded that under specific conditions even without the presence of additional risk factors, exposure to microcystins can lead to initiation and promotion of PLC (Ibelings & Havens, 2008; Svirčev *et al.,* 2010; Žegura *et al.*,2003).

These toxins are highly stable in water and are resistant to boiling thus presenting a risk to consumers in less developed regions who collect water from surface sources to drink (Dietrich & Hoeger, 2005). If these contaminated water sources are used for human consumption, there is a risk of human poisoning. Conventional drinking water treatment may not be effective under bloom conditions in removing microcystins from drinking water and hence there is a risk to consumers (Falconer & Humpage, 2005). Despite the presence of cyanobacteria blooms in the Lake Victoria water, the health risk this could pose from cyanotoxins has not been assessed. As such, the current study will evaluate the health risk of cyanotoxins in both Lake Victoria water and household drinking water for Nyanza Gulf residents.

CHAPTER THREE

STUDY METHODOLOGY

3.1 Study Design

This was a longitudinal study design. Both survey research and experimental approaches were adopted. It was conducted over a period of six months between May 2015 and October 2015. During this time the concentration of cyanobacteria and cyanotoxins in the lake water and households was monitored. Replication of samples was done in recognition that cyanobacteria are unlikely to be distributed homogeneously in natural waters.

3.2 Study Area

Nyanza Gulf is in Western Kenya whose watershed lies between 0.25N - 1.00S latitudes and 34.0E - 36.0E longitudes. It covers an approximate total drainage area of 12,300 km2. Nyanza Gulf has an area of 1400 km^2, mean depth 7 m, maximum depth 30 m and a 550 km shoreline that is located entirely in Kenya on the northeast of Lake Victoria (Misigo & Suzuki, 2018). It is connected to the open waters by Rusinga Channel. The gulf is river-fed embayment by multiple rivers largely arising from the Kenya highlands on East and South East. The average areal annual rainfall distribution of up to 1400 mm on the Easternside-1600 mm on South Eastern-South and the annual maximum and minimum temperature of the watershed area is about 30˚C and 8˚C, respectively. The catchment area experiences equatorial climate with two rainfall seasons; March to June (long rain season), September to November (short rain season), followed by a relatively dry season in December to February and July to August respectively (Misigo & Suzuki, 2018). Nyanza Gulf borders five counties namely Busia, Siaya, Kisumu, Homabay and Migori. The main economic activities among the riparian communities are fishing, farming, business and formal employment. The study site was beaches/water collection points along Lake Victoria.

Nyanza Gulf of Lake Victoria was considered for this study due to the large size of livelihood it supports. The six beaches included in the study are located in areas with calm waters which promote eutrophication attracting cyanobacterial blooms.

3.3 Target Population

The study population was beaches and households along the Nyanza Gulf that use the lake water for drinking. There are 278 beaches and approximately 94, 147 households based on the Beach Management Units (BMUs) records. Selection of beaches was based on preliminary analysis of the water samples collected. Six beaches in the Nyanza Gulf were selected to form the study population. Households living along the selected beaches exclusively using Lake Victoria water for drinking were also recruited guided by households' population for a particular beach.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

i. Only beach sites used for withdrawing drinking water were used in the study.

ii. Only consenting and households drawing their drinking water from the beach directly were included in the study.

3.4.2 Exclusion Criteria

i. Beaches with other activities such as agricultural, car washing etc. were excluded from the study.

3.5 Sampling Design

3.5.1 Sample Size Determination

The percentage of households that their drinking water drawn from the lake is likely to contain cyanotoxins is not known. Based on the assumption that the prevalence of cyanotoxins in household drinking water is 50%, the sample size was determined using the formula by Fisher's *et al.* (1998):

$$\mathrm{N} = \frac{Z^2 pq}{d^2}$$

Where:

N = the desired sample size (if the target population is greater than 10,000)

Z = the standard normal deviate 1.96 at 95% confidence interval

p = assuming the prevalence of cyanotoxins in household drinking water 50%

q = 1 – p = 0.5

d = level of statistical significance set at 0.05.

Therefore;

$$\mathrm{N} = \frac{1.96^2(0.5)(0.5)}{0.05^2} = 384$$

A further 10% of 384, which is, approximately 38 was added to cover for unresponsive subjects, bringing the total sample size to 422 subjects.

3.6 Variables

3.6.1 Independent Variable

- Concentration of cyanobacteria
- Strains of Microcystins

3.6.2 Dependent Variable

- Health risk in drinking water

3.7 Sample Collection

3.7.1 Water Samples

Water samples for analysis of microcystins in the Lake Victoria water was collected once a month for a period of 6 months beginning May to October 2015.The sample water was obtained from the exact point where the riparian communities draw water from Lake Victoria. One litre of water was collected as sample using 1.5 litre sterile plastic sampling bottles. The collected water samples were stored in an icebox for transportation to the LAVICORD laboratory at Maseno University, City Campus for storage and subsequent analysis. Standard water abstraction techniques were used to obtain water in a manner that minimized contamination during sampling. A similar amount of water was also collected from 30% (approx. 127) of all the households sampled.

3.7.2 Household Samples

For each beach selected, the detail of households was obtained from CHWs working in the villages. Based on the total households per beach village, a proportionate sample size was drawn and included in the study. A sampling interval as determined by the household population and the sample size was followed. The primary caregiver from each household was selected for participation in the study from the systematically sampled households.

Table 3.1: Household sampling procedure

Beach	Households in beach	Households to be sampled	Sampling Interval
Ogal (Kisumu)	230	61	4
Mawembe (Kisumu)	223	59	4
Alum (Homabay)	302	80	4
Rang'ombe (Homabay)	242	64	4
Olambwe (Mbita)	294	78	4
Kolunga(Mbita)	298	79	4
Total	**1589**	**422**	

3.8 Sample Analysis

The following experiments were carried out in the laboratory to the water samples collected:

3.8.1 Chlorophyll – A Analysis

200ml of sample water was vacuum-filtered using 0.45µm membrane filtersas soon as the water samples are collected (Bowles *et al.,* 1985). The filters were folded and put in a labelled aluminium foil and kept in a darkened desiccator to prevent radiation. 10 ml 90% methanol (v/v) including 2% $MgCl_2$, was added to a test-tube, filter with sample inserted and the caps closed well. The test-tubes were then heated with the samples in a water bath at 70℃ for 30 minutes followed by cooling at room temperature. The contents of each test-tube were then transferred into separate centrifuge tubes, and centrifuge for 10 minutes at 2500 rpm/min. The supernatant was carefully transferred into a glass/quartz cuvette and the absorbance of Chl-a in methanol measured at four wavelengths (750nm, 665nm, 645nm, 630nm) by a spectrophotometer using 90% methanol to adjust the baseline and calibrationas recommended in Saijo *et al*., (1975).The value of Chl-a was then calculated using the formula:

$$Chl-a(\mu g/L)=\left(11.6\times\left(A665-A750\right)-1.31\times\left(A645-A750\right)-0.14\times\left(A630-A750\right)\right)\times\frac{V}{V_f}$$

Where,

- V (ml) - Volume of 90% methanol;
- V_f (ml)- Volume of filtrated sample water;
- A750, A665, A645 and A630- Absorbance values at 750nm, 665nm, 645nm and 630nm respectively.

The Chlorophyll-a data was used to calculate the Trophic State Index (TSI) for Chl-a using the formulae below:

$$\text{TSI} = 9.81 \times \ln(Chl\ a) + 30.6$$

3.8.2 Microcystin analysis using PP2A enzymatic assay method

In the laboratory, the water samples were analyzed for presence of microcystin using Protein Phosphatase 2A (PP2A) enzyme assay method according to Heresztyn and Nicholson (2001). 2ml of water samples collected was put in a screw-capped test-tube and put in a boiling water bath for one hour. After cooling down it was centrifuged for 15 minutes at 3000rpm. 10µl of the supernatant was transferred to a 96-well microplate. 10µl of enzyme dilution (PP2A enzyme in Bovine Serum Albumin {BSA}, 0.1 M Tris, 40mM Dithiothreitol {DTT}, 10mM $MnCl_2$, 0.3M $MgCl_2$ and MilliQ water) was added to the samples, shaken and incubated for 5 minutes at 37^{o}C to bind the microcystin to the enzyme. 100µl of reaction mixture (containing 4-nitophenyl phosphate disodium salt {Sigma Aldrich}, BSA, 0.4M Tris, 10mM $MnCl_2$, 0.3 $MgCl_2$ and MilliQ water) was added to the wells containing samples. The microplate was then incubated at 60 minutes at 37^{o}C. Absorbance was measured at 405nm by a plate reader and the results

analysed in a Microsoft Excel sheet. Standards of 10µl, 5µl, 2.5µl, 1.25µl, 0.625µl, 0.3125µl and 0.15625µl of MC-LR (from Sigma Aldrich) were used.

The PP2A method is categorized as a semi-quantitative method of MC analysis. Quantification by this method is possible for concentrations lower than 10µg/L. For higher concentrations, serial dilutions of the sample were carried out, analyzed and the results extrapolated by the dilution factor. As such, the method is used prior to HPLC to detect the presence or absence of MC. The detection level of MC by PP2A is lower, meaning quite low quantities such as 0.05µg/L of MC are positively identified by this method. On the other hand, the threshold for HPLC detection is comparatively higher. In this study, PP2A method was used first and samples which recorded equal or higher than 0.25µg/L (detection limit for PP2A) were processed for analysis by HPLC.

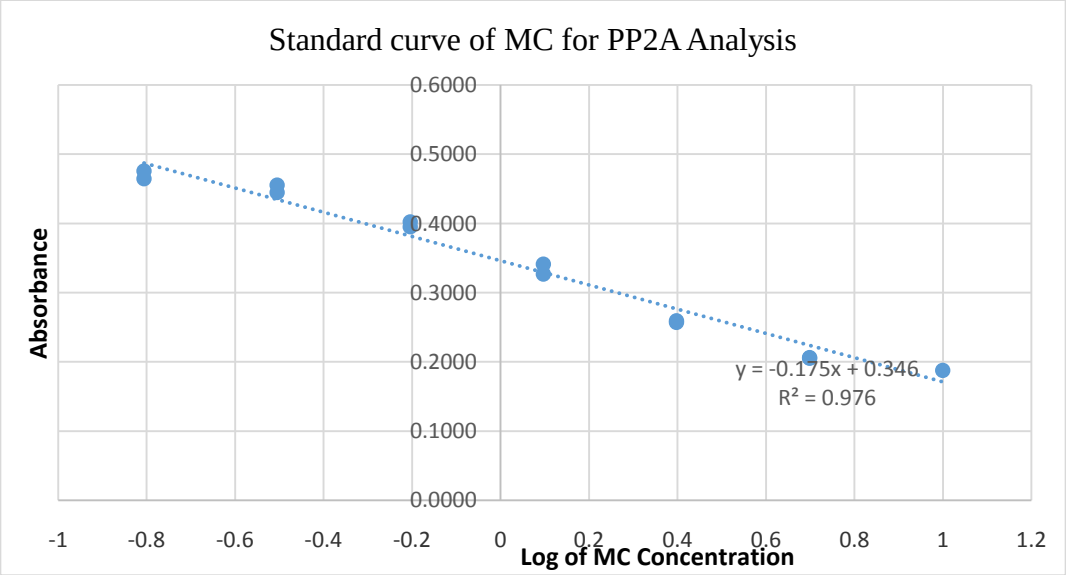

Figure 3.1: Standard curve of MC for PP2A Analysis

3.8.3 Microcystin analysis using HPLC

Microcystin characterization was done using High Performance Liquid Chromatography (Lawton *et al*., 1994; Heresztyn & Nicholson, 2001). 100ml of sample water was transferred to a conical flask sealed with aluminum foil and put in a boiling water bath for 1 hour. Once the sample had cooled down, it was vacuum filtered using 0.45µm Whatman GF/C microfiber filters. The filtrate was then passed through a pre-treated Strata-X 33µm SPE cartridge (60mg, 3ml, Phenomenex, U.S) for adsorption of microcystin. The cartridge was first washed by passing 5ml of 100% methanol followed by 20ml Milli-Q water at the rate of 5ml/min. This was done two times then the sample was allowed to pass through at the same flow-rate. After the sample, the cartridge was then washed two times using 5ml of 20% methanol and 15 ml of Milli-Q water so as to remove small-size solid material. 3ml of 100% methanol was used to re-dissolve adsorbed microcystin into a test-tube. The methanol was then evaporated by heating up to 40^{o}C combined with pumping in air for about 1 hour. Thereafter, the sample to be injected into HPLC was dissolved in 1ml of carrier solution (50% methanol in 0.05% TFA), syringe-filtered into vials using filter paper of pore size 0.45µm. in the HPLC machine samples were put alongside standards of 0.667µg/l of MC-LR, MC-YR and MC-RR. Solutions used in the HPLC were 100% methanol and TFA solution (50% methanol in 0.05% TFA). Subsequent graphs generated by the HPLC machine for samples were analysed alongside the standards and the results analysed in Microsoft Excel.All reagents used were of HPLC or analytical-reagent grade. Water used throughout was of high-purity that was produced with a Milli-Q system (Millipore, Milford, MA, USA).

HPLC method is purely quantitative and more accurate. In HPLC, different strains of MC produce peaks at specific points in the graph generated. The position and size of peak by a

sample are used to calculate the concentration against peaks of known standards. A typical chromatogram of MC-LR, MC-RR and MC-YR graph showing peaks is shown in the figure below:

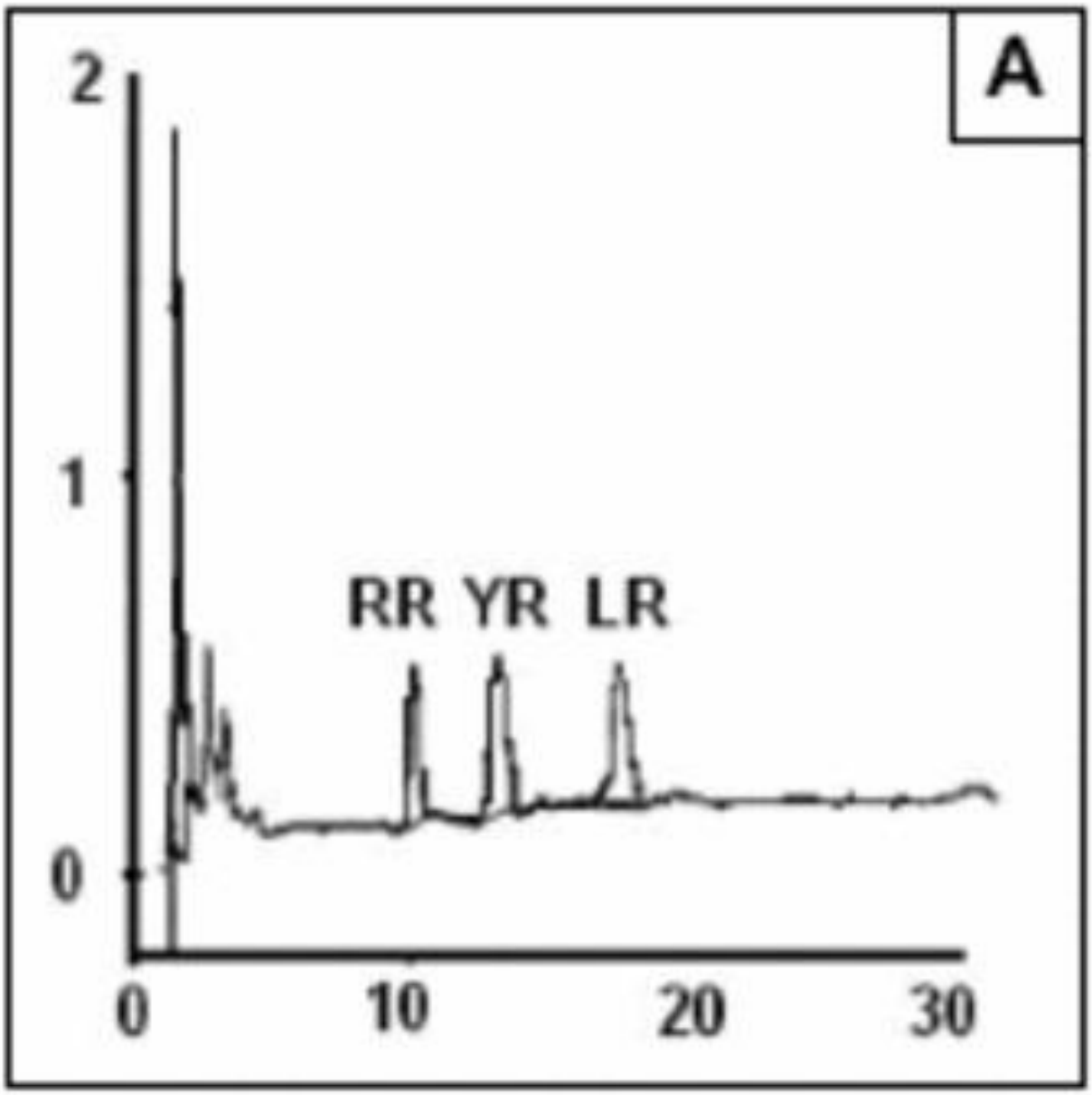

Figure 3.2: A typical chromatogram of MC-LR, MC-RR and MC-YR

3.9 Data Collection

This study adopted both survey research and experimental methods. Survey research approach was used to collect socio-demographic, water source and water handling data at the household level. Experimental methods involved identification of cyanobacteria and determination of levels of cyanotoxins in both lake and household drinking waters.Household data and water quality in terms of levels of cyanobacteria and cyanotoxins was collected as below:

3.9.1 Household Data

A structured questionnaire **(**AppendixIV) was used to obtain data from households. This information helped to enrich data from experimental i.e. cyanotoxins and microcystin levels in the drinking water.

3.9.2 Water Quality Data

For all samples collected, the level of cyanotoxins was recorded after analysis.

3.9.3 Estimation of risk for human health from data on microcystin in water

Assessment of health risk of microcystin was evaluated based on the formula (Falconer & Humpage, 2005),

$$\text{Daily Intake of Microcystin} = \frac{\text{Average Concentration of MC} \times \text{Water Ingested}}{\text{Body Weight}}$$

$$\text{Risk Factor of MC} = \frac{\text{Daily Intake of Microcystin}}{\text{Tolerable Daily Intake of Microcystin}}$$

Where:

Tolerable Daily Intake of 0.044µg/kg-bw/day (WHO, 1999)

Standard Body Weight taken to be 60kg (Falconer & Humpage, 2005)

Water Ingested is the average daily water intake in litres by an individual.

3.10 Pre-testing Study Tools

Study tools were pretested in Wichlum Beach in Siaya County which is one of the beaches along the Nyanza Gulf of Lake Victoria. This was done to ensure reliability and validity of the study tools. It was also done to give an estimate of time that was to be taken to administer a questionnaire to a respondent as well as to help to eliminate ambiguous questions. A total of 20 women were interviewed.

3.10.1 Reliability of Study Tools

The Cronbach's alpha technique which measures internal consistency was used to achieve reliability. It requires only a single administration of test to estimate internal consistency and is computed as follows:

$$\text{Alpha} = Nr / (1 - r\,(N - 1))$$

Where:

r = mean inter-item correlation

N = number of items in the scale

SPSS v20 application was used to generate inter-item correlation matrix first, then summed up and estimated the average. A coefficient of0.85was obtained which implied that there was a high degree of reliability (Gliem & Gliem, 2003).

3.10.2 Validity of Study Tools

The content validity technique was used to address the match between test questions and the content or subject area they are intended to assess, the extent to which a test or the questions on a test appear to measure a particular construct as viewed by lay persons, clients, examinees, test user. Measuring of content validity was done using C. H. Lawshe (1975) method proposed for each of the subject matter expert raters (SMEs) on the judging panel respond to the question for each item. Using Lawshe's formula termed the Content Validity Ratio (CVR):

$$CVR = (n_e - N/2)/N/2$$

Where: CVR = Content Validity Ratio

n_e = number of SME panellists indicating "essential"

N = total number of SME panelists

This formula yields values which range from +1 to -1; positive values indicate that at least half the SMEs rated the item as essential. The mean CVR across items was used as an indicator of overall test content validity (Lawshe, 1975).

Substituted as in

$CVR = (13 - 14/2)/14/2$

$= 0.8571.$

Therefore, the questionnaire passed the overall test content validity.

3.11 Data Management and Statistical Analysis

Analysis was done using Statistical Package for Social Sciences (SPSS) version 20 contained in personal computer (PC). Data was stored in both source records and computer databases accessible

only to the authorized persons throughout the study. Frequency charts, proportions and tables were used in presentation of socio-demographic data. Comparison of microcystin levels with the recommended guideline value and TDI set by WHO was made. A correlation test was conducted to find out whether there was a relationship between the levels of MC concentration in the beaches and the households. A two-way ANOVA was done on a level of MC in 128 samples drawn from the 6 beaches to determine the interaction between effect of the specific beaches and month when sample was taken on the levels of MC. A two-way ANOVA was also done to find out whether the source of the water (beaches) and the method of water treatment had an effect on the levels of MC in the samples drawn from the households. These calculations were performed using SPSS vs. 20 and the F value was set at the default value of $p<0.05$.

3.12 Ethical Approval (Appendix V)

Ethical clearance for the study was obtained from Maseno University Ethics Review Committee (MUERC). Before commencement of the study, permission to conduct the study was obtained from the Maseno University School of Graduate Studies (SGS) and also from the administrative heads of the various locations. Informed consent of participants was obtained and confidentiality of participants was maintained at all times. The questionnaires did not have any form of personal identifiers. Participation was voluntary and participants were informed that they can withdraw from the study at any stage of the interview if they so desire.

CHAPTER FOUR:

RESULTS

4.1 Socio-demographics characteristics of households in the Nyanza Gulf

A total of 421 households were visited and interviews conducted in all of them, out of which 61 (14%) were from Ogal Beach, 59 (14%) were from Mawembe Beach, 80 (19%) were from Alum Beach, 64 (15%) were from Rang'ombe Beach, 78 (19%) were from Olambwe Beach and 79 (19%) were from Kolunga Beach. The males interviewed were 74 (18%) and the majority were female i.e. 347 (82%). When the marital status of the respondents was considered, the result was that 6 (1%) were singles, 339 (81%) were married or cohabiting and 76 (18%) were divorced or widowed. All the respondents were Christians. Looking at their occupation,163 (39%) of those interviewed worked in the fishing industry, those in the business sector were 129 (64%) and respondents who engage in agriculture were 116 (27%). The remaining three (1%) worked in governmental institutions and NGOs while ten (2%) were working in other places. Averagely each household had five members and the household with the highest and lowest number of members being one and 21 respectively.

Table 4.1 Socio-demographic characteristics

Characteristic	**Number (*N=421*)**
Resident Beach	
Ogal	61 (14%)
Mawembe	59 (14%)
Alum	80 (19%)
Rang'ombe	64 (15%)
Olambwe	78 (19%)
Kolunga	79 (19%)
Gender	
Male	74 (18%)
Female	347 (82%)
Marital Status	
Single	6 (1%)
Married/Cohabiting	339 (81%)
Divorced/Widowed	76 (18%)
Religion	
Christian	421 (100%)
Occupation	
Fishing	163 (39%)
Business	129 (31%)
Agriculture	116 (27%)
Government/NGOs	3 (1%)
Others	10 (2%)

The table shows 421 respondents instead of 422. This is because of one incomplete questionnaire.

4.2 Water usage, treatment and storage households in the Nyanza Gulf

When the interviewees were asked about their water usage, treatment and storage mechanisms, they responded as follows:

4.2.1 Water usage

All 421 (100%) use the beach water for cooking, a huge percentage of them 408 (97%) respondents use the beach water for drinking including preparation of tea/coffee/porridge while 13 (3%) replied that they don't consume water from the beach. From the questionnaire responses, it was established that the respondents consumed 6 cups of beach water daily on average either directly through drinking the water or indirectly in tea and food. For this study, the cup of reference had a capacity of 500 milliliters (ml).

Table 4.2 Water usage

Characteristic	**Ogal** (n=61)	**Mawembe** (n_1=59)	**Alum** (n_2=80)	**Rang'ombe** (n_3=64)	**Olambwe** (n_4=78)	**Kolunga** (n_5=79)	**Total %**(N=421)
Cups (500 ml) of Drinking Water Consumed							
1-3	28	34	47	4	41	24	42.3(178)
4-6	27	22	32	57	31	42	50.1(211)
7-9	5	3	0	3	4	13	6.7(28)
10 or more	1	0	1	0	2	0	1(4)
Cups (500 ml) of Tea/Porridge/Coffee Consumed							
None	2	0	0	41	0	0	10.2(43)
1	21	14	33	22	7	12	25.9(109)
2-3	34	35	47	1	59	41	51.5(217)
4-5	3	7	0	0	12	23	10.7(45)
≤6	1	3	0	0	0	3	1.7(7)
Average of Total Cups Consumed							
Average	6.0	6.0	5.2	5.3	6.4	7.6	100(6.1)

4.2.2 Water treatment

Water treatment was undertaken in 324 (78%) of the homes not done in the remaining 97(23%) homes. Out of those that treat their water, 265 (82%) did chlorination which was the most commonly used method of water treatment followed by filtration 20 (6%), boiling 20 (6%), combination of boiling and filtration with 18 (5.6%) and others was 1 (0.5%) as shown in the table 4.3. In general, the results showed that chlorination is the most commonly used method.

Table 4.3 Water treatment

Characteristic	**Ogal** (n=61)	**Mawembe** (n_1=59)	**Alum** (n_2=80)	**Rang'ombe** (n_3=64)	**Olambwe** (n_4=78)	**Kolunga** (n_5=79)	**Total (%)**(N=421)
Water Treatment (Households which treat water)							
Yes	59	54	79	3	62	67	77(324)
No	2	5	1	61	16	12	23(97)

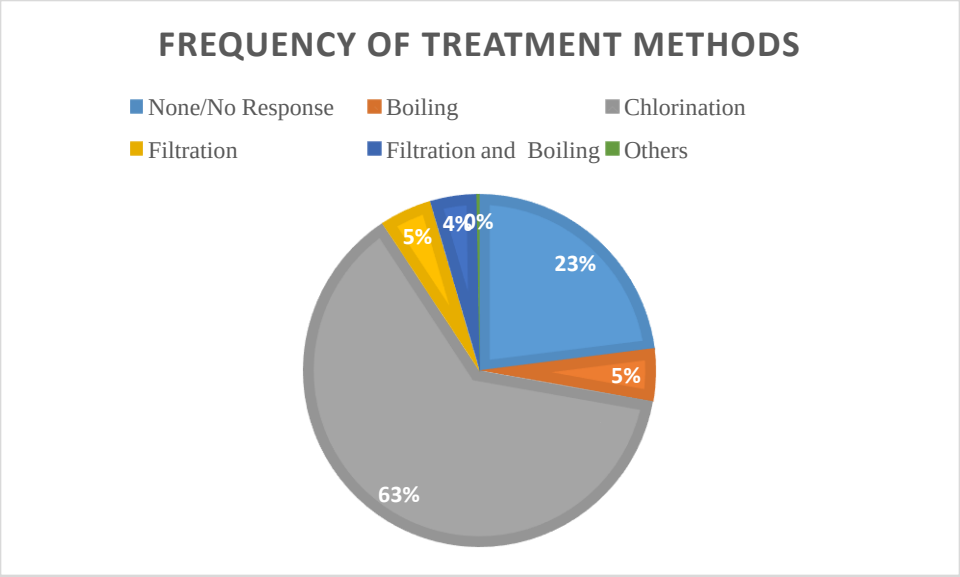

Figure 4.1: Frequency of water treatment methods used in households

4.2.3 Water storage

The beach water would be stored in most households 277 (66%) for two to four days. Fewer households i.e. 17 (4%) would store water for longer period of one week or over. 84 (20%) of the households visited store water for one day or less while 43(10%) households store water for five or six days. In 218 (52%) households earthenware containers were being used to store water while in 175 (42%) of the households plastic containers were utilized. 20 (4.8%) households used metallic containers and only eight (2%) households used glassware in water storage.

Table 4.4 Water storage

Characteristic	**Ogal** (n=61)	**Mawembe** (n_1=59)	**Alum** (n_2=80)	**Rang'ombe** (n_3=64)	**Olambwe** (n_4=78)	**Kolunga** (n_5=79)	**Total %**(N=421)
Water Storage Containers used							
Earthenware	25	46	57	1	48	41	51.8(218)
Glass	7	0	0	0	0	1	1.9(8)
Metallic	1	0	0	15	4	0	4.8(20)
Plastic	28	13	23	48	26	37	41.6(175)
Store Days (Number of Days Water is stored)							
≤ 1	9	10	10	6	3	46	20(84)
2-4	47	57	65	47	55	26	65.8(277)
5-6	3	7	5	11	11	6	10.2(43)
≥7	2	5	0	0	9	1	4(17)

4.3 Concentration of cyanobacteria in both Lake Victoria water and household drinking water for Nyanza Gulf residents

4.3.1 Chl-a levels in the Nyanza Gulf

Chl-a level fluctuated over the months in all the beaches. In Ogal and Mawembe beaches, it was relatively low between June and November 2014 before rising in January 2015 and lowering again in the subsequent months. Alum and Rang'ombe beaches had had the highest levels in June 2014 before dropping sharply in August 2014, followed by a rise in January 2015 and then dropping again. Olambwe and Kolunga beaches levels were highest in August 2014 before dropping and rising thereafter.

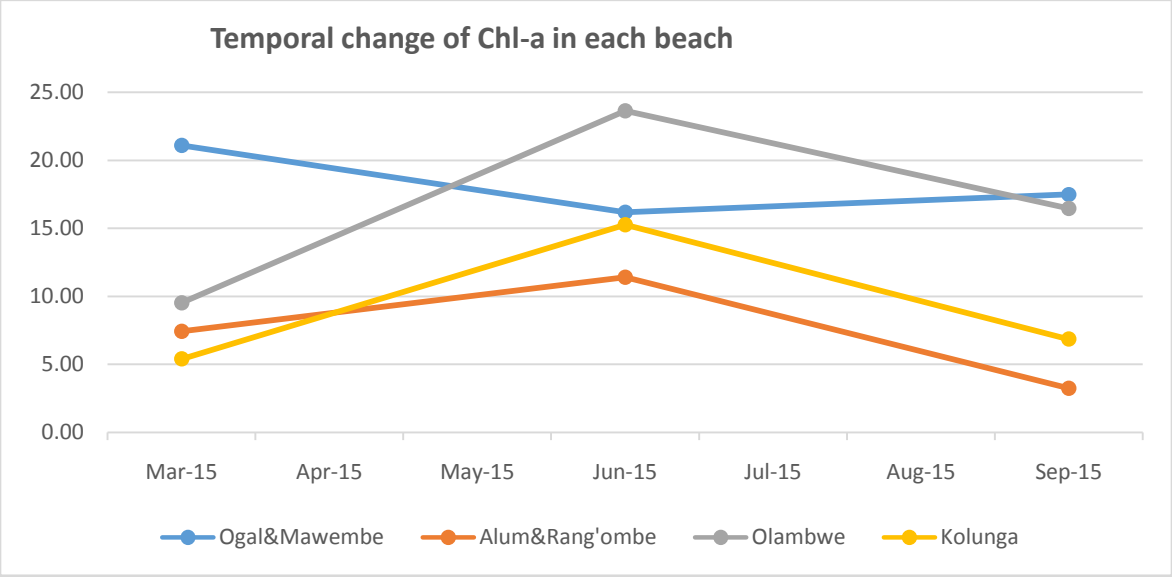

Figure 4.2: Graph showing the temporal change in Chlorophyll-a in the beaches

Using data showing the Chl-a level in the various beaches, the average TSI levels for each beach was calculated. The resultant values were then used to gauge the eutrophication levels in the beaches. All beaches were found to be eutrophic. Classification was done based on biomass-related trophic state indices (Carlson, 1996).

Table 4.5: Classification of beaches based on Trophic State Index

Beaches	Average PP2A	Average TSI	Classification
Ogal	8.14	57.86	Eutrophic
Mawembe	6.17	57.86	Eutrophic
Alum	1.46	54.09	Eutrophic
Rang'ombe	3.17	54.09	Eutrophic
Olambwe	1.39	56.79	Eutrophic
Kolunga	0.31	53.14	Eutrophic

4.3.2Microcystin in beach and household water

Out of the 127 samples collected from households, 103 (80%) samples were positive for presence of MC. For a similar number of beach samples, 112 (88%) samples had cyanotoxins. On average, 215 (84%) of samples contained MC. There was a general trend in the level of MC

in households being lower those of the respective beaches as the Figure 4.3clearly shows. Ogal and Mawembe beaches had higher values than the rest in both beach and household.

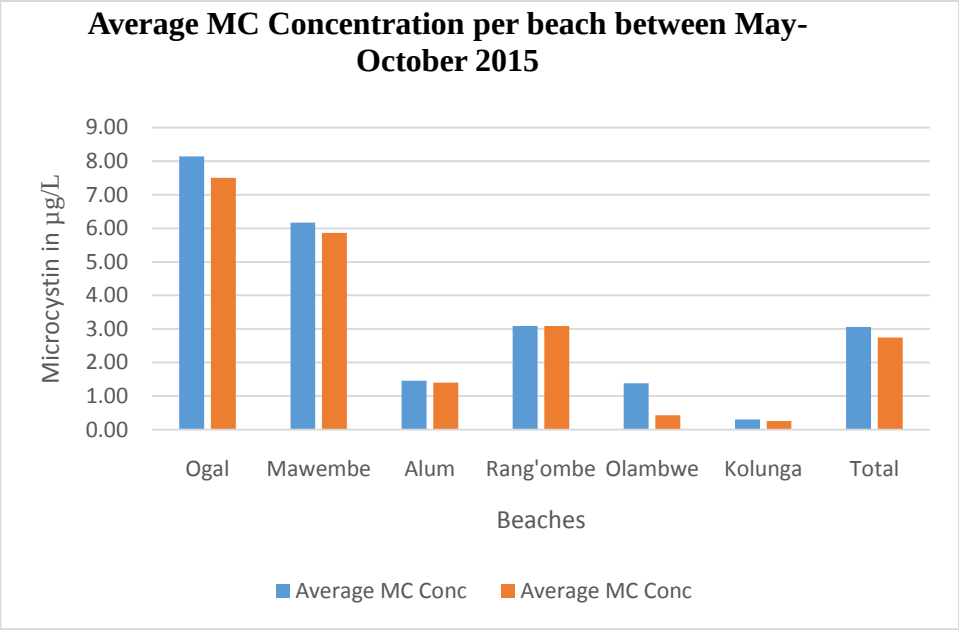

Figure 4.3: Average Concentration of Microcystin per beach for May to October 2015

When monthly comparisons among beaches were done for beach and household averages as is shown Figure4.4, the month of May presented higher concentration of MC followed by June. In Ogal beach, the concentrations of MC were very high in all months except for the month of July and August while in Olambwe and Kolunga beaches they were very low. There was a general trend of the concentration of MC dropping from the month of May through August and then rising as from August through to October 2015. Kolunga beach had a fairly constant concentration all throughout the 6 months of the study.

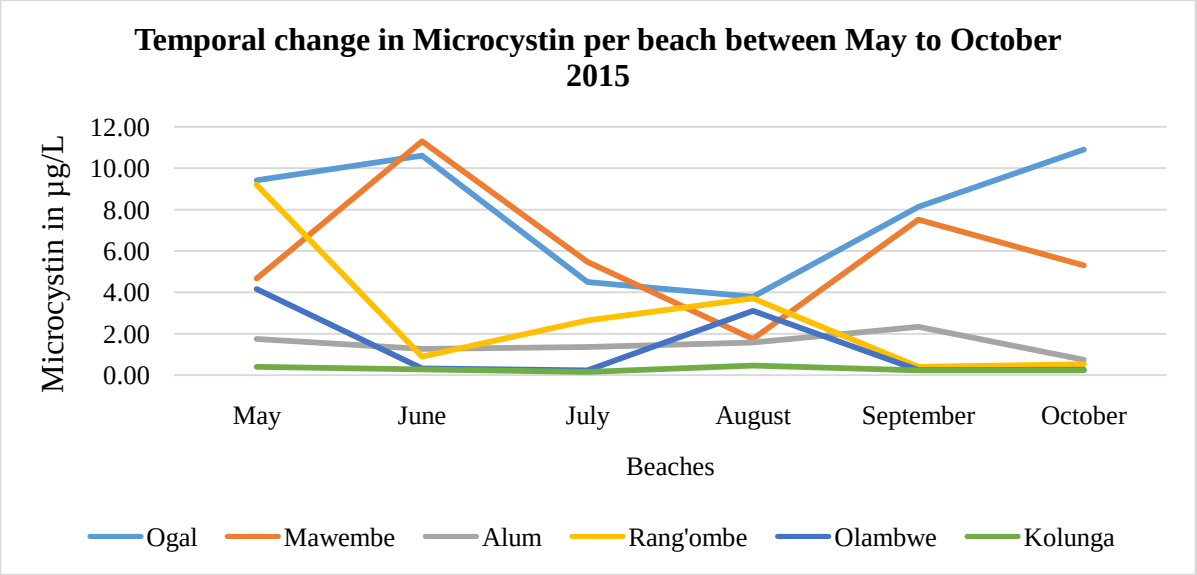

Figure 4.4: Temporal change in Microcystin per beach

When the average concentration across all the beaches was computed, the concentration of MC was highest in the month of May at 5.26µg/L, decreasing to 3.48 µg/L in June and decreasing further in July to 2.24 µg/L. In August there was a slight increase to 2.48 µg/L before decreasing to 2.1 µg/L in September and rising again in October to 2.53 µg/L.

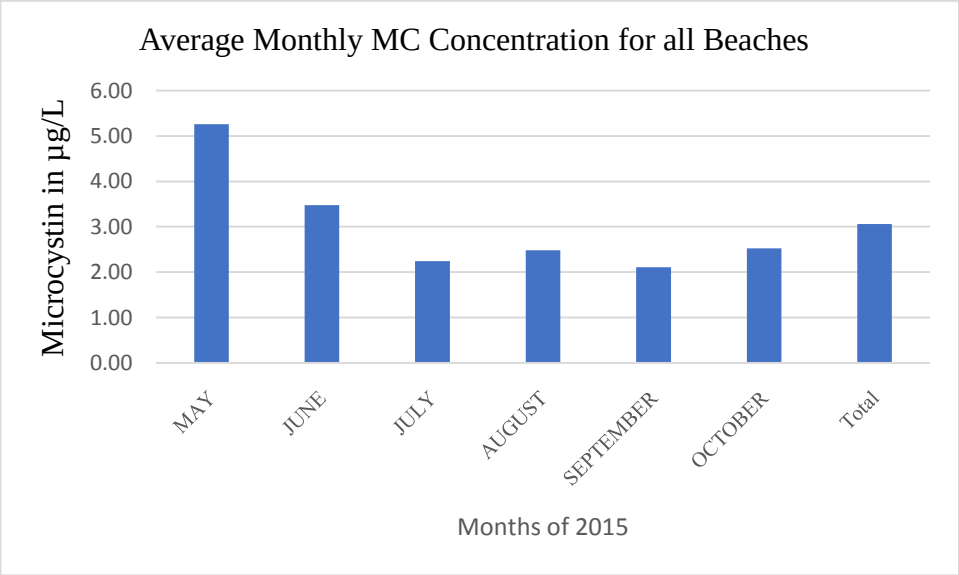

Figure 4.5: Temporal change in Microcystin per beach

4.3.3 Correlation between MC concentration in the beaches and the households

A correlation test was conducted to find out whether there was a relationship between the levels of MC concentration in the beaches and the households and found out that there was a strong positive relationship between the two ($r = 0.822$, $n=128$, $P<0.0000$, $\alpha=0.01$).

Regression analysis was thereafter done and a model fit with the R squared being 0.672 implying that 67.2 % of the levels of MC in households can be explained by levels of MC in beaches. The resulting linear equation is $Y = 0.158+0.846X$. This therefore means that an increase in the level of MC in beaches by a value of 1 increases the levels of MC in household by 0.846.

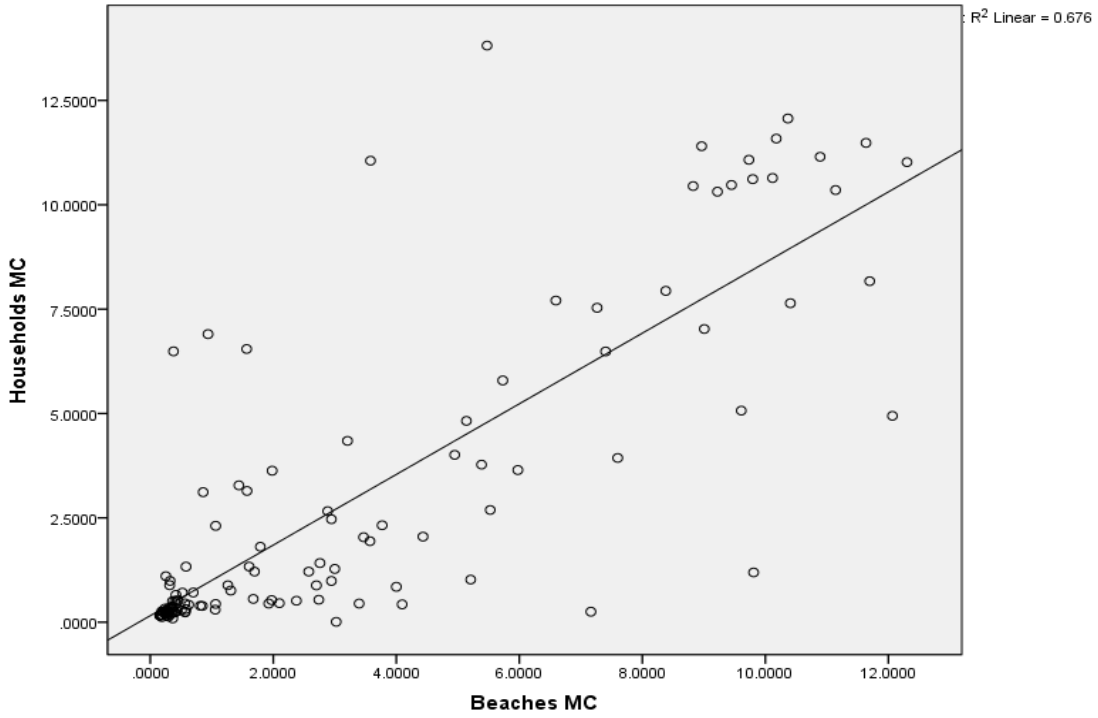

Figure 4.6: Correlation between MC concentration in the beaches and the households

A two-way ANOVA was done on a level of MC in 128 samples drawn from the 6 beaches to determine the interaction between effect of the specific beaches and month when sample was taken on the levels of MC. There was a significant interaction between the effect of beaches and months on the levels of MC in the samples taken, $F (25, 92) = 12.09$, $p < 0.0005$. There was significant variation in the level of cyanotoxins in samples drawn from the lake in different

months of the year. There was also variation in the level of cyanotoxins in samples drawn from beaches. This means different beaches had different concentrations over different months.

To find out whether the source of the water (beaches) and the method of water treatment had an effect on the levels of MC in the samples drawn from the households, a two-way ANOVA was done and it was found that there was no interaction between the effect of beaches and effect of water treatment method used on the level of MC in 128 samples drawn from the households, $F(9, 110) = 0.97$, $P>0.05(P=0.4708)$. Therefore was no significant variation in the level of cyanotoxins from samples drawn from household drinking water with regards to treatment method used and the beach from which the water in the household was drawn.

4.4 Identification of the cyanotoxins in Lake Victoria and household drinking water for Nyanza Gulf residents

Ogal beach presented the highest concentration of MC-RR, MC-YR and MC-LR followed by Mawembe beach and the lowest being Kolunga beach for the three strains. The concentration of MC-RR strain was the highest among the three strains followed by MC-LR and MC-YR in beaches 1 and 2 but was the lowest while MC-LR was highest and then MC-YR in the remaining beaches. Kolunga beach had very low concentrations of the three MC strains as shown in the figure 4.7 and figure 4.8

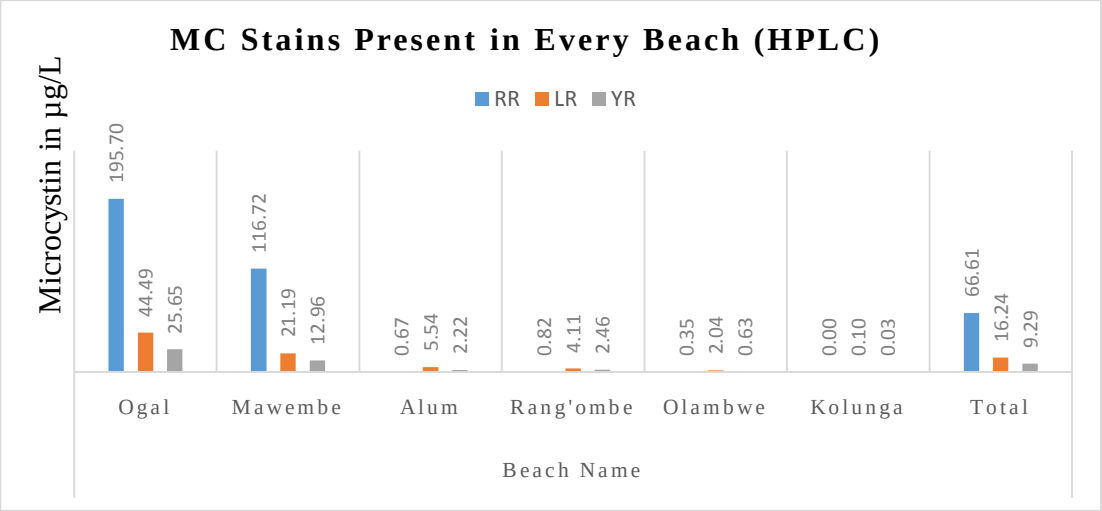

Figure 4.7: MC Stains Present in Every Beach by HPLC method

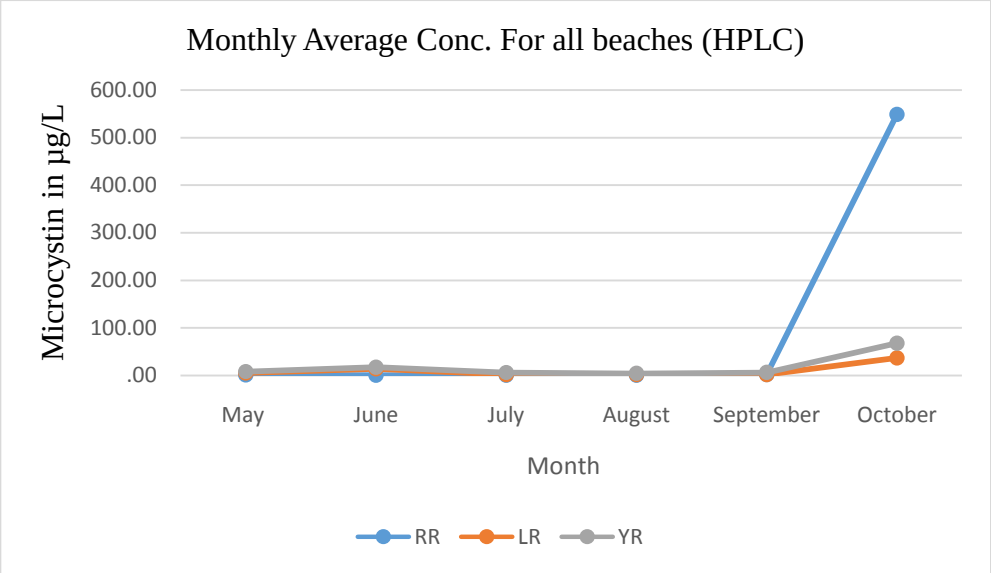

Figure 4.8: Monthly Average Concentration for all beaches using HPLC

4.5 Determination of the health risk of cyanotoxins in both Lake Victoria water and household drinking water for Nyanza Gulf residents

4.5.1 Comparison of concentration of MC against WHO standard

The WHO has set a provisional guideline value of 1µg/L for MC-LR for drinking water. Based on this, a comparison was made with water from beaches and households from the six beaches. It

was established that for the beach water, five out of six beaches, i.e. Ogal, Mawembe, Alum, Rang'ombe and Olambwe registered MC levels above the WHO guideline value. Kolunga beach was the only beach with water below the standard. For household water, Kolunga and Olambwe beaches were below the standard, whereas Ogal, Mawembe, Alum and Rang'ombe exceeded the WHO guideline value.

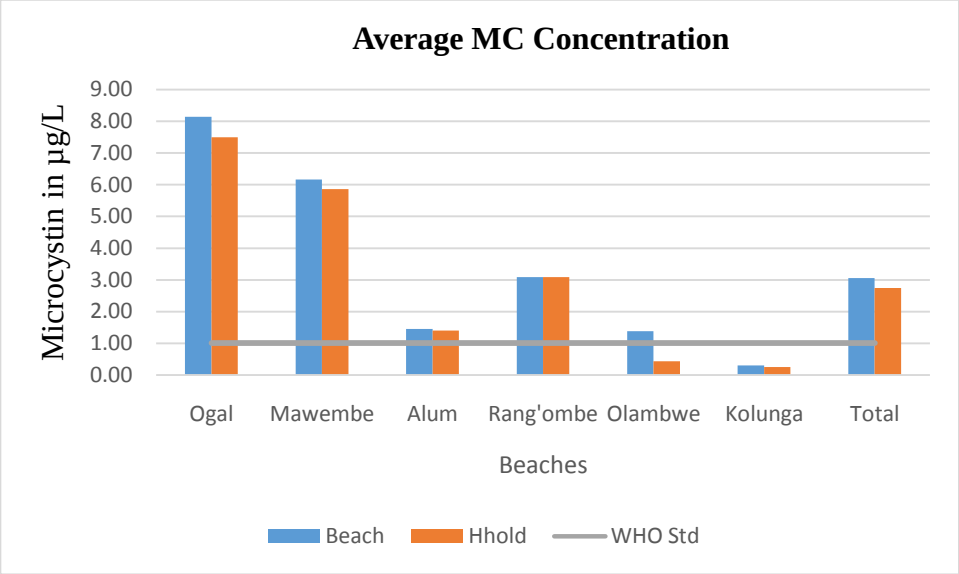

Figure 4.9: Average MC Concentration

4.5.2 Health Risk based on TDI

The health risk was calculated based on the formulae in Section 3.9.3. Daily Intake of MC was calculated and compared with the Tolerable Daily Intake by WHO set at 0.044µg/kg. The risk factor for Ogal beach was the highest at 9.32, followed by Mawembe beach at 7.05. Alum beach had a relatively low risk factor of 1.36 but Rang'ombe was much higher at 3.18. Olambwe beach had a risk factor of 1.59 whereas Kolunga beach had the lowest value at 0.45. On average, the waters of Nyanza Gulf were 3.86 times higher than the recommended TDI.

Table 4.6: Daily intake of Microcystin per beach

Beach	Average Concentration of Microcystins	Average Water Ingested	Body Weight	Daily Intake of Microcystins
Ogal	8.14	3.00	60	**0.41**
Mawembe	6.17	3.00	60	**0.31**
Alum	1.46	2.60	60	**0.06**
Rang'ombe	3.17	2.65	60	**0.14**
Olambwe	1.39	3.20	60	**0.07**
Kolunga	0.31	3.80	60	**0.02**
Average	3.44	3.04	60	**0.17**

Table 4.7: Risk Factor for MC per beach

Beach	Daily Intake of Microcystins	Tolerable Daily Intake	Risk Factor for MC
Ogal	0.41	0.04	**9.32**
Mawembe	0.31	0.04	**7.05**
Alum	0.06	0.04	**1.36**
Rang'ombe	0.14	0.04	**3.18**
Olambwe	0.07	0.04	**1.59**
Kolunga	0.02	0.04	**0.45**
Average	0.17	0.04	**3.86**

4.5.3 Effectiveness of the various water treatment methods on the level of MC in the households

In trying to answer this, levels of MC were recorded at both the beaches and the households. Difference was calculated between the level of MC in the beach and the corresponding household water samples. The average concentration of MC in beach water samples was 3.06

µg/L, the average concentration for household water samples was 2.75µg/L and the mean difference was 0.31µg/L. ANOVA was then conducted to determine if there were significant differences with regards to the change in the level of MC between the household and the beaches grouping by the various methods of water treatment. The result was that there was no significant difference given the p value of 0.456 against a significance level of 0.05.

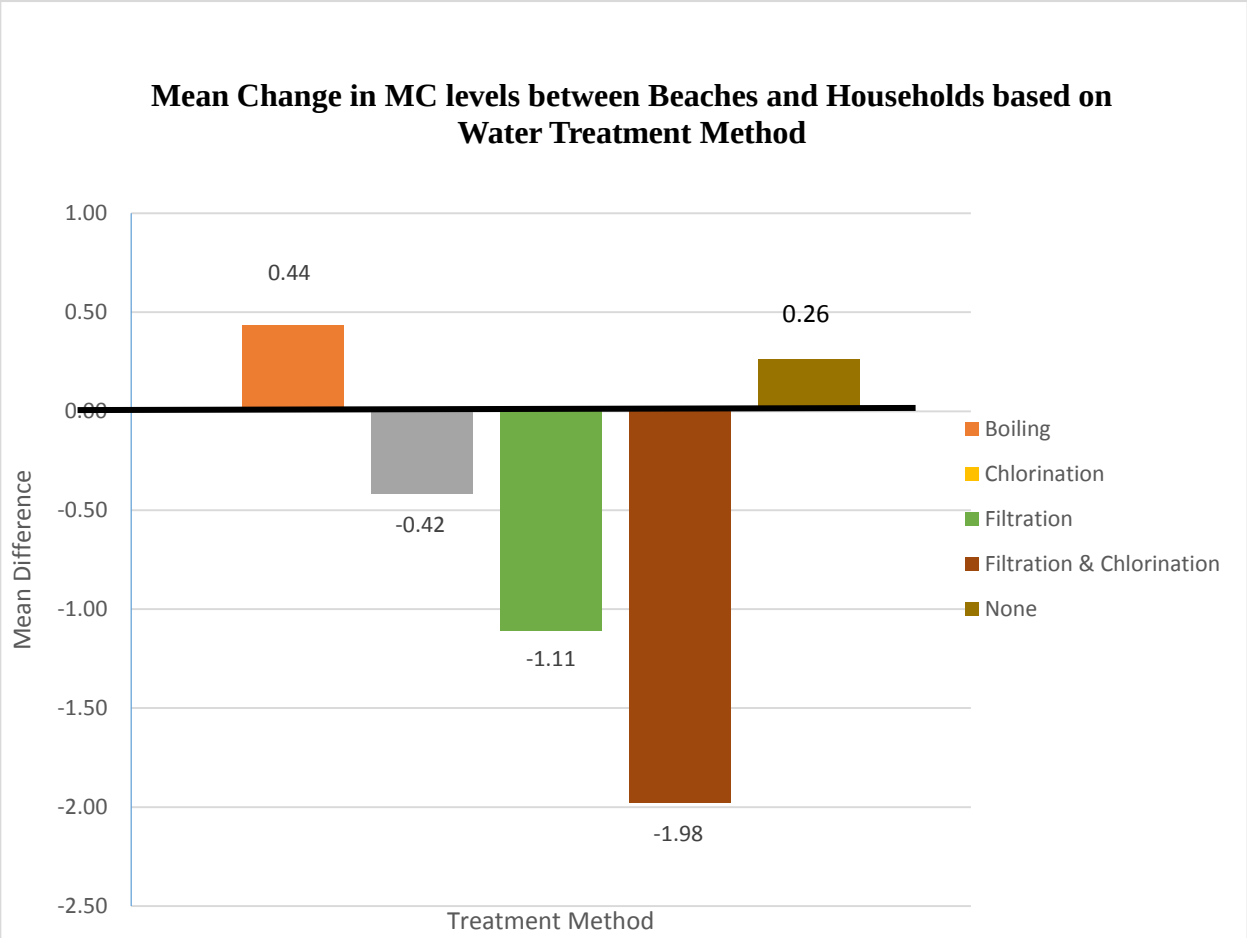

Figure 4.10: Mean Change in MC levels between Beaches and Households based on Water Treatment Method

An analysis of the mean change in the level of MC between beaches and households with regards to water treatment method gave the following results: Boiling and non-use of any treatment

method resulted into an increase in the level of MC concentration while chlorination and filtration led to the decrease in the level of MC concentration.

CHAPTER FIVE

DISCUSSION

5.1 Determination of concentration of cyanotoxins in both Lake Victoria water and household drinking water

The current study observed toxic cyanobacteria in the Nyanza Gulf. Microcystins which are cyanotoxins were present in samples collected from the beach as well as from the household samples. It has been observed that cyanobacteria occur in surface waters whose nutrient loads favor their proliferation. The Chlorophyll-a levels observed indicated the waters to be generally eutrophic which similar studies found out. One such study by Sitoki *et al.* (2012) that was conducted in Kenya in the Nyanza Gulf found out evidence of eutrophication which results in increased macro-nutrient concentration. As a result, there was enhanced cyanobacterial growth especially the Microcystis species which contributed to over 70% of cyanobacterial bio volume (Sitoki *et al*., 2012). The levels of cyanotoxins recorded in this study were over the WHO limit of 1µg/L which is similar to what two other previous studies recorded (Kotut *et al.,* 2006; Sitoki *et al*., 2012) Nutrient increase in the Nyanza Gulf has been attributed to nutrient input from agricultural and urban areas in the catchment areas and has been enhanced by heavy rainfall (Hecky *et al*., 2010; Gikuma-Njuru, 2008). River Kisat near Kisumu which carries sewage effluent from the Kisumu town empties into the Nyanza Gulf could be a major source of nutrient loading into the lake as it is not ascertained the effluent released into the lake is devoid of nutrients. This can be linked to the high levels of cyanotoxins that were recorded in the beaches within Kisumu.

In this study, the highest MC concentrations were recorded in May and October coinciding with the wet season and subsequent nutrient enrichment. Typically, the climate at the Nyanza Gulf has

two wet seasons which are between March and May and between October and December. The study conducted by Sitoki *et al*. (2012) recorded the highest MC concentrations in between November and March. From these, it is seen that cyanobacteria which produce microcystins grow abundantly during months that have high levels of rainfall coupled with the warm temperature typical of the lake basin. This has been attributed to the nutrient loading coming from water from the surrounding agricultural regions and the residential areas. Water coming from the agricultural regions will often sweep through farmlands carrying with it nutrients such as phosphorus and nitrogen which end up in the lake.

Findings from this study indicate that beaches around Kisumu Bay area, that is Ogal and Mawembe beaches, recorded the highest concentrations of Microcystins compared to the two beaches near Homabay and the two in Mbita. This might be due to the level of eutrophication in the three regions, the level of eutrophication being higher in Kisumu area than in the Homabay or the Mbita. Kisumu is more populated and wastes from the residential areas are likely to find its way into the lake from surface run-off which then flows into the lake. River Kisat which carries the sewage water from Kisumu has its mouth in Lake Victoria at Kisumu. As a result, the nutrient rich waters promote eutrophication in the lake. These findings are similar to what the study by Sitoki *et al.* (2012) conducted whereby the MC concentrations were highest near Kisumu Bay area and lowest at the Rusinga Channel. In the study of Microcystins in the Nyanza Gulf, Simiyu, *et al.* (2018) was only able to determine the presence of MC in water samples from Kisumu but none in Rusinga Channel. However, the fish samples from the same waters of Rusinga channel yielded positive results for MC. For the two beaches that were located near Rusinga Channel, the study by Simiyu, *et al.* (2018) was able to detect presence of MC albeit relatively low compared with water samples from the other beaches. Although in this study MCs were detected in the waters in Mbita which is close to Rusinga Channel, the levels were

comparatively lower than the levels recorded in Kisumu and Homabay. The little or lack of MC in water in the Rusinga channel/Mbita can be attributed to the mixing and dilution of the water with that of the open waters of Lake Victoria thus lowering the concentrations of cyanobacteria and cyanotoxins by extension.

This study also found out that the household MC concentration was slightly lower than the corresponding beach MC concentration. This can be attributed to the various water treatment procedures carried out in the households as 77% of the respondents carried out come form of treatment to water. However, the difference is very slight (0.31µg/L) and this might points to low effectiveness of the treatment methods.

5.2 Identification of cyanotoxins in Lake Victoria and household drinking water

In surface waters there are about seven genera of toxic cyanobacteria which are most likely to be encountered. Of these, Microcystin spp. have been reported to be the most abundant (Chorus & Bartram, 1999). In a study carried out in German freshwaters, MC-RR, MC -YR and MC-LR were the main toxin constituents in the Microcystis spp. (Fastner *et. al*, 1999). From a study done, where sixteen microcystins were isolated and purified by HPLC, MC-RR was found to be the most frequent in these samples as the main toxin (Luukkainen *et al.,* 1994). In a study done by Ame *et al.* (2010) to evaluate the presence of four common Microcystin (MC-RR, MC–YR, MC-LR and MC-LA) in water samples and tissues of fish collected from a lake, MC-RR was the most dominant variant in the water samples followed by MC-LA and MC-LR. MC-YR had the lowest concentrations of the congeners measured. Findings from this study similarly found MC-RR to be the most dominant, followed by MC-YR while the least abundant was MC-LR.

In a study done to compare the toxicity of the three strains to zooplanktons, MC-RR was found to be the most toxic strain tested. This was based on LC_{50} values. Furthermore, the study found

out that in some individuals, lower concentrations of this microcystin (MC-RR) are much more toxic than the other two microcystins (MC-YR and MC-LR). The high toxicity was attributed to the presence of dehydrobutyrine residue as the reason for the higher toxicity of Microcystin RR as compared to the other microcystins which contain N-methyldehydroalanine. However, a similar study done where toxicity of the three strains was evaluated in mice, MC-LR was found to be the most potent followed by MC-YR and MC-RR (Gupta *et al*., 2003).

Due to this observation, determining the structure of microcystin is essential as it has consequences for assessment of risk of the microcystin. There is no much knowledge of the possible synergistic, potentiation, antagonistic or additive effects of exposure to multiple variants of cyanobacterial toxins or about interactions between the toxins and other stressors.

5.3 Determination the health risk of cyanotoxins in both Lake Victoria water and household drinking water

The TDI is the amount of a potentially harmful substance that can be consumed daily over a lifetime with negligible risk of adverse health effects. This study found that the daily intake of microcystins in the Nyanza Gulf is way above the recommended for drinking water by WHO. The TDI was four times higher and the risk factor four. High levels of risk to human health are linked to the ingestion of large cyanotoxin quantities from water or the intake of small doses during extended chronic exposure (Svirčev *et al.*,2010). Therefore, observing that this found the levels of cyanotoxins in household drinking water way above what is recommended (2.75µg/L), this could be posing a health risk to the consumers. Given that this study only focused on drinking water, the daily intake of microcystins could be higher if other sources of microcystin exposure are factored in. For example, in a study conducted by Soares *et al*. (2004), microcystins accumulate in the liver, muscle and tissues of tilapia and can be subsequently passed to

consumers. If these two sources of ingestion of water are combined, especially given that tilapia is a common delicacy in the Nyanza Gulf, it is very likely that microcystins are consumed way more than the TDI recommended in the Nyanza Gulf. Chronic exposures to cyanobacteria and their toxins have been associated with increased occurrence of liver and colorectal cancer (Yu, 1995; Zhou *et al.*, 2002; Svircev *et al.*, 2009). This could be a potential health risk the consumers of the lake water are exposed to due to the levels of cyanotoxins recorded from the samples.

According to a survey conducted on the microcystin exposure risk from lakes in Uganda by Poste *et al*. in 2011, it was shown that more than 50% of the WHO lifetime tolerable daily intake results from consuming untreated drinking water. They recommended strategies of dealing with microcystins from the lake water used for drinking to involve regular monitoring of cell numbers of toxic cyanobacteria in the raw water. Such methods include removal of particles by flocculation and ozonation followed by activated carbon filtration or sand filtration to remove dissolved microcystins (Chorus &Bartam, 1999).

Water treatment procedures for cyanotoxins should incorporate removal of both soluble and suspended substances. Since cyanotoxins are produced within the cyanobacterial cells, removal measures should involve destruction or avoidance of the cells. Since cyanotoxins are soluble in water, procedures to reduce their toxicity or remove the toxins completely from water are essential.

Chlorination is not very effective in destroying cyanobacteria. Although there was a decrease in cyanobacteria concentrations in households where chlorination was used to treat water, the difference is slight. The efficiency of chlorination depends mainly on the chloride compounds used as well as the concentration used. Aqueous chlorine and calcium hypochlorite at greater or equal to 1 mg/L remove more than 95% of microcystins or nodularin, while sodium hypochlorite

at the same dose or chloramine achieve 40-80% removal at most (Hizfield *et al*,.2000). A chlorine residual of at least 0.5 mg/L should be present after 30 min contact time in order to destroy cyclic peptides completely. Cyanotoxins occur in two modifications: cell bound and dissolved in water. Filtration is slightly effective in removing cyanobacterial cells but dissolves toxins remain in the drinking water. Cyanotoxins occur in two modifications: cell bound and dissolved in water. Filtration is slightly effective in removing cells but dissolves toxins remain in the drinking water.

This study therefore identified a need for water treatment methods for removal of cyanotoxins. Given that a population of 94 000 inhabitants of the Nyanza Gulf depend on the lake water for drinking, development of methods that will remove cyanobacterial cells as well as get rid of cyanotoxins in the water is paramount. Although adverse health effects have not been documented from the region, were cannot rule out any effects of drinking water contaminated by cyanotoxins. In other countries where cyanobacteria and cyanotoxins occurrence in water is much studied and effects documented, chronic exposure to cyanotoxins has led to liver and neurological diseases.

Therefore this study has added knowledge about the occurrence of cyanotoxins in the Nyanza Gulf waters. Through the comparison of the beach water and household water which showed minimal difference, ways of increasing access to safe drinking water need to be developed.

5.4 Summary

In summary, findings of this study have supported that there is eutrophication in the Nyanza Gulf. All beaches sampled were found eutrophic. As a result, cyanobacteria blooms have flourished, releasing toxins in the water such as Microcystins. Cyanobacteria occur mostly in May and October, coinciding with the wet season. The microcystins are more abundant in the

more eutrophicated waters of Kisumu Bay and Homabay and less abundant in the less eutrophicated and clear waters of Rusinga Channel/Mbita. 84% of water samples contained cyanotoxins. On average the cyanotoxin levels was 3.44µg/L. There was significant variation in Microcystin levels between different beaches and different months (ANOVA: $F=12.09$, $p<0.0005$) and no variation between beaches and water treatment (ANOVA: $F=0.97$, $p=0.47$).The three types of MC investigated (MC-RR, MC-YR, MC-LR) were present in the Nyanza Gulf. MC-RR was the most common followed by MC-YR. MC-LR had the least concentration levels. The water is posing a health risk to the residents who used it for drinking and cooking. The microcystin levels are over and above the set guideline by the WHO. The TDI is three times higher than the value recommended. The methods used for water treatment have not been effective in removing the toxic cyanobacteria. Chronic exposure to low concentrations of microcystins in drinking water is a serious problem to public health in the Nyanza gulf and may contribute to promotion of cancer in humans.

5.5 Conclusions

1 Eutrophication was observed in the Nyanza gulf resulting in flourishing of cyanobacteria which release cyanotoxins in the lake water especially during the wet seasons.

2 MC-RR is the most abundant cyanotoxins followed by MC-YR and MC-LR is the least abundant in the Nyanza Gulf.

3 There is a health risk posed by cyanotoxins to the residents of the Nyanza gulf who use the lake water for drinking.

5.6 Recommendations from the Current Study

1. Nutrient loading and eutrophication should be checked in the Nyanza Gulf. The county governments need to create forums where local residents are made aware of the impact

of activities such as farming and waste management have on the ecosystem especially the lake. This can be done through promotion of responsible use of fertilizers and farming practices that prevent nutrients from being carried away. The county governments along Lake Victoria which should also ensure proper disposal of waste and sewage to reduce on the nutrient enrichment that comes from sewage effluent. Regular monitoring of the cell numbers of toxigenic cyanobacteria in the raw water should also be done.

2. Ways of getting rid of the cyanotoxins identified need to be developed. This should include the removal of both intracellular and extracellular toxins. Filtration reduces presence of cyanotoxins by a huge margin if done with medium with small pore size. The riparian communities should be educated on carrying out filtration to remove cyanobacterial cells before boiling so as to reduce the availability of cyanotoxins in the water collected for drinking.

3. There should be sensitization of the riparian communities about the health risk that comes with consuming water contaminated with cyanobacteria and cyanotoxins. The county governments should carry out advocacy sessions in the riparian communities regarding the health risk of cyanotoxins from drinking water directly from the lake.

5.7 Recommendations for Further Studies

1. Further research needs to be carried out to find out the possible presence of synergistic, potentiation, antagonistic or additive effects of the three strains of microcystins.

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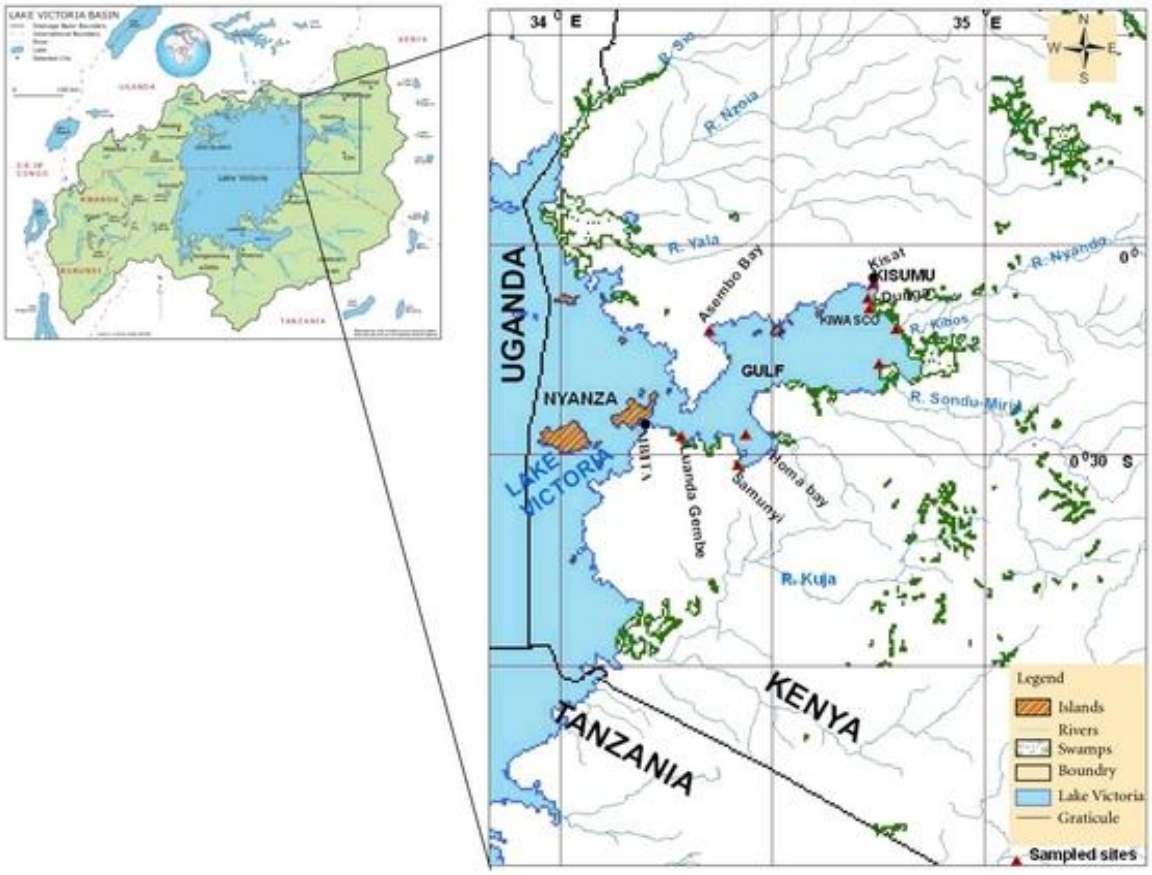
APPENDICES

Appendix I: Map of Lake Victoria Basin showing the Nyanza Gulf (Study Area)



Map of the Nyanza Gulf, Lake Victoria adapted from [*http://www.hindawi.com/journals/tswj/2012/106429/fig1/*](http://www.hindawi.com/journals/tswj/2012/106429/fig1/)

Appendix II: Letter of Introduction

Lilian Kwamboka Otoigo,

Maseno University,

P.O Box Private Bag,

Kisumu.

The Chief,

.....Location,

Kisumu/Homabay/Mbita/Siaya.

Dear sir/Madam,

Research Collaboration Request

I am Lilian Kwamboka Otoigo, a student of Master in Public Health, Maseno University - Kisumu City campus. I am conducting a research household drinking water quality among users of Lake Victoria water. I will carry my study on beach villages along Lake Victoria and your location is part of the area.

Since this research will be carried out in your area of jurisdiction, I kindly request for your permission to carry out. It will involve visiting households and obtaining water samples for analysis in the laboratory.

Any assistance from your office to assist me achieve the purpose of this research is highly appreciated.

Yours faithfully,

Lilian Kwamboka Otoigo

Appendix III: Study Participant Consent

INFORMED CONSENT FORM

Hello,

My name is …… I am a Research Assistant for a postgraduate student at Maseno University called Lilian Kwamboka Otoigo. I am conducting a research on **Assessment of health risk of toxic cyanobacteria in drinking water in the Nyanza Gulf water, Lake Victoria, Kenya**. The purpose of this research is to establish if the water drawn from the Nyanza Gulf of Lake Victoria could be posing any health risks in terms of cyanobacteria to the communities that use it for drinking. To do this, we will be asking the primary caretaker in the households of this village about water collection, usage and storage in the household. This is because this village is near one beach along the Nyanza Gulf.

I would like you to participate in this study. If you agree to be in this study you will respond to a questionnaire and your participation will take about 30 minutes. However, please note the following:

- Participation in this research may involve personal information but your records will be handled as confidentially as possible. No names will be used in any report from this study.
- There will be no direct benefit to you from participating in this study. However, your participation and the research findings will provide information on the safety of the Lake Victoria water for drinking in regards to cyanobacteria.
- A risk associated with participation in this study is the possibility that others other than the researcher may find out your responses to the survey questions are, thus loss of confidentiality. To protect this, no names will be recorded but only codes to identify the village and household.
- The findings of this research will be shared with you and your community through a community meeting before they are made widely available to the public.
- Participation in this research is voluntary. You are free to decline to be in this study, or to withdraw at any point. Your decision as to whether or not to participate in this study will have no influence on your normal activities.

I have read the consent form and/or explained to, describing the nature of the study and the benefits. I have had a chance to ask all questions regarding this study. I voluntarily agree to participate.

Date …….. Signature of the Participant ……..……...

Date ……...Signature of the Person obtaining Consent……..……

In case of any questions or clarifications the research assistant will help you. If you have further questions, feel free to contact the following:

1. Principal Investigator, Lilian Kwamboka Otoigo (School of Public Health and Community Development, Maseno University, Mobile**0722573744**)

2. The Secretary, Maseno University Ethics Review Committee (MUERC), Maseno University Main Campus, P. O. Box, Private Bag, Maseno, Kenya. Telephone Numbers: **+ 254 57 351 622 EXT. 3050**, Email address: muercsecretariate@maseno.ac.ke

Thank you**.**

Lilian Kwamboka Otoigo (Principal Investigator)

Appendix IV: Study Questionnaire

QUESTIONNAIRE

My name is Lilian Kwamboka Otoigo. I am a student from Maseno University and I am carrying out a research on the quality of water in Lake Victoria. I will appreciate your participation in this survey.

The information that you will provide will help the government and other stakeholders to plan, implement, monitor and evaluate programs on the quality of Lake Victoria water.

(Kindly request the respondent to answer this questionnaire while you indicate his/her honest response either by ticking his/her option or by filling in the blanks giving as many details as possible.)

- Where do you get your household water from?

1. Lake Victoria

2. Other (excluded from study)

(Research Assistant to obtain informed consent before proceeding to the next part.)

SECTION A: BACKGROUND INFORMATION (RA to give description)

i) Village …….

ii)Questionnaire no…….

SECTION B: SOCIO-DEMOGRAPHIC INFORMATION

i. Gender (not to be asked, to be identified by research asst.)

1. Male

2. Female

ii. What is your marital status?

1. Single

2. Married/Cohabit

3. Divorced/Widowed

iii. What is your religion?

1.Christian

2. Muslim

3. Others (e.g none, budhist, hindu, African traditional religion)

iv. What the occupation of the household head?

1.Fisherman

2.Businessman

3. Farmer

4. Employed (government officers, NGOs)

5.Others. Please specify …….

v. How many members are there in this household?.....

vi. How old are the household members? (Insert answer in the table below against each member)

vii. What is their level of education? (Insert answer in the table below against each member)

1. No formal education

2. Primary incomplete

3. Primary complete

4. Secondary incomplete

5. Secondary complete

6. Tertiary

(Research assistant to indicate who respondent is by ticking against the name).

Household members (No names included)	Age	Level of Education
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

SECTION C: WATER USE AND TREATMENT

i. Do you use the lake water for cooking?

1. Yes

2. No

ii. Do you use the lake water for drinking?

1. Yes (go to question iii,)

2. No (go to question vi)

ii. If you use Lake water for drinking, do you treat it?

1. Yes (go to question iv,)

2. No (go to question v)

iv. If you treat, how do you treat it?

1. Filtration

2. Boiling

3. Filtration and boiling

4. Chlorination (Waterguard, Aquaguard, Pur etc.)

5. Others. Please specify …….

v. How do you store the drinking water?

1. Plastic containers

2. Glass containers

3. Metallic containers

4. Earthenware containers

5. Others. Please specify …….

vi. Approximately how many cups of water do you (respondent) drink in a day?

1. 1-3

2. 4-6

3. 7-9

4. 10 or more

5. None

vii. Approximately how many cups of tea or coffee do you (respondent) drink in a day?

1. None

2. One cup

3. Two or three cups

4. Four or five cups

5. Six cups or more

viii. How long do you store lake water? (I will do a pretest and modify answer as appropriate)

1. One day or less

2. Two to four days.

3. Five or six days

4. One week or over one week

We have come to the end of this discussion.

Thank you once more for participating in this survey.

Appendix V: Research Approvals

MASENO UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: PG/MPH/00067/2012

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 06th March, 2015

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR LILIAN KWAMBOKA OTOIGO— PG/MPH/00067/2012

The above named is registered in the Master of Public Health Programme of the School of Public Health & Community Development, Maseno University. This is to confirm that her research proposal titled "An Assessment of Health Risk of Toxic Cyanobacteria in Drinking Water in the Nyanza Gulf Water, Lake Victoria Kenya" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

MASENO UNIVERSITY
DIRECTOR
06 MAR 2015
SCHOOL OF GRADUATE STUDIES

Prof. P.O. Owuor
DEAN, SCHOOL OF GRADUATE STUDIES

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221
Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 9th June 2015

TO: Lilian Kwamboka Otoigo
PG/MPH/00067/2012
Department of Public Health
School of Public Health and Community Development
P. O. Box Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/000158/15

RE: An Assessment of Health Risks of Toxic Cyanobacteria in Drinking Water in the Nyanza Gulf Water, Lake Victoria. Proposal Reference No: MSU/DRPI/MUERC/ 000158/15

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 9th day of June, 2015 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 8th June, 2016. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 6th May, 2016.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 6th May, 2016.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

Yours faithfully,

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee.

DIRECTORATE OF RESEARCH PUBLICATION & CONSULTANCIES 09 JUN 2015 MASENO UNIVERSITY

Cc: Chairman,
Maseno University Ethics Review Committee.