



Article title: Identification and quantification of Cyanotoxins in Lake and household water around Lake Victoria, Kenya

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Identification and quantification of Cyanotoxins in Lake and household water around Lake Victoria, Kenya

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Abstract

Cyanobacteria are single-celled algae that thrive in warm and nutrient rich water bodies. They can produce different kinds of toxins called cyanotoxins that can affect the liver. Cyanobacteria have been reported in Lake Victoria, which is an important source of drinking water for the riparian communities. This is as a result of eutrophication in Lake Victoria increasing the levels of cyanobacteria in the Nyanza Gulf. The purpose of this study was to identify and quantify microcystins in household and Lake Victoria water in Nyanza Gulf. In a longitudinal study adopting experimental design, six beaches were studied and 127 samples 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. 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. 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). This information provides an insight into the quality of Lake Victoria water for drinking based on quantities of microcystins in the sampled water. The study recommends regular monitoring of cyanobacterial cells in the lake water.

Keywords: Cyanobacteria, Cyanotoxins, Microcystins, PP2A, HPLC, Eutrophication

Introduction

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). Many genera of cyanobacteria are known to produce toxins, which are referred to as cyanotoxins. Microcystins are hepatotoxins commonly produced by the cyanobacteria genera *Anabaena*, *Microcystis*, *Oscillatoria*, *Planktothrix*, *Nostoc*, and *Hapalosiphon* (Szląg *et al.*, 2015). 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).

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). 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. It was therefore important to establish the presence and concentration of cyanotoxins in both Lake Victoria water and household drinking water so as to assess the potential health risks associated with cyanotoxins in drinking water. With the knowledge developed from this study and other similar ones, there will be a need to develop effective treatment procedures to remove these toxins in water. The main aim of this study was to identify and quantify the cyanotoxins in Lake Victoria and household drinking water in the Nyanza Gulf. This information will also form a basis of advising county governments and the local residents on how to treat lake water before household use.

Materials and Methods

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. It has an area of 1400 km², 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).

Samples were collected from six beaches along the Nyanza Gulf of Lake Victoria: Ogal Beach (0°9'S, 34°35'E), Mawembe (0°9'S, 34°37'E), Rang'ombe (0°25'S, 34°28'E), Alum

(0°26'S, 34°28'E), Olambwe (0°26'S, 34°15'E) and Kolunga (0°25'S, 34°8'E). Water was also collected from households along the beaches.

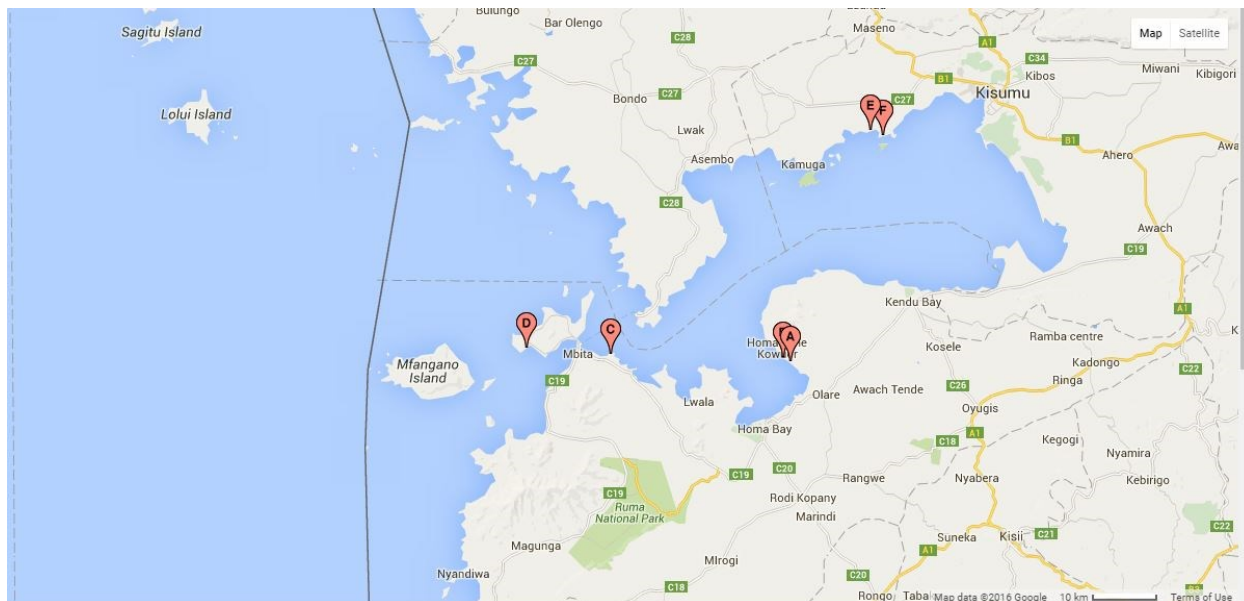


Figure 1: Map of the Nyanza Gulf, Lake Victoria with sampling points

Sample collection and Analysis

Experimental methods were adopted to identify and determine levels of microcystins in both lake and household drinking waters. Water samples for analysis of microcystins in the Lake Victoria water were collected once a month for a period of 6 months beginning May to October 2015 from the exact point where the riparian communities draw water from Lake Victoria. 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.

PP2A

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 was boiled for an hour then centrifuged for 15 minutes at 3000rpm. 10 μ l of the supernatant was transferred to a 96-well microplate. 10 μ l of enzyme dilution (PP2A enzyme in Bovine Serum Albumin {BSA}, 0.1 M Tris, 40mM Dithiothreitol {DTT}, 10mM MnCl₂, 0.3M MgCl₂ and MilliQ water) was added to the samples, shaken and incubated for 5 minutes at 37°C. 100 μ l of reaction mixture (containing 4-nitrophenyl phosphate disodium salt {Sigma Aldrich}, BSA, 0.4M Tris, 10mM MnCl₂, 0.3 MgCl₂ and MilliQ water) was added to the wells containing samples. The microplate was then incubated for 60 minutes at 37°C. Absorbance was measured at 405nm by a plate reader and the results analysed in a Microsoft Excel sheet. Standards of 10 μ l, 5 μ l, 2.5 μ l, 1.25 μ l, 0.625 μ l, 0.3125 μ l and 0.15625 μ l of MC-LR (from Sigma Aldrich) were used. For higher concentrations, serial dilutions of the sample were carried out, analyzed and the results extrapolated by the dilution factor.

HPLC

Microcystin characterization was done using High Performance Liquid Chromatography (Lawton *et al.*, 1994; Heresztyn & Nicholson, 2001). 100ml of sample water was put in a boiling water bath for 1 hour then vacuum filtered using 0.45 μ m Whatman GF/C microfiber filters. The filtrate was then passed through a pre-treated Strata-X 33 μ m 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 repeated twice before the sample was allowed to pass through at the same flow-rate. The cartridge was then washed two times using 5ml of 20% methanol and 15 ml of Milli-Q water. 3ml of 100% methanol was used to re-dissolve adsorbed microcystin into a test-tube then methanol was then evaporated

by heating up to 40°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

Results

The results revealed that in the six beaches that were sampled, the three strains of Microcystin (MC-LR, MC-RR and MC-YR) were present in different quantities as detailed here. The concentrations of MC-RR was highest at 94.37 µg/L followed by MC-LR at 10.16 µg/L and MC YR lowest at 18.16 µg/L. 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 Ogal and Mawembe. However in the other four beaches (Alum, Rang'ombe, Kolunga and Olambwe) the concentration of MC-RR was the lowest while MC-LR was highest followed by MC-YR. Kolunga beach had very low concentrations of the three MC strains as shown in the Figure 2 and Figure 3.

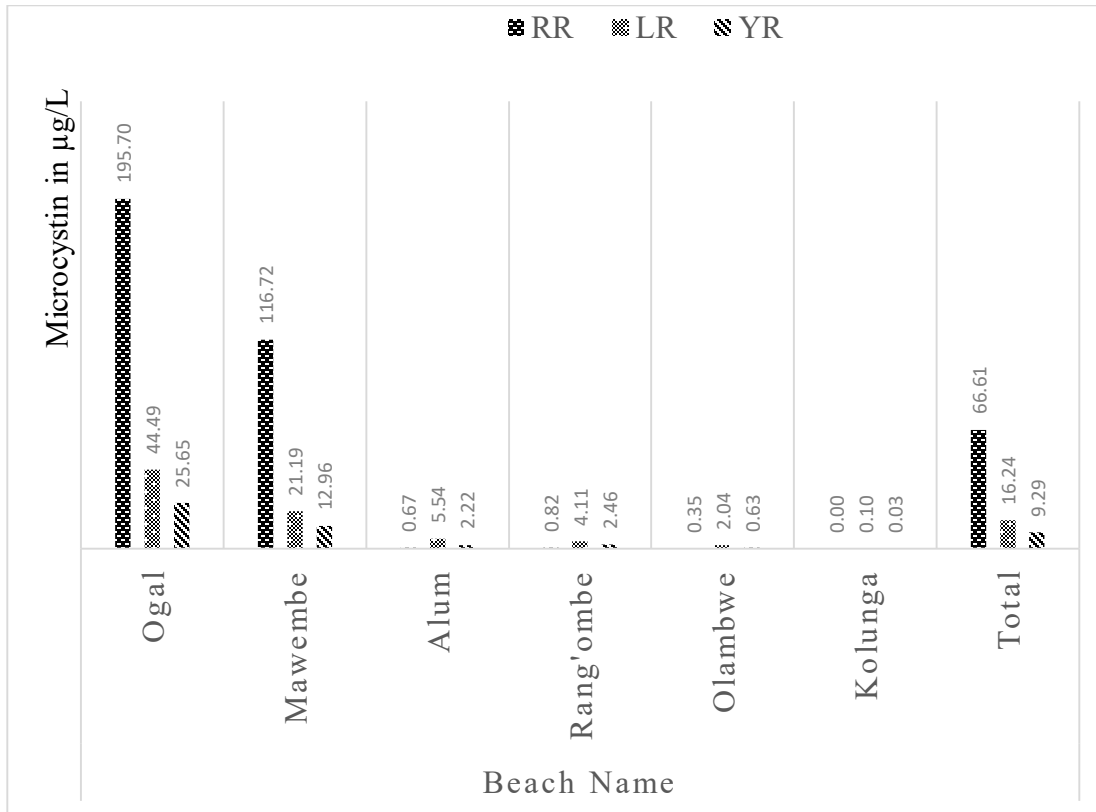
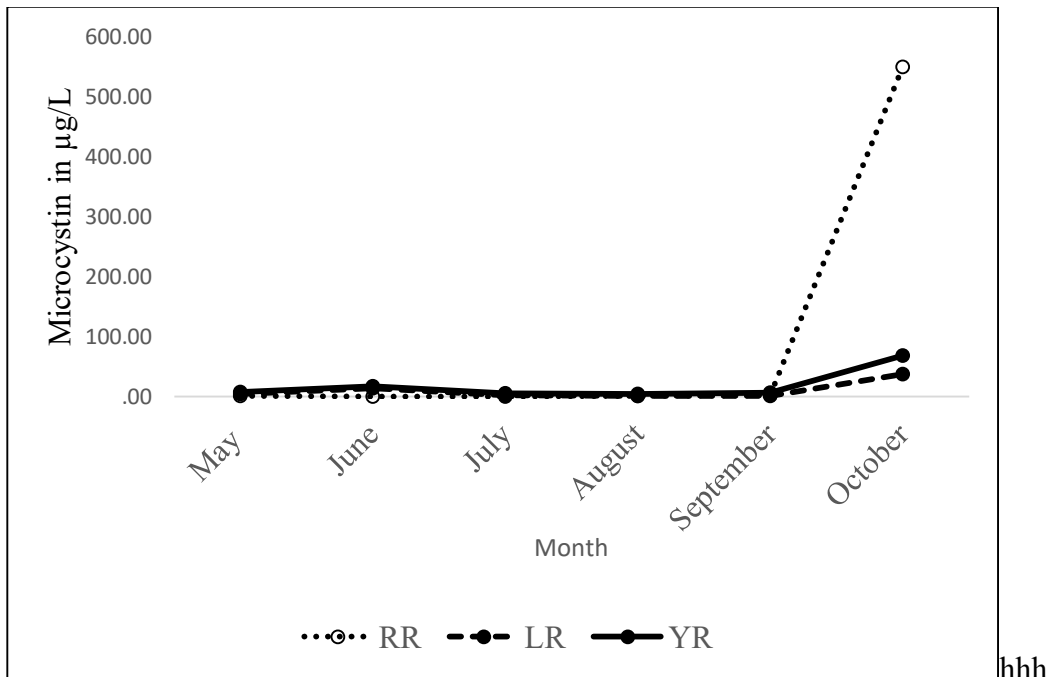


Figure 2: Microcystin stains present in every beach (HPLC)



hhh

Figure 3: Monthly average concentration for all beaches (HPLC)

Out of the 127 samples collected from households, 103 (80%) samples were positive for presence of microcystins. For a similar number of beach samples, 112 (88%) samples had microcystins. In total, 215 (84%) of samples contained microcystins. There was a general trend in the level of microcystins in households being lower those of the respective beaches as shown in

Figure 4.

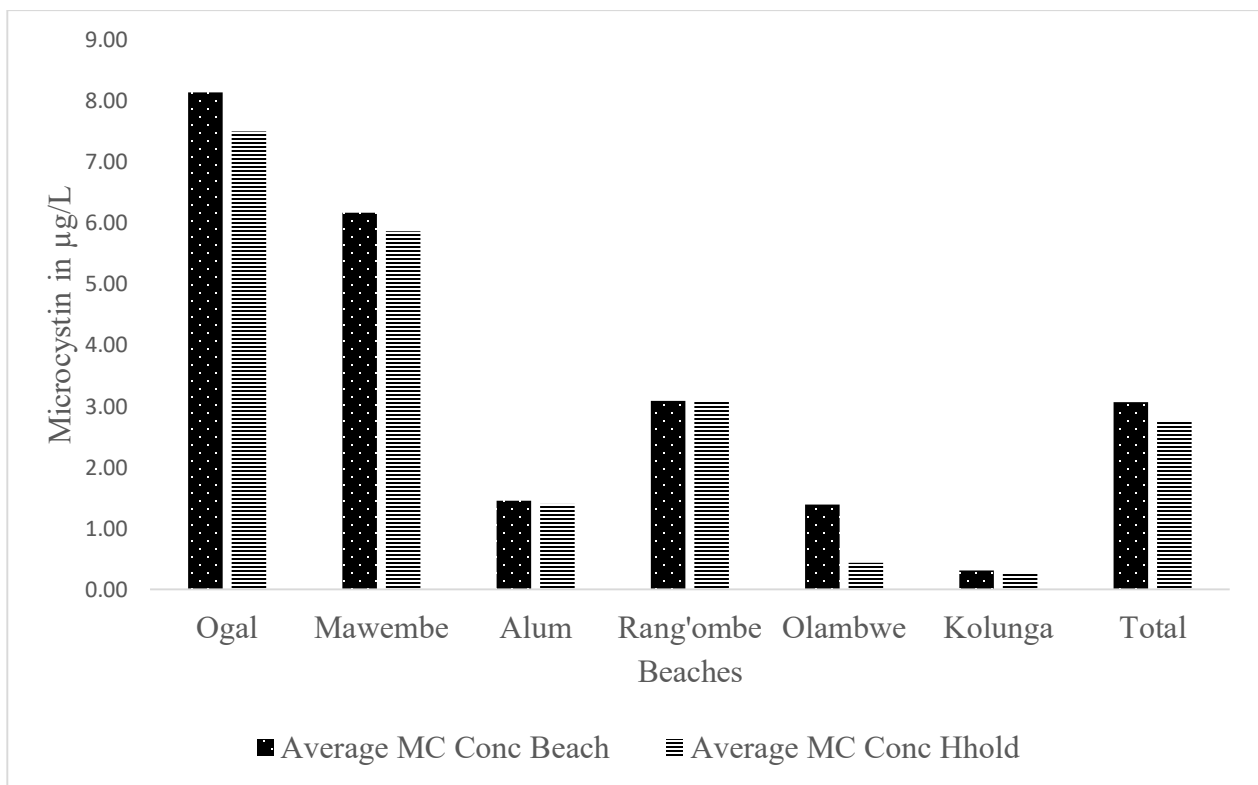


Figure 4: Average concentration of microcystins per beach for May to October 2015

When the average concentration across all the beaches was computed, the concentration of microcystins 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 $\mu\text{g/L}$ in September and rising again in October to 2.53 $\mu\text{g/L}$. This is shown in Figure 5

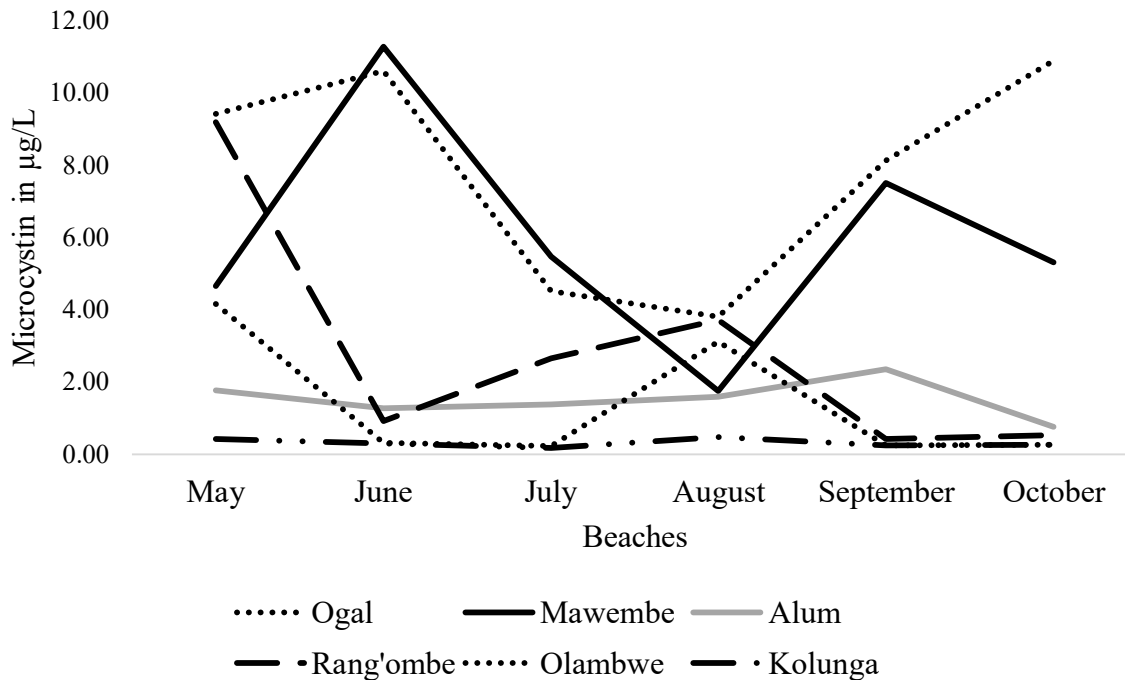


Figure 5: Temporal change in microcystin per beach between May to October 2015

Discussion

This study observed the presence of three microcystin variants in the water samples collected from households and beaches along Lake Victoria. Findings from this study found MC-RR to be the most dominant, followed by MC-YR while the least abundant was MC-LR. 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. (Fastener *et. al*, 1999). From another 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 from Los Padres Lake (Argentina), 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. This is in agreement with findings of the current study as in both, MC-RR was the most dominant strain. 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.

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. A study by Sitoki *et al.* (2012) conducted 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 1 µg/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, 2005). River Kisat near Kisumu which carries sewage effluent from the Kisumu town empties into the Nyanza Gulf and 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 microcystin 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 microcystin 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 their way into the lake from surface run-off which then flows into the lake. These findings are similar to what the study by Sitoki *et al.* (2012) conducted whereby the microcystin 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 microcystin in water samples from Kisumu but none in Rusinga Channel. The little or lack of microcystin 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 microcystin concentration was slightly lower than the corresponding beach microcystin concentration. This can be attributed to the various water treatment procedures believed to be carried out in the households. However, the difference is very slight (0.31µg/L) and this might point to low effectiveness of the treatment methods in use.

Conclusion and Recommendation

Cyanotoxins were present in the Nyanza Gulf as a result of flourishing of cyanobacteria which release cyanotoxins in the lake water especially during the wet seasons. Three strains of Microcystin (MC-RR, MC-LR and MC-YR) were confirmed to be present in both household and lake water in the Nyanza Gulf. MC-RR was found to be the most abundant cyanotoxin followed by MC-YR and MC-LR is the least abundant in the Nyanza Gulf.

Nutrient loading and eutrophication should be checked in the Nyanza Gulf. Regular monitoring of the cell numbers of toxigenic cyanobacteria in the raw water should also be done. Ways of getting rid of the cyanotoxins identified need to be developed which should include the removal of both intracellular and extracellular toxins.

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