

Microcystins contamination of surface water supply sources in Zaria-Nigeria

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Abstract Cyanobacterial contamination of public water supply systems is a worldwide problem. The present study investigated water quality and microcystins (MCs) contamination of four public water supply systems in Zaria, Nigeria. The water bodies were eutrophic in the rainy and dry season and supported high phytoplankton biomass with chlorophyll a concentrations generally higher than 20.0 μg/L. The biomass of the predominant species (Microcystis aeruginosa and Anabaena subcylindrica) of cyanobacteria had a significant positive correlation with particulate and dissolved MCs. Dissolved MCs concentrations were higher $(>1.0 \mu g/L)$ than the maximum permissible limits for drinking water in all the water bodies in the dry season and three of them in the rainy season. These results suggest that there is the need to have a regular monitoring program for these water bodies to prevent acute and chronic health hazards associated with MCs contamination of drinking and irrigation water.

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Introduction

In most tropical semiarid regions of the world, reservoirs and lakes are an integral part of societal development. The lentic systems in these regions usually have large spatial and temporal variations of physical and chemical conditions. Nutrient enrichment of these aquatic ecosystems results in the formation of cyanobacterial blooms (Xu et al. [2011\)](#page-11-0). These blooms cause serious environmental problems because of the toxic compounds cyanobacteria produce (Chen et al. [2009;](#page-9-0) Nonga et al. [2011\)](#page-10-0). The most common and studied cyanotoxins are the monocyclic heptapeptides called microcystins (MCs), which are produced through nonribosomal peptides synthases (NRPS) (Agha et al. [2013\)](#page-9-0). To date, over 80 variants of MCs have been reported, and the most commonly encountered being the MC-LR variant (World Health Organization WHO [2004](#page-11-0); Falconer [2005](#page-10-0)).

MCs are very important because of the acute and chronic effects they have on humans such as hepatocyte necrosis and pooling of blood to cause liver failure, interference with intracellular regulatory processes, and signal transduction, which may subsequently lead to death (Mackintosh et al. [1990](#page-10-0); Jochimsem et al. Jochimsen et al. [1998;](#page-10-0) Carmichael et al. [2001;](#page-9-0) Chen et al. [2009\)](#page-9-0). For chronic exposure, the World Health Organization (WHO) recommends a limit of 1 μg/L to avoid public health risks of MCs (WHO World Health

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Organization WHO [1998](#page-11-0)). Limited evidence from some studies report a correlation between liver and colorectal cancers and cyanobacteria contamination of drinking water in China (Ueno et al. [1996](#page-10-0); Ito et al. [1997](#page-10-0); Humpage et al. [2000](#page-10-0)). Animal intoxication has been frequently reported in Southern and Eastern Africa (Steyn [1945](#page-10-0); Krienitz et al. [2003;](#page-10-0) Nonga et al. [2011\)](#page-10-0).

Investigations from the African, Asian, and American continent show that as much as 90 % of all sampled water bodies contain MCs (Chorus [2001;](#page-10-0) Kotut et al. [2006;](#page-10-0) Chia et al. [2009a](#page-9-0), [b;](#page-9-0) Xu et al. [2011](#page-11-0); Bittencourt-Oliveira et al. [2014;](#page-9-0) Jancula et al. [2014\)](#page-10-0). In Nigeria, the situation is further complicated by landuse patterns surrounding these aquatic ecosystems, including intensive agricultural activities and direct discharge of raw domestic and industrial wastes into them (Chia et al. [2009a](#page-9-0), [b\)](#page-9-0). Most developing countries do not have adequate water treatment facilities, which means there is a high probability that humans and wild life are perpetually exposed to chronic cyanotoxins poisoning from rivers, lakes, and reservoirs. In addition, current evidence show that MCs bioaccumulate in living plant tissues (Codd et al. [1999](#page-10-0); Hereman and Bittencourt-Oliveira [2012](#page-10-0)). Therefore, MCs are of both economic and public health concerns to water resource managers, drinking water treatment plant operators, lake association, and local officials responsible for decision making with regards to toxic cyanobacteria.

The study area Zaria has a population of more than 1.2 million inhabitants (NPC National Population Commission – NPC [2006](#page-10-0)). As in other parts of Nigeria, Zaria has a chronic water supply problem due to inadequate and not properly treated potable water. A recent study by Chia et al. ([2013a\)](#page-9-0) revealed cyanobacterial and microalgal contamination of water obtained from aquifers as well as that from the Kubanni Reservoir (aka ABU Dam) in Zaria, Nigeria. The lentic water bodies selected in the present study (ABU Dam, Zaria Dam, Makwaye Lake, and Bomo Lake) are the principal sources of potable and irrigation water in Zaria, Nigeria. Unfortunately, studies have shown that the hygienic quality of drinking water supplied to consumers in this region is unsatisfactory (Dada et al. [1990](#page-10-0); Chigor et al. [2012\)](#page-10-0). This is because of the significantly high bacteriological loads and relatively poor physicochemical characteristics recorded for surface water bodies in Zaria (Ekanem and Irekpita [2004](#page-10-0); Adakole and Abolude [2009](#page-9-0); Abolude et al. [2009](#page-9-0)). However, very little attention has been paid to the presence and

distribution of cyanotoxins in water bodies in Zaria (Chia et al. [2009a,](#page-9-0) [b\)](#page-9-0) and Nigeria as a whole. Therefore, the objective of this study was to determine the extent of surface water contamination with particulate and dissolved MCs, as well as cyanobacterial composition and physicochemical characteristics of the water bodies in both rainy and dry seasons.

Materials and methods

Study area

Zaria is located on longitude 11° 3' N; and latitude 7° 42' E. It is situated in the Northern Guinea Savannah and has a tropical continental climate with distinct rainy and dry seasons. There are four public water supply systems in Zaria, Nigeria viz. Ahmadu Bello University (ABU) Dam (11° 08′ N, 07° 43′ E), Bomo Lake (11°12′ E, 07° 38′ W), Makwaye Lake (11° 12′ N, 07° 36′ E), and Zaria Dam (10° 38' and 7° 42'). ABU dam is the major source of drinking water to the ABU community and Samaru village (Fig. [1](#page-2-0)). The reservoir has a depth of ca. 6 m, water level of ca. 644.81 m, and surface area of 484 ha. It receives high levels of municipal waste from the surrounding university campus and Samaru village. Bomo Lake is situated near Bomo village, lying 6 km North-West of ABU Zaria, with a depth of ca. 2 m and surface area of 9.0 ha, and has evidence of human disturbance. Makwaye Lake is located north of ABU Dam and has a depth of ca. 4 m and surface area of 110 ha. There is a lot of unregulated irrigation farming and animal grazing activities around and in its catchment area. Zaria Dam is situated on River Galma in Sabon Gari and has a water depth of 8.8 m, dam height of 14.9 m, and surface area of 484 ha.

Sample collection

Plankton samples were collected during the dry (March) and rainy (May) seasons of 2013 using a 10-μm mesh phytoplankton net. Twelve fixed sampling points divided into three sampling points per sampling station were selected and maintained per water body throughout the study. Samples were preserved and transported on ice so that the algae will be maintained at low metabolic states until they were properly processed in the laboratory. Subsamples were preserved with Lugol's iodine solution for morphological identification and quantification.

Fig. 1 Map showing the location of different lentic water bodies investigated for cyanobacteria and microcystins contamination in Zaria-Nigeria

Samples for physicochemical parameters were analyzed in situ in the field where possible, while the remainder was kept refrigerated until analyses in the Hydrobiology Laboratory, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

Analysis of physicochemical parameters

Total dissolved solids (TDS), electrical conductivity, temperature, and pH of the water were determined in real-time in the field using the multi-parameter water quality portable Hanna's instrument (model No. H1991300). Alkalinity and water hardness were determined following the procedures described by Lind [\(1979\)](#page-10-0). Dissolved oxygen (DO) and biochemical oxygen demand $(BOD₅)$ were determined using the modified Winkler azide method (Lind [1979;](#page-10-0) APHA American Public Health Association: APHA [1998\)](#page-9-0). The phenoldisulphonic acid method (Mackereth [1963](#page-10-0)) was used for nitrate-nitrogen analysis, while the Denige's method (APHA American Public Health Association: APHA [1998\)](#page-9-0) was employed for phosphate phosphorus and total phosphorus determination.

Biological analysis

Cyanobacteria identification was done using online identification resources coupled with identification keys

provided by Prescott [\(1977](#page-10-0)) and APHA (American Public Health Association: APHA [1998](#page-9-0)). Cell counts were determined microscopically with the aid of an improved bright-lined neubaeur counting chamber.

The extraction and analysis of chlorophyll a concentrations were carried out according to Chia et al. ([2013b,](#page-9-0) [c\)](#page-9-0). The phycocyanin-to-chlorophyll a (PC:Chl- a) ratios of the environmental samples were determined as the ratio between the light absorption by phycocyanin (PC) $(OD_{627 \text{ nm}})$ and the light absorption by the second chlo-rophyll a (Chl-a) peak (OD_{680 nm}) (Briand et al. [2012\)](#page-9-0).

Extraction and quantification of microcystins

The transported samples were filtered using Millipore GF/C filter papers of 1 μm pore size (Millipore, USA). The residues were used for particulate MCs determination, while the filtrates were used for dissolved MCs determination. Cell lysis of particulate (intracellular) MCs was done by re-suspending the biomass residue in double distilled deionized water, freezing and thawing it five times. The samples for MC quantification were preserved in 75 % methanol to avoid adsorptive losses which could lead to errors in analytical procedures. Both intracellular and extracellular MCs concentrations were determined using the Beacon's ELISA tube kit specific to MC (Beacon Analytical Systems Inc. UK). The assay was performed with high protein binding tubes pre-coated with anti-rabbit immunoglobin G (IgG). The kit uses a polyclonal antibody that binds both MCs and a microcystin-enzyme conjugate. The coated tubes were read at 450 nm and corrected with the absorbance at 605 nm within 20 min of stopping the reaction according to the manufacturer's instruction.

Statistical analyses

The data collected were first analyzed using Levene's homogeneity of variance. Where the results were significant and the variance homogenous, the data were subjected to analysis of variance (ANOVA) to determine significant differences between the means of measured parameters per time and sampling station. The Tukey's Post-Hoc test was used to separate significantly different means. The correlation between the physicochemical parameters was established using a correlation based principal components analysis (PCA). Statistical analyses were carried out using Statistica 8.0 for windows. All analyses were done at 5 % significance level.

Results

Physicochemical parameters results are given in Table [1.](#page-4-0) pH was fairly constant (6.93–8.06) throughout the study period. Water temperature ranged from 26.15 to 28.85 °C, where it was the lowest in the dry season and the highest in the rainy season. Total dissolved solids (TDS) varied significantly ($p < 0.05$) between the water bodies. The highest value was recorded in Bomo Lake (87 mg/L) in the rainy season, while the lowest was in Makwaye Lake (43.50 mg/L). In the dry season, the highest TDS was obtained in ABU dam (73.00 mg/L), while the lowest was in Makwaye Lake (41.00 mg/L). Electrical conductivity and water hardness increased significantly ($p < 0.05$) from dry to rainy season in all four aquatic ecosystems. Maximum conductivity was found in Bomo Lake (173 μ S cm⁻¹) and the highest water hardness was recorded in Makwaye Lake (5.10 mg/L) during the rainy season, whereas in the dry season, the least conductivity was obtained in Makwaye Lake (74 μ S cm⁻¹) and the lowest water hardness in ABU and Zaria Dams (1.7 mg/L). Alkalinity was the lowest in Makwaye Lake (3.80 mg/ L) and the highest in ABU dam (5.45 mg/L) during the dry season, while in the rainy season, it was the highest in Makwaye Lake (5.50 mg/L) and the lowest in Zaria Dam (3.85 mg/L). Significantly higher dissolved oxygen concentrations were recorded in the dry season than the rainy season (Table [1](#page-4-0)).

The highest dissolved oxygen concentration was observed in Zaria Dam in both dry (7.25 mg/L) and rainy (5.98 mg/L) seasons, while the lowest was in Bomo Lake during the dry (4.25 mg/L) and rainy (2.85 mg/H) L) seasons. Biochemical oxygen demand was significantly ($p < 0.05$) different between the seasons, where the highest values were found in the rainy season in all the sampled water bodies (Table [1](#page-4-0)). Nutrient (nitrate nitrogen, phosphate phosphorus, and total phosphorus) levels were generally the highest in the dry season than the rainy season. However, nitrate-nitrogen concentration was lower in ABU dam in the dry season (0.10 mg/L) than the rainy season (0.90 mg/L). Seasonal changes in nitrate nitrogen and total phosphorus concentrations in the different water bodies were significant ($p < 0.05$) (Table [1](#page-4-0)). Turbidity of the lakes significantly ($p < 0.05$) increased in the rainy season with Zaria Dam having the highest value (126.50 mg/L), and Bomo Lake having the lowest value (8.08 mg/L) in the dry season.

Table 1 Physicochemical characteristics of public water supply lentic systems in Zaria, Nigeria Table 1 Physicochemical characteristics of public water supply lentic systems in Zaria, Nigeria

*Means are significantly ($p < 0.05$) different between March and May for the respective water bodies

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Values are mean \pm standard deviation for $n = 12$

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Chlorophyll a concentrations were the highest in the dry season in ABU Dam (52.00 µg/L) and Bomo Lake $(50.00 \mu g/L)$, while in the rainy season, Makwaye Lake (39.00 µg/L) and Zaria Dam (46.00 µg/L) had the highest concentrations (Table 2). Throughout the study, phycocyanin:chlorophyll ratios were high but not significantly different between the water bodies and seasons. In the dry season, ABU and Zaria Dams had the highest dissolved MCs concentrations, while in the rainy season, ABU Dam and Bomo Lake had the highest concentrations. The rainy season was characterized by the absence of dissolved MCs in Zaria Dam and generally lower concentrations in all the water bodies than in the dry season. For particulate MCs, the highest concentrations in both dry (3.16 µg/L) and rainy (3.02 μg/L) seasons were observed in ABU Dam. Similar to dissolved MCs, lower particulate MCs concentrations were obtained in the rainy season in the water bodies compared to the dry season. *Microcystis* aeruginosa and Anabaena subcylindrica had the highest cell densities and were the dominant species in the water bodies in the dry season, while both species were only dominant in ABU Dam in the rainy season (Table [3](#page-6-0)). In addition, M. aeruginosa had high cell density and was dominant in Makwaye Lake in the rainy season. The changes in cell densities of M. aeruginosa and A. subcylindrica between the two seasons were significant ($p < 0.05$) (Table [4](#page-7-0)). The lowest cyanobacterial cell densities were obtained in the rainy season in all the water bodies compared to the dry season. In the dry season, Makwaye Lake had the highest cyanobacterial cell density, while ABU Dam had the lowest. However, in the rainy season, ABU Dam had the highest cyanobacterial cell density, while Makwaye Lake had the lowest.

Principal components analysis results showed a significant positive correlation of M. aeruginosa and A. subcylindrica biomass with particulate and dissolved MCs concentrations (Fig. [2](#page-8-0)). Significant positive correlations between total phosphorus, phosphate phosphorus and MCs (particulate and dissolved) concentrations were observed. A negative correlation of nitrate nitrogen with MCs content was obtained.

Discussion

The changes in most of the physicochemical characteristics of the water bodies investigated are in agreement

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Table 3 Distribution and abundance $(x10⁴$ cell per mL) of cyanobacterial species in public water supply systems in Zaria, Nigeria

Table 3 Distribution and abundance $(x10^4$ cell per mL) of cyanobacterial species in public water supply systems in Zaria, Nigeria

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Table 4 ANOVA summary for the different parameters measured in the water bodies in Zaria, Nigeria

Values in italics are significant $(p \le 0.05)$

with those previously recorded of lentic aquatic ecosystems in Zaria (Abolude et al. [2009;](#page-9-0) Chia et al. [2009a](#page-9-0),[b](#page-9-0)). However, total phosphorus concentrations exceeded the boundary set between mesotrophy and eutrophy by the Organization for Economic Cooperation and Development (OECD) for aquatic ecosystems (OECD Organization for Economic Cooperation and Development [1982\)](#page-10-0). Based on the results obtained in the present study, the public water supply systems were eutrophic $(>0.1$ mg L⁻¹) according to OECD (Organization for Economic Cooperation and Development [1982](#page-10-0)).

The pigment chlorophyll a is possessed by all photosynthetic organisms and is a good biomass estimator of phytoplankton in aquatic ecosystems (Xu et al. [2011\)](#page-11-0).

Chlorophyll a concentrations in these water bodies fell within the range reported for eutrophic water bodies, where values are more than 25 μ g L⁻¹. This is because the lowest value recorded in the present study was 20 μg L^{-1} . According to Gurbuz et al. [\(2009](#page-10-0)) and Xu et al. [\(2011](#page-11-0)), chlorophyll a concentrations can be used as a biomarker for potential MCs contamination of water bodies, in the event of cyanobacterial contamination. This explains the significant positive correlation observed between chlorophyll *a* concentrations and total particulate and dissolved MCs. Toxic cyanobacterial blooms with more than 50 μg L^{-1} chlorophyll a can cause severe health effects such as liver damage (Falconer et al. [1999](#page-10-0); Meriluoto and Codd [2005\)](#page-10-0). In the present study, Bomo Lake and ABU Dam had

chlorophyll *a* concentrations $\geq 50 \mu g L^{-1}$. A further support to this is the high phycocyanin-to-chlorophyll ratios recorded in all the water bodies. This is a cause of concern especially to water managers as it implies that cyanobacterial population in the lentic water bodies was dominant. As phycocyanins are associated with cyanobacteria, the higher ratios to chlorophyll a levels recorded in the present study implies the chances of finding significant MCs concentrations or cyanobacterial contamination were high. The presence and extent of cyanobacterial and MCs contamination of the water bodies were dependent on prevailing physicochemical conditions, especially nutrient concentrations. This is supported by the significant positive correlation of total phosphorus and phosphate phosphorus with A. subcylindrica and M. aeruginosa biomass and MCs concentrations. The biomass of some cyanobacterial species are also good predictors of MCs concentration (Bittencourt-Oliveira et al. [2015](#page-9-0)). Rolland et al. ([2005\)](#page-10-0) and Giani et al. [\(2005](#page-10-0)) have linked biomass composition and in particular Microcystis sp. to MCs concentration. Furthermore, M. aeruginosa which is a known high MCs producer and one of the most widespread hepatotoxic species in freshwaters (Chorus and Batramm, Chorus and Bartram [1999\)](#page-10-0) was found in all the water bodies.

Long term population health can only be guaranteed by the provision of safe drinking water. It is in line with this that the World Health Organization (WHO World Health Organization WHO [1998\)](#page-11-0) provided a guideline that limits the concentration of MC-LR in drinking water

at 1.0 μg L^{-1} . Most countries around the world either adopt this guideline or formulate their own according to local conditions. Unfortunately, the results of the present study showed that in virtually all cases of detection, MCs concentrations exceeded the limits provided by WHO. This problem is further complicated by the fact that water treatment plants in Zaria and most parts of Nigeria use the conventional single train and one disinfection segment water treatment system. This system is not capable of removing dissolved MCs (USEPA US Environmental Protection Agency [2015\)](#page-11-0). However, it can remove some particulate MCs by sedimentation and coagulation. Prior to the present study, in 2008, reconnaissance samples were collected from some of these water bodies in the dry season, which presented the first detection of total MCs in northern Nigeria (Chia et al. [2009a](#page-9-0)). In the present study, we observed that all the water bodies (100 %) had significant toxin concentrations during the dry season, while in the rainy season, all except Zaria Dam had significantly high toxin concentrations. Also for the first time, MCs were detected in Zaria Dam during the dry season. Furthermore, it can be seen that the concentrations detected 5 years after the previous reconnaissance sampling in all the reservoirs were significantly higher. These changes can be related to the increase in nutrient loads that have been recorded in the present study, which are higher than previously recorded. These results highlight the risk to public water supply in the investigated water bodies and should be a cause of concern to water managers in the region. This is because the

Fig. 2 PCA biplot showing the correlation between cyanobacterial species, microcystins production, and physicochemical parameters

people living in and around the catchment of these water bodies sometimes drink the water from them without prior treatment. In addition, the traditional methods that are used for water treatment like filtration/sieving and boiling are not effective in the removal of dissolved MCs from water (USEPA US Environmental Protection Agency [2015](#page-11-0)).

The growth of cyanobacteria in the environment is regulated by light quality, temperature, resource availability, and interspecific competitions (Paerl and Huisman [2009](#page-10-0)). While it is not possible to control light quality and temperature, as they are characteristic of the tropical climate of Nigeria, nutrient enrichment of the water bodies can be significantly reduced to prevent excessive proliferation of toxic cyanobacteria. The regulation of agricultural activities in and around the catchment will be a good first step (Lurling and Roessink Lürling and Roessink [2006](#page-10-0); Cecchi et al. 2009). Cecchi et al. suggest that watershed management and nutrient control, through the provision of buffer zones around reservoirs made up of riparian vegetation, are very crucial in reduction of risks associated with high toxin levels, such as those detected in the present study. These buffer zones will reduce the concentration of nutrients that finally gets into the water bodies from surface runoff.

Conclusion

The detection of MCs in the lakes and dams meant for public water supply in Zaria is a serious public health concern. The detected MCs concentrations were significantly higher than previously detected and the limits set by WHO. The presence of this toxin in the water supply sources should alert water managers on the need to control the establishment and excessive proliferation of toxic cyanobacteria in these water bodies.

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