Cyanobacteria and cyanobacterial toxins in the alkaline-saline Lakes Natron and Momela, Tanzania

H.E. Nonga[1, 3], R.H. Mdegela[1], M. Sandvik[2], E. Lie[3, 4], C.O. Miles[2] and J.U. Skaare[2, 3]

[1]Sokoine University of Agriculture, P. O. Box 3021 Morogoro, Tanzania
[2]Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway
[3] Norwegian University of Life Sciences, Campus Adamstuen, P.O. Box 8146 Dep, N-0033 Oslo, Norway
[4]Norwegian Institute for Water Research, Gaustadalléen 21, N-0349 Oslo, Norway
Email: nongahezron@yahoo.co.uk / hezron@suanet.ac.tz

SUMMARY

Physicochemical parameters, phytoplankton communities, microcystin (MC) concentrations and potential MC-producing cyanobacteria were investigated in Lakes Natron and Momela, Tanzania. In Lake Big Momela, concentrations of soluble reactive phosphorus, nitrate and ammonia were 7.1, 2.6 and 0.9 µg/L, respectively, while dissolved oxygen, salinity, conductivity and pH were 9.4 mg/L, 19‰, 30 mS/cm and 9.7, respectively. The concentrations of soluble reactive phosphorus, nitrate and ammonia in Lake Natron were 129.4, 8.1 and 58 µg/L, respectively, while dissolved oxygen, conductivity and pH were 8 mg/L, 52 mS/cm and 9.5 respectively. The phytoplankton communities in both lakes were dominated by cyanobacteria, particularly *Arthrospira fusiformis*. *Navicula* and *Nitzschia* diatoms, and *Chlorella*, *Chlorococcum* and *Scenedesmus* green algae were common in Lakes Momela and Natron. Liquid chromatography–mass spectrometry (LC-MS) analysis phytoplankton detected four microcystin variants namely MC-RR, -YR, -LR and -RY. The total MC concentrations in Lake Natron were 0.1–4.5 µg/mL of phytoplankton scum and in Lake Momela were below quantifiable levels. Polymerase chain reaction analysis of phytoplankton revealed presence of *Microcystis* and the *Microcystis mcy*B gene in some samples. Finding of potential MC-producing cyanobacteria and MCs in study lakes poses a health risk to Lesser Flamingo which feed on cyanobacteria.

Key words: alkaline-saline lakes, microcystins, phytoplankton, physicochemical parameters

INTRODUCTION

The Rift Valley Lakes of East Africa are situated within the eastern arm of the Gregory Rift Valley System which is associated with widespread of volcanic activity thought to be responsible for the alkaline-saline characteristics of the lakes (Baker *et al.*, 1972; Hecky and Kilham, 1973). The lakes have low phytoplankton species diversity, and they support growth of specific saline tolerant phytoplankton, in particular cyanobacteria like *Arthrospira fusiformis* (Melack, 1988). The *A. fusiformis* constitutes the main feed for the Lesser Flamingos (*Phoeniconaias minor* Geoffroy) which are the common waterbirds in the lakes (Melack, 1988). Several studies in alkaline-saline lakes have indicated the presence of potentially toxic cyanobacteria, which occasionally assume dominance, displacing the *A. fusiformis* populations (Ballot *et al.*, 2009; Krienitz *et al.*, 2005; Krienitz and Kotut, 2010). For example, high concentrations of MCs (4593 µg/g dry weight) and anatoxin-a (223 µg/g dry weight) have been reported in phytoplankton samples from Kenyan alkaline-saline lakes dominated by *A. fusiformis*, *Anabaenopsis* spp., *Chrooccocus* spp. and *Anabaena* spp. (Ballot *et al.*, 2004). In addition, MCs and anatoxins have also been detected in *A. fusiformis* culture from Kenyan alkaline-saline lakes (Ballot *et al.*, 2004). Anthropological factors due to human activity in the lake ecosystems are thought to account for the changes in phytoplankton composition (Ndetei and Muhandiki, 2005).

The presence of cyanotoxins in saline lakes has been associated with the recent frequent mortality of Lesser Flamingos in most of the East Africa alkaline-saline lakes (Krienitz *et al.*, 2003; Lugomela *et al.*, 2006; Nonga *et al.*, 2011). However, in Lake Natron which is the only breeding site for the East African Lesser Flamingos population (Childress *et al.*, 2008), the phytoplankton community and MCs status has not been studied. The purpose of this cross-sectional study was to establish the baseline information on: (i) physicochemical parameters and phytoplankton communities in Lake Natron and Lakes Momela; (ii) MC concentrations in phytoplanktons and; (iii) the potential MC-producing cyanobacteria in the phytoplankton of Lake Natron and Lakes Momela, Tanzania.

MATERIALS AND METHODS

Study areas, physicochemical parameter assessment and identification of phytoplankton

This study was conducted during November and December 2010 in alkaline-saline Lake Natron and Lakes Momela. The Momela lakes constitute a series of several lakes namely Lake Big Momela, Lake Small Momela, Lake Tulusia and Lake Lekandiro, which are all located in Arusha Region, Tanzania (Figure 1). Seven sampling sites were established at Lake Big Momela, two at Lake Small Momela, one site each at Lake Tulusia and Lake Lekandiro (Figure 1). Samples were collected at around 5–10 m from the shoreline at a depth of around 1–2 m. In Lake Natron, six sampling sites were established at 10–20 m distance from the shoreline with a depth of around 0.5 m (Table 1).

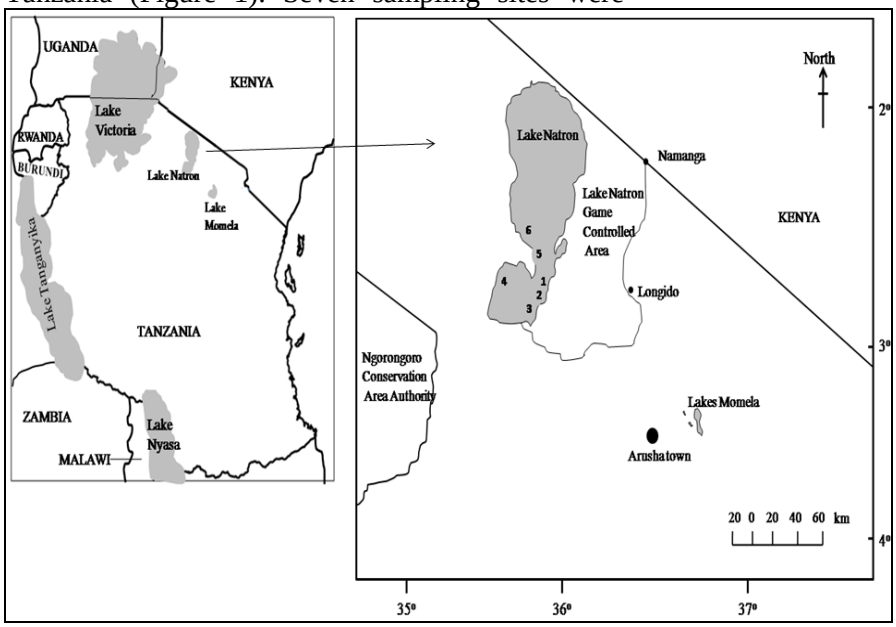

Figure 1. Maps showing the location of Lakes Momela and Lake Natron in Tanzania. The sampling sites at Lake Natron numbered 1, 2, 3, 4, 5 and 6 are Maasai Manyata, Gadaffi camp, Ngelai falls, Moinik, Namarkaun and Peninj area respectively.

Water temperature, pH, conductivity and dissolved oxygen were measured *in situ* using a portable water quality checker (Horiba U-10, Japan). Salinity was measured using a hand refractometer while water transparency was measured using a white Secchi disc with 20 cm diameter. Water samples for inorganic nutrient (nitrate, ammonia, and soluble reactive phosphorus) analysis were filtered using GF/C filters and the nutrients analysis was done according to APHA (2005).

For qualitative phytoplankton analysis, 5 L of water was concentrated through a plankton net (20 µm mesh size), to a final volume of 50 mL and fixed with formalin to a final concentration of 3%. Phytoplankton identification was done on a light microscope following morphological descriptions given by Compère, (1976), Komárek and Anagnostidis (1986; 1999; 2005), Talling (1987), Anagnostidis and Komárek (1988), and Komárek and Kling, (1991). Proportional estimation of the phytoplankton distribution on the slide was done during the identification so as to establish the predominant genera. Phytoplankton samples for MC and molecular analysis were collected by concentrating 20 L of water through a plankton net (20 µm mesh size) to about 500 mL and stored at -20 °C until analysis.

Microcystins analysis in phytoplankton scum

MCs extraction was done as described by Fastner *et al.* (1998) and the analysis was done using liquid chromatography–mass spectrometry (LC–MS/MS). Seven MC standards (MC-LR, -RR, -YR, -LA, -LF, -LY and –LW; Alexis Corporation Biochemicals Grünberg, Germany) were used. For the MC variants detected which had no standards; their identification was tentatively done based on their reaction with 2-mercaptoethanol, molecular ions and MS/MS fragmentation patterns.

Phytoplankton genetic analysis

Presence of the possible MC-producing genotypes, a quantitative real-time PCR method was applied as described by Kurmayer and Kutzenberger (2003). Each sample was analysed for the presence of the *Microcystis* genotype containing *mcy*B gene (Kurmayer and Kutzenberger, 2003) as well as for the presence of the *Anabaena* genotype containing *mcy*E gene (Vaitomaa *et al.,* 2003). Phytoplankton net sample material (100 µL) was frozen and thawed twice and then boiled for 5 min before genomic DNA was extracted using the MoleStrips DNA blood kit and the DNA-Cyano protocol on GeneMole (Mole Genetics, Lysaker, Norway) according to the manufacturer's instructions. The primers 30F and 108R were used for *Microcystis* (Kurmayer and Kutzenberger, 2003) and the primers McyE-F2 and AnamcyE-12R were used for *Anabaena* (Vaitomaa *et al.,* 2003). The qPCR was performed with 5 µL of DNA, 1 µL of each primer (Eurofins MWG Operon, Ebersberg, Germany), 12.5 µL of master mix (MESA blue qPCR Master mix for SYBR green assay with low rox; Eurogentec, Seraing, Belgium) in a total volume of 25 µL. Amplifications were performed as follows: an initial preheating for 2 min at 50 ºC and 10 min at 95 ºC, followed by 40 cycles with one cycle consisting of 15 sec at 95 ºC and 1 min at 60 ºC. In order to determine the melting temperatures for the amplification products, a melt curve stage was started after qPCR and the temperature was changed from 95 to 60 ºC and fluorescence was detected continuously. All the analyses were done in triplicate using an ABI 7500 mastercycler (ABI, Carlsbad, CA). To quantify the total population of *Microcystis* cells containing the *mcy*B gene, the linear regression curve was y = -4.965 + 50.218 (n=5, R^2 = 0.994), where y was the cycle of threshold (C_t value) at the set fluorescence threshold level obtained for the *mcy*B gene and x was the amount of starting DNA (given as $\log_{10}$ cell number equivalents of *Microcystis* strain NIVA-CYA 228/1). To quantify the total population of *Anabaena* cells containing the *mcy*E gene the linear regression curve was y = -2,677 + 32,961 (n=5, R^2=0.951), where y was the cycle of threshold (C_t value) at the set fluorescence threshold level obtained for the *mcy*B gene and x was the amount of starting DNA (given as log10 cell number equivalents of *Anabaena* strain NIVA-CYA 83/1).

RESULTS

Physicochemical parameters and phytoplankton community

Results for physicochemical parameters are presented in Table 1. Water temperature was high especially in Lake Natron (33 ± 4^{o}C) while for Lakes Momela, the temperature was relatively low (24 ± 0.5^{o}C). High pH (Lakes Momela 9.4–9.9, Lake Natron 8.7–10.1), salinity (Lake Momela 12–38‰) and conductivity (Lakes Momela 18–98, Lake Natron 11–77) were also recorded. The water transparency of Lakes Momela was low (23–35 cm) while the mean concentration of dissolved oxygen in both study lakes (Lake Momela 9.3 mg/L, Natron 7.8 mg/L) was relatively high. The nitrate levels in the study lakes were relatively low (Lakes Momela 1.8 - 80 µg/L, Natron 5.6–11.7 µg/L). Higher levels of soluble reactive phosphorus (53–196 µg/L) were recorded in Lake Natron compared to Lakes Momela (5.2–25.8 µg/L). High levels of ammonium were also recorded in Lake Natron (43–67 µg/L) compared to Lakes Momela (0.6–3.6 µg/L).

Table 1. Physicochemical characteristics of Lakes Momela and Lake Natron in November and December 2009

Lakes	Sampling sites	Date	Coordinates		Temp (ºC)	Secchi (cm)	Dissolved Oxygen (mg/L)	pH	Salinity (‰)	Conductivity (mS/cm)	PO4 (µg/L)	NO3 (µg/L)	NH4 (µg/L)
			Latitudes	Longitudes									
Big Momela	1	November	3º 13.486'	36º 54.250'	24.0	23	10.3	9.7	22	26	8.4	0.9	2.1
	2	November	3º 13.315'	36º 54.114'	24.3	23	9.3	9.7	20	31	7.6	0.8	2.5
	3	November	3º 13.650'	36º 54.532'	24.8	24	9.3	9.7	12	24	7.5	0.8	3.0
	4	November	3º 13.411'	36º 54.418'	23.8	23	8.9	9.7	22	40	6.3	1.1	1.8
	5	November	3º 13.320'	36º 54.668'	23.5	23	9.5	9.7	18	28	5.9	0.6	3.2
	6	November	3º 13.042'	36º 54.065'	23.4	23	8.7	9.6	20	32	6.8	1.2	2.8
	7	November	3º 13.348'	36º 54.416'	23.4	23	9.2	9.6	20	30	7.2	0.8	2.3
Small	1								10				
Momela		November	3º 13.458'	36º 53.118'	25.2	51	14.3	9.7		18	5.6	1.8	4.2
	2	November	3º 13.684'	36º 53.671'	25.2	37	15.2	9.4	10	21	5.2	1.4	4.8
Lekandiro	1	November	3º 12.358'	36º 53.950'	24.5	54	5.1	9.5	30	85	25.8	2.2	12.5
Tulusia	1	November	3º 12.406'	36º 54.885'	25.0	35	3.6	9.9	38	98	20.2	3.6	80
Natron	Maasai												
	Manyata	December	2º 34.584'	35º 54.759'	37.6	nd	14.9	10.1	nd	62	53	62	5.6
	Moinik	December	2º 30.728'	35º 52.899'	28.5	nd	8.5	8.7	nd	11	70	43	7.2
	Namarkaun	December	2º 29.130'	35º 53.261'	35.0	nd	5.2	8. 8	nd	37	161	56	8.4
	Gadaffi camp	December	2º 34.943'	35º 55.050'	31.1	nd	3.4	9.9	nd	58	113	61	11.7
	Ngelai falls	December	2º 35.331'	36º 00.382'	35.1	nd	10.6	10.1	nd	67	183	58	6.9
	Peninj area	December	2º 26.145'	35º 53.799'	29.7	nd	4.4	9.1	nd	77	196	67	8.8

Temp, temperature; SRP, soluble reactive phosphorus; NH_4+, ammonia; NO_3-, nitrate; nd, not determined

Phytoplankton analysis revealed that total of 18 genera belonging to 3 taxa, were identified. These included cyanobacteria (*A. fusiformis, Anabaenopsis, Anabaena, Microcystis, Chroococcocus, Nostoc, Aphanothece, Oscillatoria, Spirulina, Phormidium* and *Aphanocapsa*), Bacillariophyta (*Navicula, Nitzschia, Anomoeoneis, Anomoeoneis* and *Cyclotella*), and Chlorophyta (*Chlorella, Scenedesmus* and *Chlorococcum*). The phytoplankton communities in Lakes Momela and Lake Natron were dominated by cyanobacteria, in particular *A. fusiformis*. Polymerase chain reaction analysis of phytoplankton samples observed the presence of *Microcystis* and the *Microcystis mcy*B genes in some samples.

Microcystin analysis results

LC-MS analysis of samples established a total MC concentration in Lake Natron to be 0.1–4.5 µg/mL of phytoplankton scum and in Lake Momela were below quantifiable levels (Table 2). MC-RR was the only variant detected in Lake Momela and three MC variants (MC-LR, -YR and -RY) were detected in Lake Natron (Figure 2). MC-RY detected in phytoplankton samples from Lake Natron accounted for more than half of the total MC concentrations recorded. Polymerase chain reaction analysis indicated presence of *Microcystis* with the mcyB gene indicative of MC production (Table 2). In 3/11 of the phytoplankton samples from Lake Momela and 4/6 samples from Lake Natron *Microcystis* and the *Microcystis* genotype containing the *mcy*B gene encoding for MC synthesis were detected. None of the samples had PCR products indicating the presence of the *Anabaena* or the *Anabaena mcy*E gene.

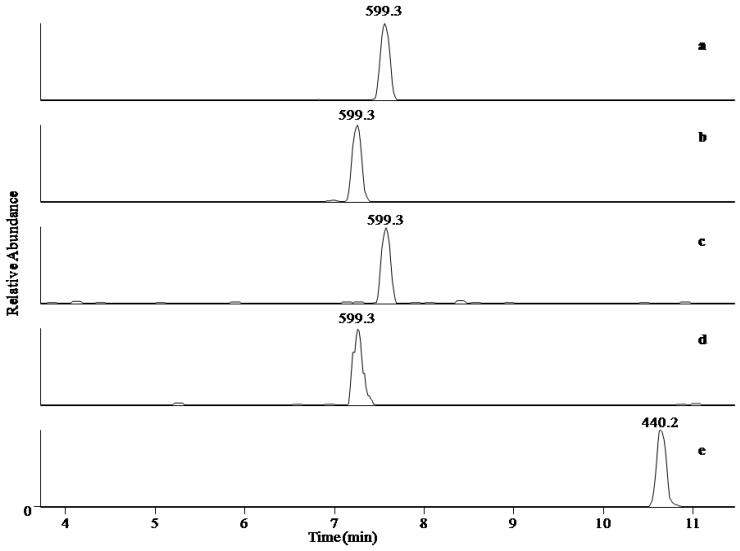

Figure 2. LC-MS^2 Chromatograms. (a) MC-LR (*m/z* 995.5) standard, (b) MC-YR (*m/z* 1045.5) standard, (c) MC-LR (*m/z* 995.5) in phytoplankton sample, (d) MC-YR (*m/z* 1045.5) in phytoplankton sample, (e) MC-RY (*m/z* 1045.5) in phytoplankton sample from L. Natron

Table 2. Microcystins and *Microcystis mcy*B and *Anabaena mcy*E genes analysis in phytoplankton samples from Lakes Momela and Lake Natron

Study lakes	Sample ID	Microcystin concentrations (µg/g wet weight)				mcy genes	
		MC-YR	MC-LR	MC-RR	MC-RY	*Microcystis mcy*B	*Anabaena mcy*E
Big Momela	BMA1-1	nd	nd	nd	nd	-	-
	BMA1-3	nd	nd	bql	nd	-	-
	BMA2-1	nd	nd	bql	nd	-	-
	BMA2-5	nd	nd	bql	nd	+	-
	BMA3-1	nd	nd	bql	nd	-	-
	BMA3-4	nd	nd	nd	nd	-	-
	BMA5-1	nd	nd	bql	nd	+	-
	BMA6-1	nd	nd	bql	nd	-	-
	BMA7-1	nd	nd	bql	nd	-	-
Small Momela	SMA1-1	nd	nd	nd	nd	-	-
	SMA1-2	nd	nd	nd	nd	+	-
Tulusia	TMA2-1	nd	nd	nd	nd	-	-
Lekandiro	LMA4-1	nd	nd	nd	nd	-	-
Natron	NA1	0.1	0.2	nd	0.4	+	-
	NA2	0.2	0.2	nd	0.3	+	-
	Ma A1	1.2	1.1	nd	2.2	+	-
	Ma A2	1.2	0.8	nd	2.1	+	-
	Mo A2	nd	nd	nd	nd	-	-
	Mo A1	0.1	nd	nd	nd	-	-

bql, below quantifiable level; nd, not detected

DISCUSSION

This study observed high water temperature especially in Lake Natron (33 ± 4^{o}C) suggestive of the drought condition that was prevailing in the area during the study period. However, for Lakes Momela, the temperature was relatively low (24 ± 0.5^{o}C) probably because the lakes are relatively deep (up to 31 m) and are surrounded by thick forests. The recorded water temperature levels in both lakes were favourable for cyanobacterial bloom formation (Paerl, 1996). The high pH, salinity and conductivity recorded were thought to be influenced by the factors of internal drainage, chemical weathering of the surrounding volcanic rocks and a semi-arid climatic regime (Baker *et al.,* 1972; Hecky and Kilham, 1973). In addition, the observed drought conditions, in particular at Lakes Natron, may cause evaporative concentration and reduction of the lake surface areas which could account for the high values of pH, conductivity and salinity.

The water transparency of Lakes Momela was low resulting in a narrow euphotic zone as previously reported by Kaaya (2007). The low water transparency was thought to be influenced by high solute concentrations and phytoplankton blooms. Our study further found a relatively high mean concentration of dissolved oxygen in both study lakes which was comparable to values reported by Kaaya (2007) in Lake Momela. The levels of dissolved oxygen in water bodies may be influenced by the rate of photosynthetic activity, wind action, depth, dissolved solute and decomposition of organic matter (Boyd, 2000).

The nitrate levels recorded in the study lakes were relatively low, which were comparable to the values recorded in alkaline-saline Lakes Manyara (Lugomela *et al.,* 2006) and Momela (Kaaya, 2007). Large cyanobacterial blooms and other phytoplankton, which were commonly observed in the lakes, may have caused high biological uptake of nutrients (Dokulil and Teubner, 2000). Furthermore, higher levels of soluble reactive phosphorus were recorded in Lake Natron compared to Lakes Momela. This may be accounted by the amount of phosphate that enters the lake from anthropogenic and natural sources. Absence of an outflow in the studied lakes may be another cause of elevating phosphorus concentrations in the lakes. Similarly, the high levels of ammonium recorded in Lake Natron (43–67 µg/L) may be caused by regeneration of nutrients from the sediments under

reduced environment, decomposition of organic matter under anaerobic conditions and, runoff containing agrochemicals, livestock manure and organic matter. In addition, the high population of Lesser Flamingo and other aquatic birds may add ammonium in water through their faecal droppings (Ganning and Wulff, 1969).

A total of 18 phytoplankton genera belonging to 3 taxa, were identified being dominated by cyanobacteria, in particular *A. fusiformis*. Studies in other East African alkaline-saline lakes show that *A. fusiformis* predominates due to its ability to tolerate a wide range of salinities via osmotic adjustments and mechanisms of internal pH regulation (Vareschi, 1982; Vonshak and Tomaselli, 2000). In addition, the higher diversity and dominance of cyanobacterial species in alkaline-saline lakes compared to other phytoplankton may be caused by the extreme high salinity and pH which possibly limit the growth of other aquatic species (Melack, 1988; Vareschi, 1982). Other probable causes for cyanobacterial dominance in alkaline-saline lakes include the high availability of dissolved inorganic carbon, elevated temperatures, and low water transparency (Scheffer *et al.,* 1997; Oduor and Schagerl, 2007).

The current study identified *Microcystis* in Lake Small Momela and Lake Natron, which concurs with other studies reporting occurrence of *Microcystis* in alkaline-saline lakes of East Africa (Ndetei and Muhandiki, 2005; Kaaya, 2007; Stewart *et al.,* 2008). Polymerase chain reaction analysis of phytoplankton samples from Lakes Momela and Lake Natron in the present study also revealed the presence of *Microcystis* and the *Microcystis mcy*B genes in some samples. This further confirms the microscopical observation of *Microcystis* in alkaline-saline lakes. Furthermore, a study by Dadheech *et al.* (2009) and Mwirichia *et al.* (2011) detected DNA and 16S rRNA gene for *Microcystis* in alkaline-saline Lake Elmenteita and Bogoria. However, Krienitz and Kotut (2010) and Kotut and Krienitz (2011) suggested that the reported

The MC levels recorded during the present study are low (0.1–4.5 µg/mL) compared to levels previously published for Kenyan alkaline-saline lakes. A study by Ballot *et al.* (2004) in Lake Bogoria and Lake Nakuru reported MC concentrations of 155 and 4,593 µg/g dry weight respectively. In addition, Ballot *et al.* (2005) reported MC concentrations of 12 and 39 µg/g dry weight in Lake Sonachi and Lake Simbi, respectively. Although high numbers of potential MC-producing species were identified during the current study, the low concentration of *Microcystis* could be due to errors during microscopic identification, since the *Microcystis* morphology can easily be confused with *Anabaenopsis abijatae* (Ballot *et al.,* 2008). During the current study, the presence of *Microcystis* in Lake Small Momela may be due to low levels of salinity (10 ‰) and pH (9.4) because of several freshwater streams feeding into the lake. Similarly, in Lake Natron, *Microcystis* was observed in samples from sampling sites located near to entry points of some rivers like at Moinik (with conductivity 11 mS/cm, pH 8.7) and Namarkaun (with conductivity 37 mS/cm, pH 8.8). A study by Hammer *et al.* (1983) reported that *Microcystis* can grow under hyposaline (salt conc. 3–20 ‰) to mesosaline (salt conc. 20–50 ‰) conditions provided that the alkalinity is low.

The current study further revealed that the cyanobacterial communities in Lakes Momela and Natron produce MCs since a concentration of up to 4.5 µg/mL of phytoplankton scum was recorded in Lake Natron. MC-RR was the only variant detected in Lake Momela while MC-LR, -YR and -RY) were detected in Lake Natron. MC-RY detected in phytoplankton samples from Lake Natron accounted for more than half of the total MC concentrations recorded. This is the first report on occurrence of MC-RY in alkaline-saline lakes of East Africa. The possible sources for the production of MCs detected may be *Microcystis* spp. The real-time PCR results further confirms presence of *Microcystis* with the mcyB gene indicative of MC production. This suggests that *Microcystis* may be among the MC-producing genus in Lakes Momela and Lake Natron. Other microcystin producing cyanobacteria detected microscopically during this study *Anabaena, Aphanocapsa, Oscillatoria, Nostoc, Anabaenopsis* and *Phormidium* spp. These cyanobacterial genera are known to be the potential MC-producers (Codd *et al.,* 1999, Chorus, 2001; van Apeldoorn *et al.,* 2007). However, *Arthrospira*, which was the most dominant cyanobacterial genus in all the alkaline-saline lakes, was also recently been reported to produce MCs (Ballot *et al.,* 2004).
MCs recorded in phytoplankton may be caused by several factors. MC production in cyanobacterial strains depends on whether or not it contains the gene for toxin production and on environmental factors promoting the gene's expression (Dittman *et al.,* 1996; Chorus, 2001). Potential MC-producing species like *M. aeruginosa* can co-exist with both MC-producing and non-producing strains (Kurmayer *et al.,* 2003). Studies have shown that MC concentration and composition, and physiological status of cyanobacterial cells are affected by factors like light energy, temperature,

pH, primary production, oxygen saturation and nutrient status (Watanabe *et al.*, 1989; Wicks and Thiel, 1990; Oberholster *et al.*, 2004). However, differences in toxin production may also occur in different growth phases and with seasonal changes in the predominant toxic or non-toxic species of cyanobacteria (Park *et al.*, 1998).

It is concluded that presence of hepatotoxic MCs in the alkaline-saline lakes have ecological impacts on aquatic food webs of lakes and cause serious health problems to aquatic life. Microcystins may be the contributory factors for the mass mortality of Lesser Flamingos which frequently occurs in the lakes.

ACKNOWLEDGEMENTS

This study was kindly supported by The Norwegian Programme for Development, Research and Higher Education (NUFU) PRO 07/10224. The permission to conduct this study was given by Tanzania National Parks (TANAPA). Delphe British Council Field-It-Eafrica Project is thanked for the kind support during field work at Lake Natron**.**

REFERENCES

Anagnostidis K, Komárek J. Modern approach to the classification system of cyanophytes. 2 – Oscillatoriales. *Algol Stud*, 50–53, 327–472, 1988.

APHA (American Public Health Association). Standard methods for the examination of water and wastewater. 21st edn. American Public Health Association, Washington DC, 2005.

Baker BH, Mohr PA, Williams LAJ. Geology of the Eastern Rift system of Africa. Geological Society of America Special Paper 136. 1–67, 1972.

Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf JS, Codd GA, Pflugmacher S. Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya-lakes Bogoria, Nakuru and E Bartram lmentaita. *J Plank Res*, 26, 925– 935, 2004.

Ballot A, Krienitz L, Kotut K, Wiegand C, Pflugmacher S. Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. Harmful Algae 4, 139–150, 2005.

Ballot A, Dadheech PK, Haande S, Krienitz L. Morphological and phylogenetic analysis of *Anabaenopsis abijatae* and *Anabaenopsis elenkinii* (Nostocales, Cyanobacteria) from tropical inland water bodies. *Microb Ecol* 55, 608–618, 2008.

Ballot A, Kotut K, Novelo E, Krienitz L. Changes of phytoplankton communities in Lakes Naivasha and Oloidien, examples of degradation and salinization of lakes in the Kenyan Rift Valley. *Hydrobiologia*, 632, 359 – 363, 2009.

Boyd CE. Water quality. An introduction. Kluwer Academic Publishers, Boston, 2000.

Childress B, Nagy S. Hughes B. (Eds.). International Single Species Action Plan for the Conservation of the Lesser Flamingo (*Phoeniconaias minor*). CMS Technical Series No. 18, AEWA Technical Series No. 34. Bonn, Germany, 2008.

Chorus, I. Introduction: Cyanotoxins – research for environmental safety and human health. In: Chorus, I. (ed.). Cyanotoxins – occurrence, causes, consequences. *Springer-Verlag, Berlin*. pp. 1–4, 2001.

Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS. Cyanobacterial toxins, exposure routes and human health. European Journal of Phycology 34, 405–415, 1999.

Compère P. Algues de la région du lac Tchad. V Chlorophycophytes (1re parties) (1). Cahiers ORSTOM. *Série Hydrobiologie*, 11, 77–177, 1976.

Dadheech PK, Krienitz L, Kotut K, Ballot A, Casper P. Molecular detection of uncultured cyanobacteria and aminotransferase domains for cyanotoxin production in sediments of different Kenyan lakes. *FEMS Microb Ecol*, 68, 340–350, 2009.

Dittman E, Meissner K, Börner T. Conserved sequences of peptide synthetase genes in the cyanobacterium *Microcystis aeruginosa*. *Phycologia*, 35, 62–67, 1996.

Dokulil MT, Teubner K. Cyanobacterial dominance in lakes. *Hydrobiologia*, 438, 1–12, 2000.

Fastner J, Flieger I, Neumann U. Optimised extraction of microcystins from lyophilised field samples – a comparison of different solvents and procedures. *Water Res*, 32, 3177–3181, 1998.

Ganning B, Wulff F. The effects of bird droppings on chemical and biological dynamics in brackish water rockpools. *Oikos*, 20, 274–286, 1969.

Hammer UT, Shamess J, Haynes RC. The distribution and abundance of algae in saline lakes of Saskatchewan, Canada. *Hydrobiologia*, 105, 1–26, 1983.

Hecky RE, Kilham P. Diatoms in alkaline, saline lakes: ecology and geochemical implications. *Limn Oceanogr*, 18, 53–71, 1973.

Kaaya LT. Phytoplankton diversity and productivity with emphasis on the bloom dynamics of the potentially toxic cyanobacterium *Arthrospira fusiformis* in Momela Lakes, Arusha, Tanzania. MSc. Thesis, University of Dar es salaam, Tanzania, 2007.

Komárek J, Anagnostidis K. Modern approach to the classification system of cyanophytes. 2 – Chroococcales. *Algol* Stud, 43, 157–226, 1986.

Komárek J, Kling H. Variation in six planktonic cyanophyte genera in Lake Victoria (East Africa). Archiv für Hydrobiologie. Supplement volume 88. *Algol* Stud, 61, 21–45, 1991.

Komárek J, Anagnostidis K. Chroococcales. *In:* Ettl, H., Gärtner, G., Heynig, H. and Mollenhauer, D. (eds.). Cyanoprokaryota Teil 1. Süwasserflora von Mitteleuropa. Gustav Fischer, Jena, 1999.

Komárek J, Anagnostidis K. Cyanoprokaryota Teil 2. Süwasserflora von Mitteleuropa. Volume 19, Elsevier Spektrum Akademischer Verlag, Heidelberg, 2005.

Kotut K, Krienitz L. Does the potentially toxic cyanobacterium *Microcystis* exist in the soda lakes of East Africa? *Hydrobiologia*, 664, 219-225, 2011.

Krienitz L, Kotut K. Fluctuating algal food populations and the occurrence of Lesser Flamingos (*Phoeniconaias Minor*) in three Kenyan rift valley lakes. *J Phycology*, 46, 1088–1096, 2010.

Krienitz L, Ballot A, Kotut K, Wiegand C, Pütz S, Metcalf JS, Codd GA, Pflugmacher S. Contribution of hot

spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS Microb Ecol, 43, 141–148, 2003.

Krienitz L, Ballot A, Casper P, Codd GA, Kotut K, Metcalf JS, Morrison LF, Pflugmacher S, Wiegand C. Contribution of toxic cyanobacteria to massive deaths of Lesser Flamingos at saline-alkaline lakes of Kenya. *Proc Intern Ass Theo Applied Limn*, 29, 783–786, 2005.

Kurmayer R, Kutzenberger T. Application of real-time PCR for quantification of microcystin genotypes in a population of the toxic cyanobacterium *Microcystis* sp. *App Environ Microb*, 69, 6723–6730, 2003.

Kurmayer R, Christiansen G, Chorus I. The abundance of microcystin-producing genotypes correlates positively with colony size in *Microcystis* sp. and determines its microcystin net production in Lake Wannsee. *App Environ Microb*, 69, 787–795, 2003.

Lugomela C, Pratap HB, Mgaya YD. Cyanobacteria blooms – A possible cause of mass mortality of Lesser Flamingos in Lake Manyara and Lake Big Momela, Tanzania. *Harmful Algae*, 5, 534–541, 2006.

Melack JM. Primary producer dynamics associated with evaporative concentration in a shallow, equatorial soda lake (Lake Elementaita, Kenya). *Hydrobiologia*, 158, 1–14, 1988.

Mwirichia R, Cousin S, Muigai AW, Boga HI, Stackebrandt E. Bacterial diversity in the haloalkaline Lake Elmenteita, Kenya. *Current Microb*, 62, 209–221, 2011.

Ndetei R, Muhandiki VS. Mortalities of Lesser Flamingos in Kenyan Rift Valley saline lakes and the implications for sustainable management of the lakes. *Lakes, Reservoirs: Res. Manag*, 10, 51–58, 2005.

Nonga HE, Sandvik M, Miles C, Lie E, Mdegela RH, Mwamengele GL, Semuguruka WD Skaare JU. Possible involvement of microcystins in the unexplained mass mortalities of Lesser Flamingo (*Phoeniconaias minor Geoffroy*) at Lake Manyara in Tanzania. *Hydrobiologia*, 678(1): 167-178, 2011.

Oberholster PJ, Botha AM, Grobbelaar JU. *Microcystis aeruginosa*: source of toxic microcystins in drinking water. *Afric J Biotechnol*, 3, 159–168, 2004.

Oduor SO, Schagerl M. Temporal trends of ion contents and nutrients in three Kenyan Rift Valley saline–alkaline lakes and their influence on phytoplankton biomass. *Hydrobiologia* 584, 59–68, 2007.

Paerl HW. A comparison of cyanobacterial bloom dynamics in freshwater, estuarine and marine environments. *Phycologia*, 35, 25–35, 1996

Park HD, Iwami C, Watanabe MF, Harada KI, Okino T, Hayashi H. Temporal variabilities of the concentrations of intra- and extracellular microcystin and toxic *Microcystis* species in a hypertrophic lake, Lake Suwa, Japan (1991–1994). *Environ Toxicol Water Qual*, 13, 61–72, 1998.

Scheffer M, Rinaldi S, Gragnani A, Mur LR, van Nes EH. On the dominance of filamentous cyanobacteria in shallow turbid lakes. *Ecology*, 78, 272–282, 1997.

Stewart I, Seawright AA, Shaw GR. Cyanobacterial poisoning in livestock, wild mammals and birds – an overview. *Adv Experim Med Biol*, 619, 613–637, 2008.

Talling JF. The phytoplankton of Lake Victoria (East Africa). *Arch Hydrob Res Supp Limn*, 25, 229–256, 1987.

Vaitomaa J, Rantala A, Halinen K, Rouhiainen L, Tallberg P, Mokelke L, Sivonen K. Quantitative real-time PCR for determination of microcystin sythetase E copy numbers for *Microcystis* and *Anabaena* in lakes. *App Environ Microb*, 69, 7289–7297, 2003.

van Apeldoorn ME, van Egmond HP, Speijers GJA, Bakker GJI. Toxins of cyanobacteria. *Molec Nutr Food Res*, 51, 7–60, 2007.

Vareschi E. The ecology of Lake Nakuru (Kenya) III. Abiotic factors and primary production. *Oecologia*, 55. pp. 81–101, 1982.

Vonshak A, Tomaselli L. *Arthrospira* (*Spirulina*): systematic and ecophysiology. In: Whitton, B.A., Potts, M. (Eds.), The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer Academic Publishers, The Netherlands, pp. 505–522, 2000.

Watanabe MF, Harada KI, Matsuura K, Watanabe M, Suzuki M. Heptapeptide toxin production during the batch culture of two *Microcystis* species (Cyanobacteria). *J Appl Phycol*, 1, 161–165, 1989.

Wicks RJ, Theil PG. Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir. *Environ Sci Technol*, 24, 1413–1418, 1990.