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The rise of potentially toxin producing cyanobacteria in Lake Naivasha, Great African Rift Valley, Kenya

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ABSTRACT

Lake Naivasha, an important inland water ecosystem and a crucial freshwater resource in the Great African Rift Valley, has displayed clear signals of degradation in recent decades. We studied the phytoplankton composition and biomass levels in the period 2001–2013 and noted a progressive increase in the occurrence of potentially toxic cyanobacteria. Analyses for the presence of cyanotoxins such as microcystins (MC), cylindrospermopsin (CYN) and anatoxin-a (ATX-a) were carried out on samples collected in 2008–2013. Among the cyanotoxins tested, low concentrations of MC were detected in the lake. This is the first record of the occurrence of MC in Lake Naivasha. For the first time, molecular phylogenetic investigations of field clones of cyanobacteria from Lake Naivasha were carried out to establish the taxa of the dominant species. Amplification of the aminotrasferase (AMT) domain responsible for cyanotoxin production confirmed the presence of the *mcyE* gene belonging to the microcystin synthesis gene cluster in field samples containing *Microcystis* and *Planktothrix* species. These findings suggest that toxin producing cyanobacteria could become a threat to users of this over-exploited tropical lake in the near future.

1. Introduction

Lake Naivasha is – beside Lake Victoria – the second largest freshwater body in Kenya. It is the coolest and the freshest of the smaller lakes in the Gregory Rift Valley (Worthington and Worthington, 1933; Harper and Mavuti, 2004). Some four decades ago, Lake Naivasha was praised as a crystal clear gem in the floor of the Great African Rift Valley. Enthusiastic naturalists described the lake as a "bird-watcher's and fisherman's paradise near Nairobi" (Brown, 1971, loc. cit. p. 82; Willcock, 1974). However, in the last 70 years, the lake's water quality has deteriorated significantly. At the end of the 1930s, a higher sediment accumulation rate induced by increased human activities in the catchment of the lake was recorded (Stoof-Leichsenring et al., 2011). In this phase, a shift in the diatom assemblage from littoral and periphyton to planktonic taxa has taken place, indicating a changing light regime characterized by loss of water transparency (Stoof-Leichsenring et al., 2012). Furthermore, the lake ecosystem of Naivasha was considerably degraded by the introduction of alien species, and a multitude of impacts leading to eutrophication. All in all, during the last century, about 23 exotic species, fishes, invertebrates and macrophytes entered the lake (Gherardi et al., 2011). The invading species established a complicated network of interactions, which led to considerable fluctuations in the population density of the primary producers. Notable among the invasive species is the water hyacinth, which has the ability to outcompete other macrophytes, and dominant phytoplankton.

The presence of microphytes, such as colonial and filamentous cyanobacteria, in Lake Naivasha were recorded by early surveys of the Cambridge Expedition to East African lakes in 1930 (Rich, 1933). However, first mass developments of cyanobacteria were witnessed in 1980 (Kalff and Watson, 1986) and subsequently in 2005 and 2006 (Harper, 2006). Nowadays, mass developments of cyanobacteria are common components of the phytoplankton communities in Lake Naivasha and hence influence the lake's water quality.

Lake Naivasha is located in a tropical semi-arid zone and subjected to dramatic fluctuations in lake level. Water level changes covering or exposing several metres of shoreline within a period of a few months occur in response to drought or flood events

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(Becht et al., 2006). These fluctuations have been exacerbated by excessive abstraction of lake water to support the geothermal power industry, the horticulture industry and water supply to human settlements in the catchment area (Harper et al., 2011). Consequently the lake looses much more water than it receives from rainfall and other inflows. During periods of low water level, the swamp vegetation found along the shoreline is exposed and this results in a dramatic decline of macrophyte community dominated by papyrus *Cyperus papyrus* L. Presently, only 10% of the area previously inhabited by papyrus remains available as the natural filter of sediments and eroded materials from the catchment (Morrison and Harper, 2009). The inflowing rivers, especially the Malewa, transport large quantities of silt and nutrients from the deforested agricultural land into the unprotected lake. Surface runoff from urban settlements, untreated wastewater from horticultural farms, wildlife and domestic animal droppings also contribute to nutrient loading of the lake.

One major consequence of the sustained degradation of the lake's environment is the progressive eutrophication, which makes the lake more vulnerable to cyanobacterial blooms (Kitaka et al., 2002; Harper et al., 2011). The occurrence of dense blooms of colonial coccoid cyanobacteria is indicative of the potential production of cyanotoxins in this lake. Cyanotoxins create health hazards both for humans (through the consumption of drinking water and fish from the lake), to livestock and wild animals watering at the lake shore.

In this paper, we present data on; (i) abundance of cyanobacteria in comparison to the entire phytoplankton community in Lake Naivasha between 2001 and 2013, (ii) characterization of uncultured field clones of the dominant cyanobacteria, (iii) detection of toxin genes in field samples, and (iv) toxin content in field samples.

2. Materials and methods

2.1. Lake Naivasha

Lake Naivasha is a eutrophic freshwater lake located 1890 m above sea level in the Gregory Rift Valley approximately 80 km North of Nairobi, the capital of Kenya. The lake has a surface area of 100–150 km^2 and its main basin is ±6 m deep (Harper et al., 2011). Detailed characteristics of the lake as well as a comprehensive picture of its present ecological challenges are provided in the proceedings of an international conference on Lake Naivasha held in 1999 (Harper et al., 2002) and a review by Harper et al. (2011).

2.2. Sampling

The sampling point was at distance of 20 m from the shore near the jetty of the Elsamere Field Study Centre in the south-western bay of the lake (00°45′24″ S, 36°17′07″ E). Phytoplankton samples for this study were taken on 21 different dates at irregular intervals spread over the period 2001–2013. The samples were collected directly with a sample bottle from a depth of about 10 cm below the water surface. An aliquot was fixed with Lugol's solution for cell counting. Samples for morphological analyses were concentrated with a plankton net having a mesh aperture of 25 μm and fixed with formaldehyde. For molecular analyses, 1 L of the fresh sample was filtered through membrane filters with a pore-size of 0.6 μm (Schleicher & Schuell GmbH, Dassel, Germany). To determine the toxin content of the water, 125 mL was filtered through pre-weighed glass fibre filters (Whatman GF/C, Whatman International Ltd, Maidstone, England). After drying the filter with sample, it was weighed again, and the dry weight of the phytoplankton on the filter calculated. A polycarbonate filter holder SM 16510 (Sartorius AG, Göttingen, Germany) and a manual vacuum pump VacuMan (Bürkle GmbH, Bad Bellingen, Germany) were used to carry out filtration in the field.

2.3. Microscopy

Phytoplankton was counted according to Utermöhl (1958) in sedimentation chambers (Hydro-Bios Apparatebau GmbH, Kiel, Germany) under an inverted microscope Eclipse TS 100 (Nikon Corporation, Tokyo, Japan). The phytoplankton biomass was calculated by geometric approximations using the computerized counting program OPTICOUNT (Opticount, 2008). The specific density of phytoplankton cells was taken as 1 g cm^{-3}. The phytoplankton were photographically documented under a Nikon Eclipse E 600 light microscope using a Nikon digital camera DS-Fi1, and Nikon software NIS-Elements D (Nikon Corporation, Tokyo, Japan).

2.4. Molecular analyses

Two samples containing the dominant populations of potentially toxic cyanobacteria were collected from the pelagic habitat of Lake Naivasha. Under the light microscope, the sample collected on 14 November 2010 was confirmed to be dominated by *Microcystis* while the sample of 19 November 2011 was mainly composed of *Planktothrix*. The genomic DNA was extracted from field samples using Dynabead DNA Direct System I (Invitrogen/Dynal Biotech, Oslo, Norway) following the steps outlined in the manufacturer's manual. The polymerase chain reactions (PCR) were performed in a Peltier Thermal Cycler PTC 200 (MJ Research Inc., San Francisco, USA). The volume and concentrations of PCR cocktail used were as described by Dadheech et al. (2012). Primers CYA361f and CYA785r (Mühling et al., 2008) were employed for amplification of V3-V4 regions of 16S rRNA gene. Amplification of 16S rRNA gene fragment was carried out as follows: initial 3 min at 94 °C; 30 cycles of 30 s at 94 °C; 30 s at 55 °C; 45 s at 70 °C: and a final elongation step at 72 °C for 3 min. The primers PCβf and PCαr (Neilan et al., 1995) were used for amplification of beta and alpha subunits including intergenic spacer (*cpc*BA-IGS) of the phycocyanin operon using the PCR protocol described by Ballot et al. (2008). The amplification of the aminotrasferase (AMT) domain responsible for cyanotoxin production was done using HEPF and HEPR primers (Jungblut and Neilan, 2006) and a PCR protocol described earlier (Dadheech et al., 2009). The amplified products were cleaned using QIAquick PCR purification column according to manufacturer's protocol and examined on a 1% agarose gel. Cleaned PCR products were cloned using the Zero Blunt® Topo® PCR cloning kit (Invitrogen, Germany) according to manufacturer's instructions. The positive clones selected were PCR amplified and then cycle sequenced to retrieve the sequence of 16S rRNA gene fragment, *cpc*BA-IGS and AMT domain. The uncultured *Microcystis* sp. clones sequenced were designated as nav_16S_ micro (16S rRNA gene), nav_cpc_micro and nav_ mcyE _micro. Similarly, uncultured *Planktothrix* sp. clones sequenced were designated as nav_16S_plank, nav_cpc_plank and nav_mcyE_plank. Both strands were sequenced on ABI 3100 Avant Genetic Analyzer using BigDye Terminator v3.1 using Cycle Sequencing Kit (Applied Biosystems, Applera, Deutschland, GmbH, Darmstadt, Germany) as described in the manufacturer's manual.

The sequences of 16S rRNA gene, *cpc*BA-IGS and AMT domain belonging to *Microcystis* and *Planktothrix* taxa were retrieved from nucleotide NCBI database and aligned with sequences obtained in the present study using MUSCLE software (Edgar, 2004). Alignment was checked visually using the Manual Sequence Alignment Editor, Align v05/2008 (Hepperle, 2008). The phylogenetic trees were constructed by the maximum likelihood (ML) method using the program MEGA v5.0 (Tamura et al., 2011) with default settings,

applying a suitable model of nucleotide substitution. Confidence values for the edges of the maximum-likelihood tree were computed by bootstrapping of 1000 replications. The nucleotide sequences reported in this study have been deposited in the NCBI database under the GenBank accession numbers JX988500–JX988501 (16S rRNA gene), JX988502–JX988503 (*cpc*BA-IGS) and JX988504–JX988505 (*mcyE* gene).

2.5. Toxin analyses

Dried filters were wetted with water, frozen and thawed prior to extraction. One set of filters was extracted twice with 75% aqueous methanol for microcystins (MC) while the other was extracted twice with 0.1% formic acid for CYN, Deoxy-CYN and ATX-a. Each extraction step included ultrasonication for 15 min followed by shaking for 1 h. After centrifugation at 13,000 rpm for 10 min the supernatants were combined, dried by vacuum-centrifugation and frozen at −20 °C. Prior to analysis, samples were re-dissolved in 50% aqueous methanol (for MC) or 0.1% formic acid (for CYN, Deoxy-CYN and ATX-a) (Fastner et al., 1998; Selwood et al., 2007).

Microcystins were analyzed by high-performance photodiode array detection (HPLC-PDA) as described by Fastner et al. (1999). MCs, CYN and ATX were analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The analyses were carried out on an Agilent 2900 series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a API 5500 QTrap mass spectrometer (AB Sciex, USA) equipped with a turbo-ion spray interface.

MCs were analyzed according to Spoof et al. (2003). The extracts were separated using a Purospher STAR RP-18 end-capped column (30 mm × 4 mm, 3 μm particle size, Merck, Germany) at 30 °C. The mobile phase consisted of 0.5% formic acid (A) and acetonitrile with 0.5% formic acid (B) at a flow rate of 0.5 mL min^{-1} with the following gradient program: 0 min 25% B, 10 min 70% B, 11 min 70% B. The injection volume was 10 μL. Identification and quantification of the MCs ([Asp3]-MC-RR, MC-RR, MC-YR, [Asp3]-MC-LR, MC-LR, MC-LW, MC-LF, MC-LA, standards purchased at Enzo Life Sciences, Germany) was performed in the MRM (Multiple Reaction Monitoring) mode. The detection limits of different congeners ranged from 0.0003 to 0.003 μg L^{-1} based on a sample volume of 125 mL.

For the LC–MS/MS analysis of CYN, Deoxy-CYN and ATX-a, the extracts were separated using a 5 μm Atlantis C18, 2.1 mm × 150 mm column (Waters, USA) at 30 °C. The mobile phase consisted of water (A) and methanol (B) both containing 0.1 formic acid, and was delivered as a linear gradient from 1% to 25% B within 5 min at a flow rate of 0.25 mL min^{-1} (Haande et al., 2008). The injection volume was 10 μL. Identification of CYN, Deoxy-CYN and ATX-a was performed in the MRM (Multiple Reaction Monitoring) mode with the following transitions: CYN *m/z* 416.1 ($M+H^+$) to 194 and 416.1/176, D-CYN *m/z* 400.1 ($M+H^+$) to 194 and 400.1/320, ATX-a *m/z* 166.1 ($M+H^+$) to 149, 166.1/131, 166.1/91, 166.1/43. Quantification of CYN (National Research Council, Canada), D-CYN (Novakits, France) and ATX-a (Tocris, UK) was performed with the transitions *m/z* 416.1 ($M+H^+$) to 194, 400.1/194 and 166.1/43, respectively. The detection limits were 0.1 μg g^{-1} dw for CYN and D-CYN and 0.3 μg g^{-1} dw for ATX-a. The detection limits were 0.0002 μg L^{-1} for CYN and D-CYN and 0.001 μg L^{-1} for ATX-a based on a sample volume of 125 mL.

3. Results

3.1. Phytoplankton

The phytoplankton biomass in Lake Naivasha fluctuated widely from >5 mg L^{-1} to <70 mg L^{-1} (Fig. 1). The period 2001–2005 was characterized by considerably lower phytoplankton biomass compared to the period after 2006. The main phytoplankton groups were cyanobacteria, chlorophytes, desmids and diatoms. Other groups included the flagellated lineages of cryptophyes, dinophytes, and euglenophytes. During the period of our study, the dominance patterns among the cyanobacteria varied widely with tendency towards an increase in the populations of *Microcystis* and *Planktothrix* (Fig. 2).

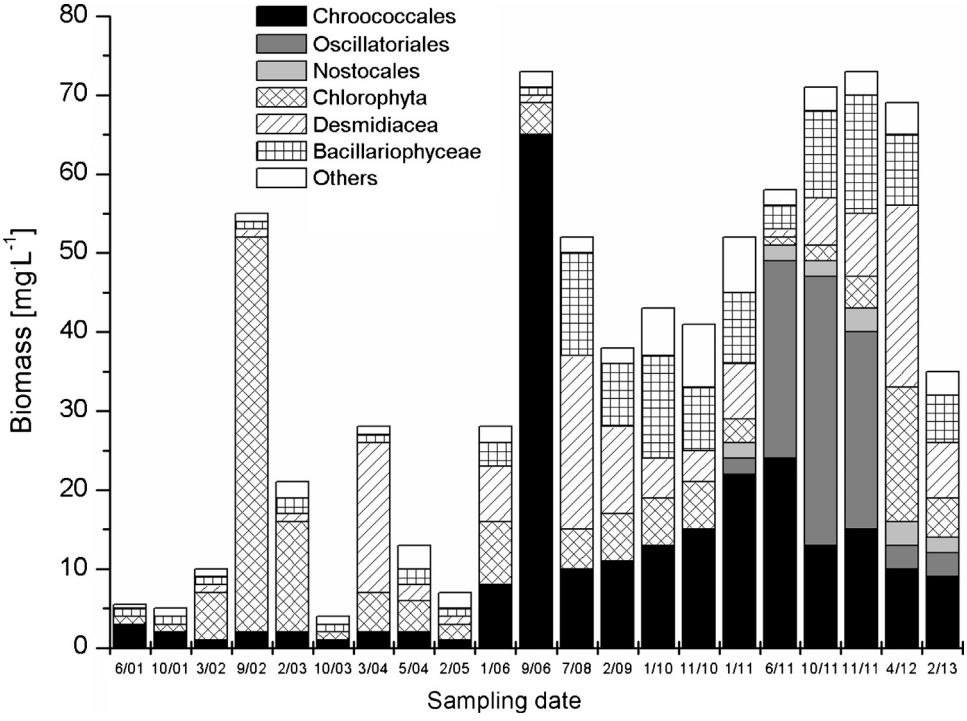

Fig. 1. Biomass and succession of main groups of phytoplankton in Lake Naivasha, 2001–2013.

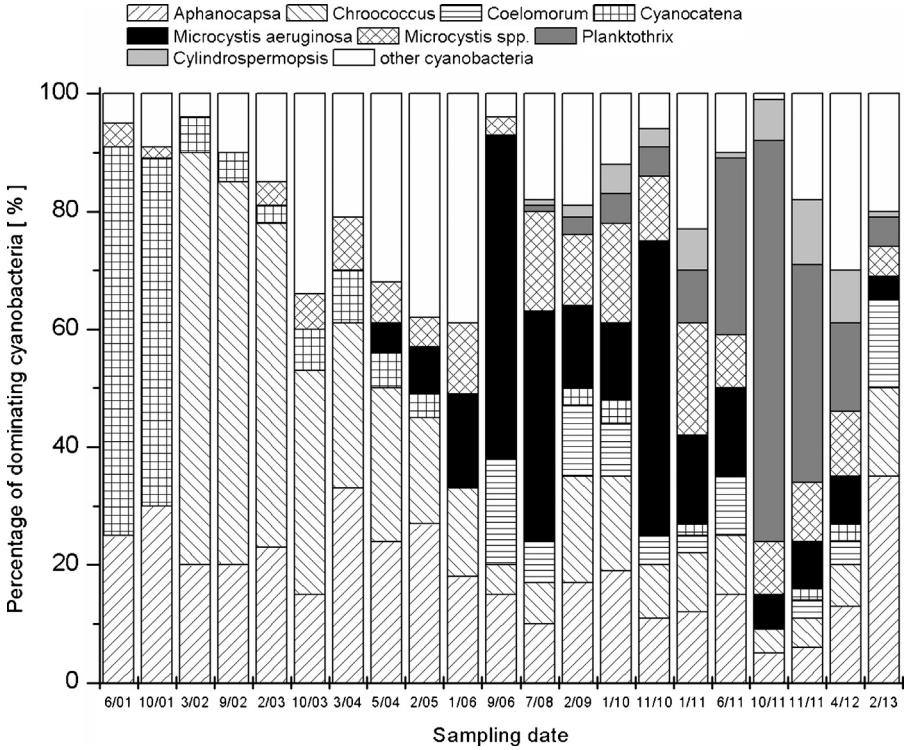

Fig. 2. Percentage contribution of members of cyanobacteria to the total cyanobacterial fraction of the phytoplankton in Lake Naivasha, 2001–2013.

The phytoplankton community of Lake Naivasha was generally very diverse with large differences between and within years. In most cases, the phytoplankton community was dominated by one species accompanied by three to six subdominants and about 50 other abundant species. In 2001, the characteristic taxon was the picoplanktonic cyanophyte *Cyanocatena planktonica* Hindák, which occurred in association with *Aphanocapsa elachista* West et West, the filamentous diatom *Aulacoseira granulata* (Ehrenberg) Simonsen and the chlorophyte *Pediastrum* div. spec. In 2002, dense blooms of the green "oil-alga" *Botryococcus terribilis* Komárek et Marvan established in the lake with a high biomass contribution of >50 mg L^{-1}, accompanied by *Chroococcus limneticus* Lemmermann. *Botryococcus* was also found in the subsequent years but its abundance was lower. In the period 2003–2005, the phytoplankton biomass was relatively low with a range from 5 to 28 mg L^{-1}. Comparatively higher biomass values were reached by the tychoplanktonic desmid *Gonatozygon* sp. whose biomass attained its maximum in March 2004 with >15 mg L^{-1}. In 2006, the cyanobacteria biomass rose to much higher levels reaching a maximum of 65 mg L^{-1} in September. *Microcystis aeruginosa* (Kützing) Kützing (Fig. 3A and B) contributed to about 55% of the biomass while the rest was due to other coccoid cyanobacteria that lacked aerotopes, especially *Aphanocapsa planctonica* (G.M. Smith) Komárek et Anagnostidis (Fig. 3A and B) and *Coelomoron pusillum* (Van Goor) Komárek (Fig. 3B). Interestingly, the masses of coccoid cyanobacteria that accumulated near the shoreline did not form the dense scums that are characteristic of *Microcystis*, but formed separated, knop-like grains.

In the following years (2008–2012), the biomass of coccoid cyanobacteria remained higher than 10 mg L^{-1}. However, they were accompanied by a diverse flora of other phytoplankton groups, especially desmids, green algae and diatoms. After 2008, in addition to *Microcystis aeruginosa*, which was still common in the lake, the species *Microcystis novacekii* Komárek & Compère (Fig. 3C) became prevalent in the lake. However, in November 2010, 50% of the cyanobacterial biomass was once again due to *M. aeruginosa* (Figs. 1 and 2). Furthermore, Oscillatoriales (*Planktothrix* sp.) and Nostocales (*Cylindrospermopsis* sp. and *Anabaenopsis* sp.) were observed in higher numbers. In November 2011, *Planktothix* cf. *mougeotii* (Fig. 3D) dominated the phytoplankton with a biomass contribution of 25 mg L^{-1}, and was accompanied by *Aphanocapsa planctonica*. However, the situation changed in 2012/2013 when heavy rains resulted in a rise in water level by 4 m. The rains diluted the lake water resulting in the partial replacement of the cyanobacteria by desmids, coccoid green algae and diatoms. Nevertheless, despite the lower phytoplankton biomass, coccoid cyanobacteria remained and were dominated by *Aphanocapsa planctonica* (Fig. 2).

3.2. Molecular analyses

Partial sequences of the 16S rRNA gene (V3 and V4 region, 390 bp), the phycocyanin operon (521–605 bp) and the AMT domain (421–471 bp) were obtained from uncultured field clones. The 16S rRNA gene tree of *Microcystis* showed a compact clade of sequences originating from various geographical regions and belonging to different species (Fig. 4). The Naivasha phylotype occurred in a sub-clade consisting of taxa from different continents. However, an earlier reported phylotype (FJ764655) from Lake Elmenteita, Kenya, formed a cluster in another subclade. The phylogenetic tree prepared with sequences of *cpc*BA-IGS exhibited different clades (Fig. 5). The main clade contained sequences of *Microcystis aeruginosa* from East Africa. However, the sequence of the Naivasha phylotype formed a separate lineage.

The phylogenetic tree of *Planktothrix* constructed with partial sequences of the 16S rRNA gene was divided into two main clades (Fig. 6). A tight clade comprised sequences from different species of *Planktothrix* except *P. pseudagardhii* Suda et M.M. Watanabe. The other clade was divided into two distinct sub-clades. Sub-clade IIa consisted of strains belonging to *P. pseudagardhii* and the sub-clade IIb was represented by *P. mougeotii* (Kütz.) Suda et M.M. Watanabe. The sequence of the clone from Naivasha occurred in sub-clade IIb

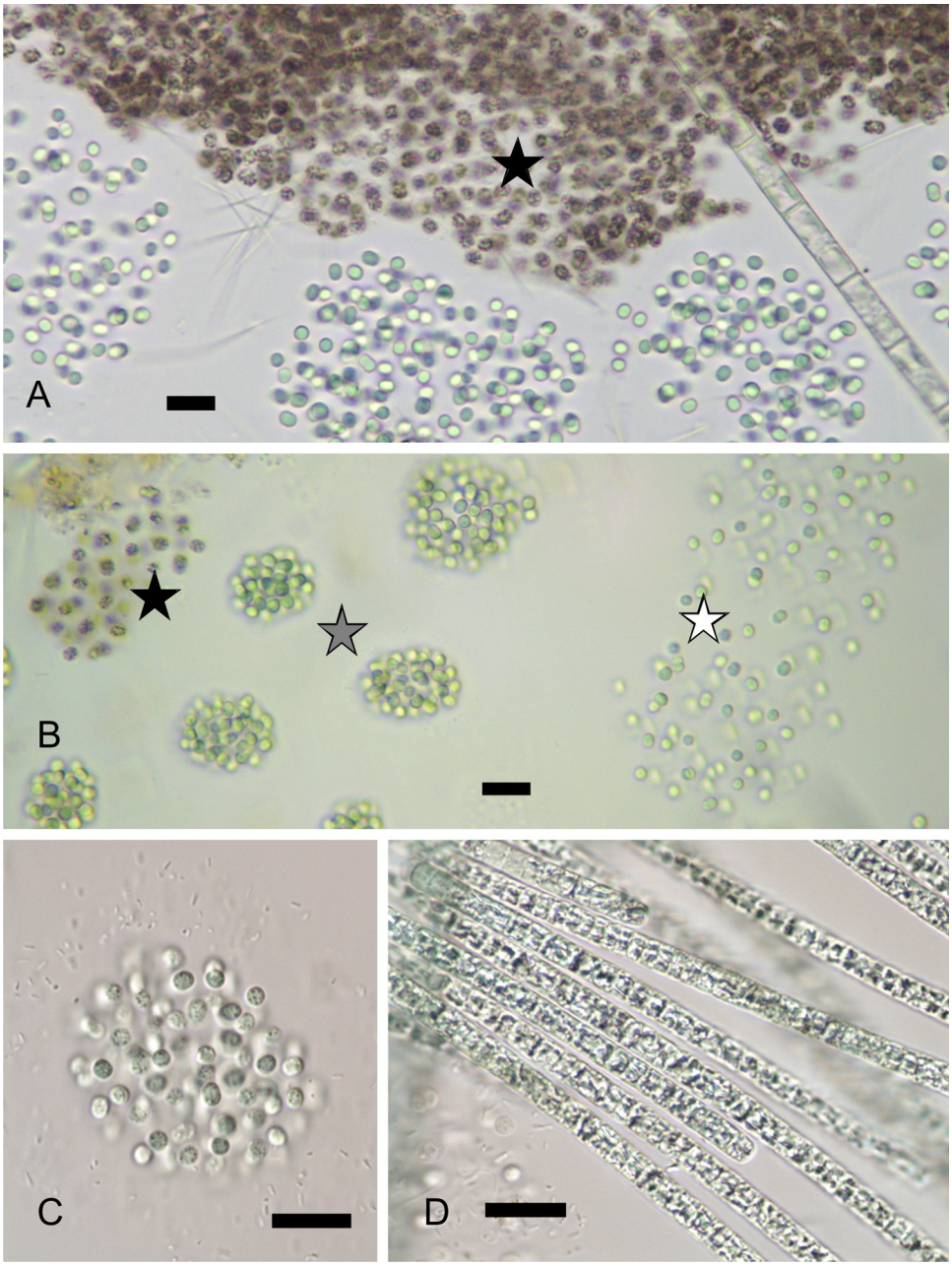

Fig. 3. Recent cyanobacterial key players in phytoplankton of Lake Naivasha. Scale bars = 10 μm). (A) *Micocystis aeruginosa* (black star), and *Aphanocapsa planctonica*, September 2006. (B) *Microcystis aeruginosa*, *Coelomoron pusillum* (grey star), and *Aphanocapsa planctonica* (white star), September 2006. (C) *Microcystis novacekii*, January 2011. (D) *Planktothrix* cf. *mougeotii*, and *Aphanocapsa planctonica*, November 2011.

with a high bootstrap support (Fig. 6). In the phylogenetic tree of the *cpc*BA-IGS, most of the sequences of strains originated from Europe as no sequence from the African continent was available in GenBank. The sequence of the Naivasha clone was divergent from available sequences and formed a separate lineage in the tree (Fig. 7).

The sequences of the AMT domain for *mcyE* gene obtained in this study from samples collected in 2010 and 2011 showed the highest similarity to *Microcystis* and *Planktothrix* respectively in BLASTN analysis. These sequences, together with retrieved closely related sequences from GenBank, were aligned, and the inferred phylogeny was determined. The phylogenetic tree exhibited a clear bifurcated topology with a clade including sequences mostly from *Microcystis* while the other clade was dominated by sequences of *Planktothrix* (Fig. 8). Both clades were supported by 100% bootstrap. The sequence of Naivasha clone belonging to *mcyE* gene of *Microcystis* occurred in the clade comprising sequences of *Microcystis* strains from several continents including the African continent (Kenya, Uganda and South Africa). The *mcyE* sequence of the *Planktothrix* clone fell into a clade with sequences of different species of *Planktothrix* and other member of Oscillatoriales. A sequence of the *mcyE* gene of *Planktothrix* from the African continent was not available for comparison.

3.3. Toxin analyses

While no microcystins could be detected by HPLC-PDA, LC–MS/MS analyses revealed the presence of low amounts of microcystins in the range of 0.001–0.041 $\mu g\,L^{-1}$ in all samples from 2008 to 2013 (Fig. 9, Table 1). Calculated per dry weight of phytoplankton

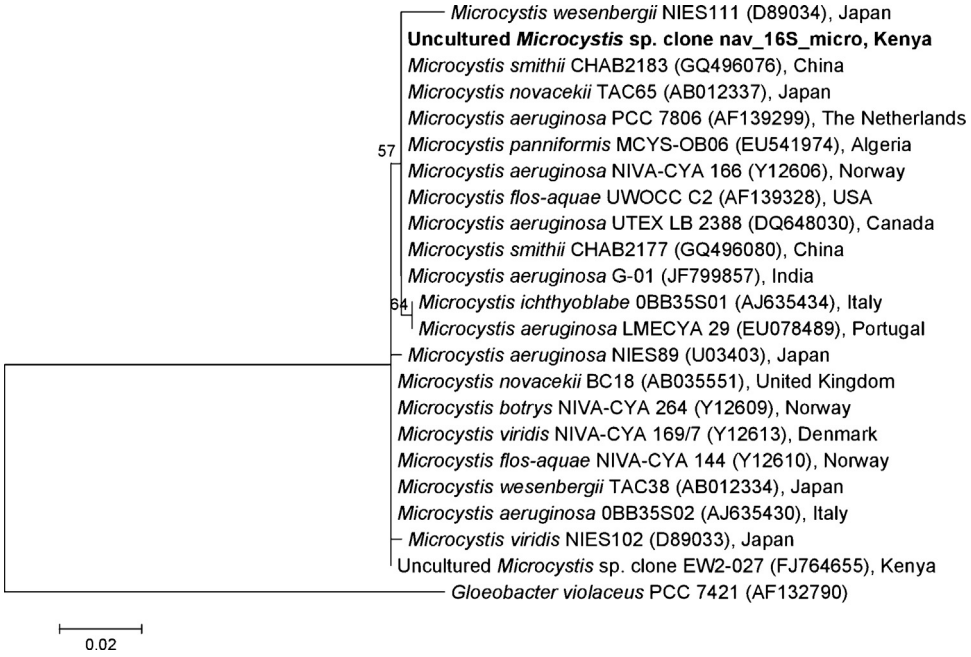

Fig. 4. Maximum likelihood tree of *Microcystis* sp. inferred from the partial nucleotide sequences of 16S rRNA gene (V3–V4 region). *Gloeobacter violaceus* PCC 7421 was used as an outgroup. The ML tree was derived using the K 2-parameter model. The numbers above branches indicate bootstrap support (>50%) from ML (1000) replicates.

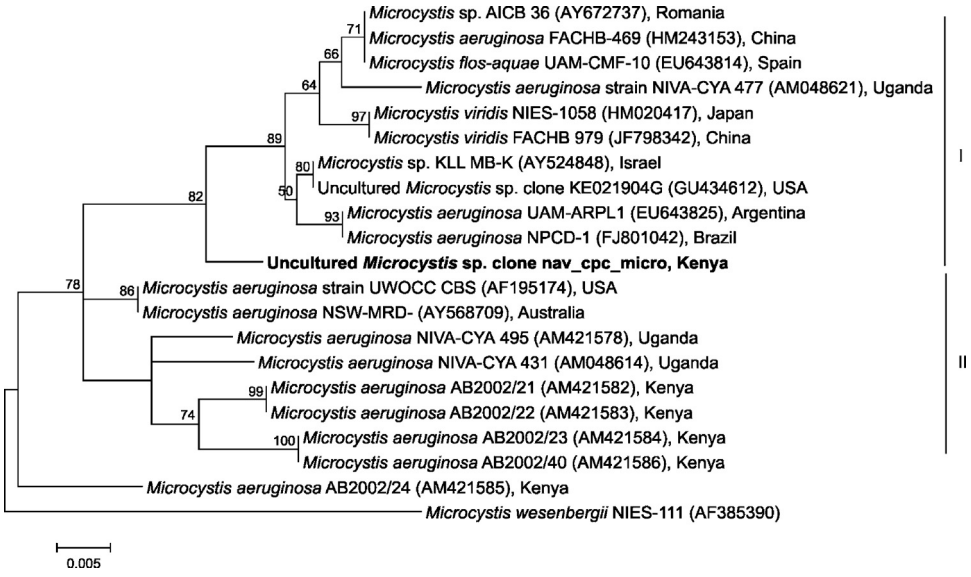

Fig. 5. Maximum likelihood tree of *Microcystis* sp. inferred from nucleotide sequences of the *cpc*BA-IGS region. *Microcystis wesenbergii* NIES-111 was used as an outgroup. The ML tree was derived using the K 2-parameter model. The numbers above branches indicate bootstrap support (>50%) from ML (1000) replicates.

Table 1
Toxin concentration [$\mu g\,L^{-1}$] in phytoplankton samples of Lake Naivasha.

Date	MC-LR	MC-RR	MC-LA	MC-YR
10.07.2008	<LOQ	n.d.	n.d.	n.d.
13.11.2010	0.041	<LOQ	<LOQ	n.d.
23.01.2011	0.026	0.004	<LOQ	n.d.
13.06.2011	0.030	0.011	<LOQ	<LOQ
30.10.2011	0.007	0.001	<LOQ	n.d.
19.11.2011	0.017	0.002	0.003	0.013
24.04.2012	0.006	0.001	<LOQ	n.d.
03.02.2013	0.015	0.008	<LOQ	n.d.

Limit of Quantification (LOQ, based on a sample volume of 125 mL): MC-LR 0.006 $\mu g\,L^{-1}$, MC-RR 0.001 $\mu g\,L^{-1}$, MC-YR 0.01 $\mu g\,L^{-1}$, MC-LA 0.002 $\mu g\,L^{-1}$.

biomass microcystins reached concentrations up to 0.1–0.3 $\mu g\,g^{-1}$ dw. Among the microcystins MC-LR, MC-RR, MC-YR and MC-LA were identified with MC-LR being the most abundant congener (Table 1). CYN, Deoxy-CYN and ATX-a were not detected in any of the samples.

4. Discussion

4.1. Phytoplankton

Earlier studies in Lake Naivasha identified more than 150 different phytoplankton species (Kalff and Watson, 1986; Hubble and Harper, 2002). A pattern characterized by a progressive

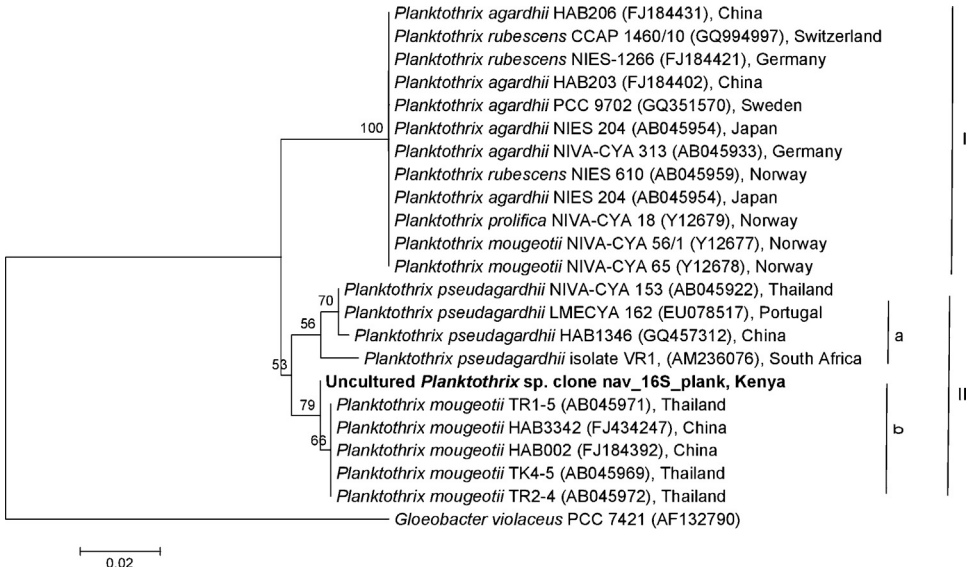

Fig. 6. Maximum likelihood tree of *Planktothrix* sp. inferred from the partial nucleotide sequences of 16S rRNA gene (V3–V4 region). *Gloeobacter violaceus* PCC 7421 was used as an outgroup. The ML tree was derived using the Jukes-Cantor model. The ML tree was derived using the K 2-parameter model. The numbers above branches indicate bootstrap support (>50%) from ML (1000) replicates.

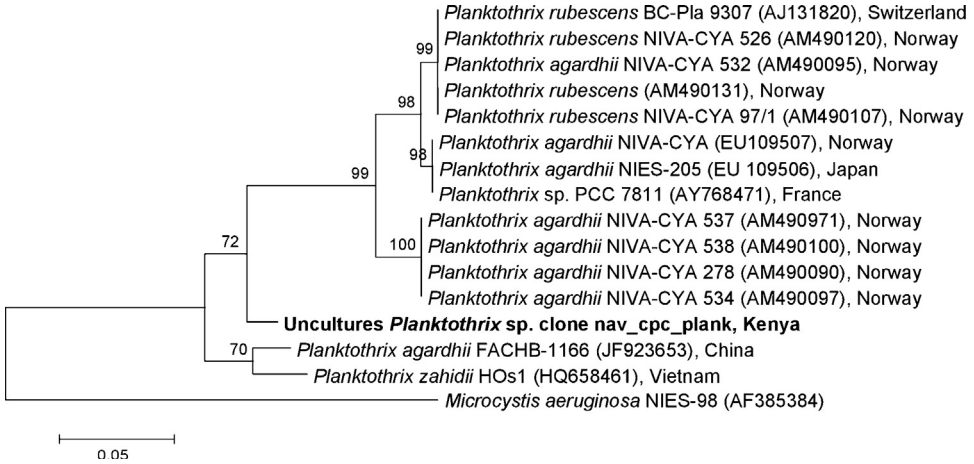

Fig. 7. Maximum likelihood tree of *Planktothrix* sp. inferred from nucleotide sequences of the *cpc*BA-IGS region. *Microcystis aeruginosa* NIES-98 was used as an outgroup. The ML tree was derived using the K 2-parameter model. The numbers above branches indicate bootstrap support (>50%) from ML (1000) replicates.

decrease of diatoms and increase of cyanobacteria has been demonstrated (Ballot et al., 2009). Based on the analysis of samples collected by Jenkin in 1929, Beadle in 1931 and Lind in 1964, Lind (1968) described Lake Naivasha as a *Melosira* lake (syn. of *Aulacoseira*). Although, *Microcystis aeruginosa* was present in these early samples, it occurred in low numbers. The first mass development of this species (14–53% of the total phytoplankton biomass of 10–20 mg L^{-1}) was recorded in March–June 1980 by Kalff and Watson (1986). Later in April 2006, Harper (2006) recorded the presence of masses of a *Microcystis*-like cyanobacterium in the southern bay of Lake Naivasha. In September 2006, we also observed a bloom of *Microcystis* and associated chroococcoid cyanobacteria which produced a biomass of 65 mg L^{-1}. However, no cyanotoxins were detected in the bloom. The first indication of the existence of toxic cyanobacteria in the lake was recorded in a culture of a toxic strain of *M. aeruginosa* from Lake Naivasha (Haande et al., 2007; Miles et al., 2012).

The process of eutrophication enhances mass developments of the potentially toxic cyanobacteria in water bodies worldwide (Heisler et al., 2008; Joung et al., 2011; O'Neil et al., 2012). Eutrophication in Lake Naivasha has resulted from a combination of different factors (Harper et al., 2011): numerous invasive animals and plants destabilize the food web, excessive water abstraction reduces the volume of water in the lake and concentrates the nutrients, and loss of the filter macrophytic swamp vegetation system resulting in enrichment through inflows and internal nutrient loads. The cyanobacteria responded to the eutrophication processes by mass developments in the lake.

4.2. Molecular analyses

The observed microscopic dominance of cyanobacterial taxa in Lake Naivasha in 2010 and 2011 is supported by evidence from molecular markers (16S rRNA gene and *cpc*BA-IGS region). In our study, the 16S rRNA gene phylogeny of *Microcystis* based on the variable regions V3–V4 showed a congruent topology of the tree to earlier phylogenetic trees with long sequences as different species of *Microcystis* formed a tight cluster (Otsuka et al., 1998; Neilan et al., 1997). A proposal to unify the five species of *Microcystis* into *M. aeruginosa* on the basis of molecular studies has been made

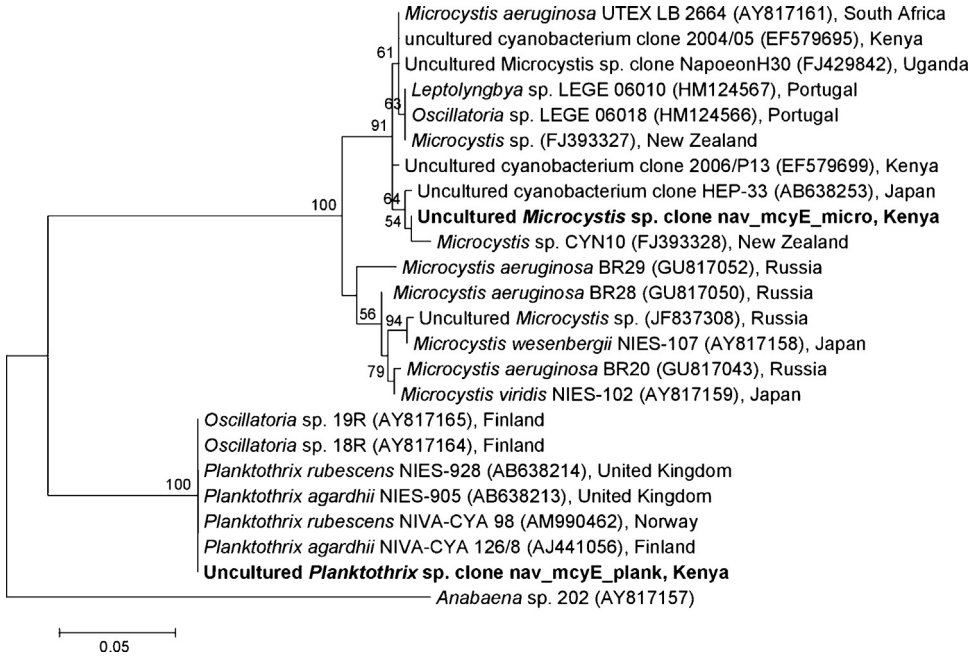

Fig. 8. Maximum likelihood tree based on partial nucleotide sequences of *mcyE* gene. *Anabaena* sp. 202 was used as an outgroup. The ML tree was derived using the K 2-parameter model. The numbers above branches indicate bootstrap support (>50%) from ML (1000) replicates.

(Otsuka et al., 2001). Phylogenetic analyses using 16S rRNA gene and 16S–23S internal transcribed spacer sequences have not identified any clear separations corresponding to the morphospecies concepts (Nguyen et al., 2012). Recently, the *cpc*BA-IGS locus was used as an effective molecular marker for the characterization of *M. wesenbergii* and other species of *Microcystis* (Tan et al., 2010). The *cpc*BA-IGS sequences of *Microcystis* formed two distinct clades (Fig. 5). Clade I was composed of different morphospecies, *M. aeruginosa*, *M. flos-aquae* (Wittr.) Kirchn., *M. viridis* (A. Braun) Lemm. and the clone from Naivasha, whereas clade II was composed exclusively of *M. aeruginosa*. The phylogenetic position of the clone obtained in this study is distant from previously investigated *Microcystis* isolates from Kenyan lakes including Lake Naivasha (Haande et al., 2007). Our study revealed the presence of genetic diversity within *M. aeruginosa* isolates in Kenyan water bodies. In the case of *Microcystis* sp., previous studies have also found different genotypes in a single water body (Bolch et al., 1997; Bittencourt-Oliveira et al., 2001; Haande et al., 2007). In contrast, several *Microcystis* sp. contained a single ITS genotype in reservoirs of Northern Ethiopia (Van Gremberghe et al., 2011a). The clustering of the Naivasha phylotypes with strains from other continents show that *Microcystis* sp. strains have no geographic restriction. The high overall diversity and wide global distribution of common ITS types in combination with the lack of phylogeographic structures suggest that this species might have a truly cosmopolitan distribution (Van Gremberghe et al., 2011b).

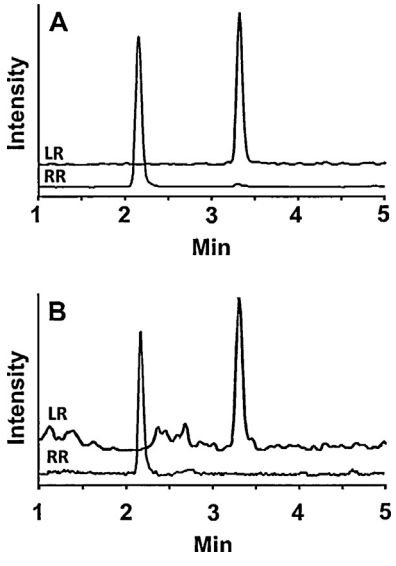

Fig. 9. Reconstructed chromatogram of (A) standard microcystin-LR and microcystin-RR and (B) the sample from January 2011.

Our study provides additional information on the phylogeny of the genus *Planktothrix* and reveals for the first time the presence of this taxon in Lake Naivasha. The *Planktothrix* clone obtained from Lake Naivasha clustered with *P. mougeotii* from China and Thailand. In the 16S rDNA phylogenetic tree, *P. mougeotii* formed a separate clade. The phylogenetic trees based on three gene regions (16S rRNA, rbcLX and rpoC1 genes) also exhibited a distinct clade represented by *P. mougeotii* (Lin et al., 2010). The *cpc*BA-IGS region phylogentic tree clearly indicated divergence between the Naivasha clone and other *Planktothrix* species from different parts of the world. The sequence obtained in this study may belong to *P. mougeotii* as *cpc*BA-IGS locus sequence of this species is not available in GenBank. Further molecular studies on isolated strains of this genus from different lakes of Kenya will be required to provide a clear picture.

The *mcyE* region has been extensively used for screening cyanobacterial strains and/or field samples (Vaitomaa et al., 2003; Rantala et al., 2006; Hodoki et al., 2012) and is considered to be the most robust predictor of toxicity in *Planktothrix* strains (Mbedi et al., 2005). We could get *mcyE* gene fragment sequences of two dominant cyanobacterial taxa from environmental samples collected in 2010 and 2011, and those are related to *Microcystis* and *Planktothrix* respectively. The sequences that can be related to the AMT domain for *mcyE* of *Microcystis* and *Dolichospermum* have

been detected from other Kenyan lakes (Dadheech et al., 2009). The risk of cyanotoxin producing strains of *Microcystis* and *Planktothrix* in Lake Naivasha is evident from our study.

4.3. Toxin analyses

Both *Microcystis* and *Planktothrix* were identified as potential microcystin producers in Lake Naivasha (*mcyE*) in this study. Globally, *Microcystis* strains and populations have been reported to contain chiefly microcystin-LR, -RR and -YR, with some strains showing similar amounts of all three variants, and some being dominated by one of them (e.g. Vasconcelos et al., 1995; Fastner et al., 1999). In our study, we found that MC-LR was the most abundant congener with MC-RR, MC-YR and MC-LA being less abundant as is the case with microcystin profiles for other African lakes (Willén et al., 2011; Sitoki et al., 2012). In contrast, Okello et al. (2010) recorded an unusual pattern with congeners such as MC-RY and [NMeSer7]-MC-YR being the most abundant microcystins in Ugandan lakes. Since no commercial standards exist for such unusual microcystins, they are usually not picked up by routine LC–MS/MS methods. We therefore analyzed all samples by HPLC-PDA as this method allows for the identification as well as quantification of microcystins for which no standards are available due to their characteristic adsorption spectra. However, no other microcystins variants were detected in our samples.

The detection of microcystins in the range from 0.001 to 0.041 $\mu g\,L^{-1}$ and 0.1 to 0.3 $\mu g\,g^{-1}$ dw by this study provides the first evidence of toxins presence in field samples from Lake Naivasha. Compared to other cyanotoxin findings from East Africa, these values are very low. Mwaura et al. (2004) measured 0–2.85 $\mu g\,L^{-1}$ microcystins in Kenyan reservoirs. In Lake Victoria maximum microcystin concentrations of 0.992 $\mu g\,L^{-1}$ (Sekadende et al., 2005) and 81 $\mu g\,L^{-1}$ (Sitoki et al., 2012) were detected. Microcystin concentrations ranging from 39 to 41 $\mu g\,g^{-1}$ dw, 310 to 19 800 $\mu g\,g^{-1}$ dw and 0 to 280 $\mu g\,g^{-1}$ dw were measured in Lake Victoria, Lake Baringo and sewage oxidation ponds of Nakuru respectively (Krienitz et al., 2002, Ballot et al., 2003, Kotut et al., 2010). In Ethiopian freshwaters, concentrations of 0–42 000 $\mu g\,g^{-1}$ dw were detected (Willén et al., 2011).

The microcystin concentration in a water body is determined by (i) the biovolume or biomass of the toxic cyanobacteria, (ii) the abundance of toxic genotypes and (iii) the toxin content of the toxic genotypes. In Lake Naivasha, the most probable reason for the comparatively low microcystin concentrations is a low abundance of microcystin-producing genotypes in the populations of *Microcystis* and *Planktothrix*. Several studies have demonstrated a high variability (0.01–100%) in the abundance of toxic genotypes in field populations of *Microcystis* and *Planktothrix* both over time within a lake as well as between lakes (e.g. Briand et al., 2008; Davis et al., 2009). Okello et al. (2010) showed a positive linear relationship between the microcystin content and the proportion of microcystin-producing (*mcyB*) genotypes in *Microcystis* populations from five African lakes. In contrast to this, the influence of environmental factors on the cellular microcystin production, which is mostly not more than a factor 2–3, appears to be of minor importance for the actual microcystin concentration in a water body (Kardinaal and Visser, 2005).

The toxin concentrations recorded in Lake Naivasha between 2010 and 2012 were too low to pose a serious threat to animals and humans. However, the presence of toxigenic genotypes in the populations of *Microcystis* and *Planktothrix* in the lake as well as an increase in their biomass in the last decade suggests that higher microcystin concentrations may occur any time soon. We therefore suggest that the monitoring of toxin levels in the lake should continue. The public should also be made aware of the potentially harmful cyanobacterial blooms.

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