Occurrence of Microcystin-Producing Cyanobacteria in Ugandan Freshwater Habitats

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ABSTRACT: Microcystins (MCs) are cyclic heptapeptides, which are the most abundant toxins produced by cyanobacteria in freshwater. The phytoplankton of many freshwater lakes in Eastern Africa is dominated by cyanobacteria. Less is known, however, on the occurrence of MC producers and the production of MCs. Twelve Ugandan freshwater habitats ranging from mesotrophic to hypertrophic conditions were sampled in May and June of 2004 and April of 2008 and were analyzed for their physicochemical parameters, phytoplankton composition, and MC concentrations. Among the group of the potential MC-producing cyanobacteria, *Anabaena* (0–10^7 cells ml^{-1}) and *Microcystis* (10^3–10^7 cells ml^{-1}) occurred most frequently and dominated in eutrophic systems. A significant linear relationship ($n = 31$, $r^2 = 0.38$, $P < 0.001$) between the *Microcystis* cell numbers and MC concentration (1.3–93 fg of MC $cell^{-1}$) was observed. Besides [MeAsp^3, Mdha^7]-MC-RR, two new MCs, [Asp^3]-MC-RY and [MeAsp^3]-MC-RY, were isolated and their constitution was assigned by LC-MS^2. To identify the MC-producing organism in the water samples, (i) the conserved aminotransferase domain part of the *mcy*E gene that is indicative of MC production was amplified by general primers and cloned and sequenced, and (ii) genus-specific primers were used to amplify the *mcy*E gene of the genera *Microcystis*, *Anabaena*, and *Planktothrix*. Only *mcy*E genotypes that are indicative of *Microcystis* sp. were obtained via the environmental cloning approach (337 bp, 96.1–96.7% similarity to the *Microcystis aeruginosa* strain PCC7806). Accordingly, only the *mcy*E primers, which are specific for *Microcystis*, revealed PCR products. We concluded that *Microcystis* is the major MC-producer in Ugandan freshwater. Environ Toxicol 25: 367–380, 2010.

Keywords: eutrophication; *Anabaena*; *Microcystis*; toxicity; genetic diversity; *mcy*E; *mcy*B gene; microcystin-RY

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INTRODUCTION

Uganda, with a total area of 241 000 km^2 astride the equator between latitude 1°30′S and 4°N and longitude 29°30′E and 35°E, has a significant amount of freshwater resources covering ~16% of its surface area (World Water Assessment Programme, UNESCO, 2006). Some of these freshwater bodies are either naturally eutrophic, e.g. L. George (Viner and Smith, 1973), or are becoming increasingly eutrophic due to human activities, e.g. L. Victoria (Verschuren et al., 2001). In general, cyanobacteria have often been found to increase under eutrophic conditions.

Blooms formed by cyanobacteria have recently been reported throughout many parts of L. Victoria (Alweny, 2007). These blooms are a cause of concern to local water consumers, policy makers, as well as the National Water and Sewerage Cooperation (NW&SC) that supplies treated water nationally. In their statement to the Ugandan parliament, NW&SC indicated that 11 billion Ugandan shillings (7 million US dollars) would be required in 2008 to treat green water. The fishermen reported that areas covered by cyanobacteria accumulating on the surface could not be used for fishing anymore. Small dead fish were also seen floating close to the shoreline, while the larger fish caught within the area appeared weak and stressed. In East Africa, massive fish kills were observed in the Nyanza Gulf of Lake Victoria (Kenya) in 1984, coinciding with the occurrence of cyanobacterial blooms (Ochumba, 1990). The toxins produced by cyanobacteria include neurotoxins, hepatotoxins, and irritant-dermal toxins. All of these cause acute harm to humans, animals, and wildlife after exposure (Chorus et al., 2000; Carmichael, 2001).

The most widely distributed hepatotoxins in freshwater are the microcystins (MCs). They are produced by the planktonic genera *Anabaena*, *Anabaenopsis*, *Microcystis*, *Nostoc*, and *Planktothrix* (Sivonen and Jones, 1999). MCs are cyclic heptapeptides and share the common structure cyclo(–D-$Ala^{(1)}$–$X^{(2)}$–D-$MeAsp^{(3)}$–$Z^{(4)}$–$Adda^{(5)}$–D-$Glu^{(6)}$–$Mdha^{(7)}$), where X and Z are variable L-amino acids (e.g., MC-LR refers to leucine and arginine in the variable positions of this peptide), D-MeAsp is D-erythro-ß-iso-methyl-aspartic acid, Adda is (2*S*, 3*S*, 8*S*, 9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4, 6-dienoic acid, and Mdha is *N*-methyl-dehydroalanine. Structural variation has been reported most frequently at positions 2, 4, and 7 of the molecule resulting in over 60 structural variants that are characterized from field samples or isolated strains (Diehnelt et al., 2006). The elucidation and characterization of the gene cluster that is involved in MC synthesis significantly increased our understanding of the regulation of toxin synthesis in cyanobacteria (Dittmann et al., 1997; Tillett et al., 2000). It was only then that the investigation of the genetic basis of MC production both in the laboratory and in the environment became possible.

Considering the repetitive occurrence of algal blooms reported in Ugandan lakes, understanding the distribution of toxin-producing cyanobacteria in Ugandan freshwaters is essential. This will form the basis of estimating the health risks associated with cyanobacterial occurrence. A previous study reported the isolation of four MC-producing *Microcystis* sp. strains from Kenya and Uganda (Haande et al., 2007). Since it is generally known that the bias introduced by strain isolation may be substantial (Wilson et al., 2005), we believe that field studies are required to provide the information that is necessary to estimate a potential health risk. This article reports the first results on the occurrence of MC-producing cyanobacteria in relation to the environmental conditions and the production of MC in twelve Ugandan freshwaters ranging from mesotrophic to hypertrophic conditions.

MATERIALS AND METHODS

Sampling and Nutrient Analyses

Depth integrated water samples were taken in May and June 2004 from twelve freshwater bodies (site nos. 1–12, Table I, Fig. 1) using a 2-L horizontal van Dorn sampler by sampling the water column every meter. To confirm the results obtained during this period, the site nos. 4, 5, 7, 10, and 11 were resampled in April 2008. In parallel, algae were concentrated by taking vertical hauls with a phytoplankton net (30 μm mesh size). Samples were filtered using Whatman GF/C filters. For chlorophyll *a* analysis, filters were stored frozen (−20°C). For MC and DNA analysis, filters were dried overnight (50°C) and stored frozen (−20°C). The filtrates were subsequently filtered through membrane filters (0.45 μm) for the analyses of dissolved nutrients. Soluble reactive phosphorus (SRP), nitrate (NO_3-N), and ammonia (NH_4-N) were determined by using the ammonium molybdate method (Wetzel and Likens, 2000), sodium-salycilate method (Müller and Wiedemann, 1955), and indophenol blue method (Krom, 1982), respectively. Soluble reactive silica was determined as yellow molybdate-silicic acid (Wetzel and Likens, 2000). The total phosphorus (TP) was determined by persulphate digestion from the aliquots of the samples and analyzed as SRP. The study (EC650) was approved by the Uganda National Council for Science and Technology in conjunction with the Uganda Wildlife Authority.

Lake Trophic States Definition

The trophic state was assigned according to Vollenweider and Kerekes (1982): Mesotrophic: TP (10–35 $\mu g\ L^{-1}$), chlorophyll *a* (3–8 $\mu g\ L^{-1}$), secchi disc depth (3–1.5 m); eutrophic: TP (35–100 $\mu g\ L^{-1}$), chlorophyll *a* (8–25 $\mu g\ L^{-1}$), secchi disc depth (1.5–0.7 m); hypertrophic: TP ($\geq$100 $\mu g\ L^{-1}$), chlorophyll *a* ($\geq$25 $\mu g\ L^{-1}$), secchi disc depth ($\leq$0.7 m).

TABLE I. Environmental parameters in Ugandan freshwaters sampled during May and June 2004

Site	Site No.	Coordinates		Area (km^2)	Depth (m)	Temperature (°C)		Conductivity ($\mu S\ cm^{-1}$)		pH		Secchi (cm)		SRP ($\mu g\ L^{-1}$)		TP ($\mu g\ L^{-1}$)		NH_4-N ($\mu g\ L^{-1}$)		NO_3-N ($\mu g\ L^{-1}$)		SRSi ($mg\ L^{-1}$)		Chl *a* ($\mu g\ L^{-1}$)		Trophic State
		Latitude	Longitude			May	June	May	June	May	June	May	June	May	June	May	June	May	June	May	June	May	June	May	June	
Swamp (between Kiranzi and Nabingora)	1	00°29.533′N	31°09.800′E	0.001	0.5	28	20	66	93	7		10		26	15	167	109	18	17	290	59	27.3	7.8	0	0	–
Nyabikere Crater Lake	2	00°29.990′N	30°19.729′E	0.04	20	25	24	287	288	7		110		119	70	425	335	1729	1983	40	24	28.4	17.7	8	13	E
Nkuruba Crater Lake	3	00°31.020′N	30°18.178′E	0.03	16	24	24	387	373	7	8		314	41	34	82	71	506	850	20	11	4.3	5.7	7	9	E
Lake George (Kahenge)	4	00°03.026′N	30°03.274′E	270	2.4	28	25	343	345	10	10	10	20	14	41	138	191	18	52	205	71	34.9	27.2	128	78	H
Lake Edward (Katwe)	5	00°09.096′S	29°53.073′E	11.5	4	28	26	590	592	10	9	30	40	12	8	132	115	26	10	80	64	8.6	10.5	44	23	H
Nkugute Crater Lake	6	00°33.086′S	30°10.362′E	0.025	20	25	25	103	120	9	8			16	7	57	23	24	8	20	11	0.7	0.1	5	5	M
Lake Mburo	7	00°39.106′S	30°56.537′E	158	4	26	24	122	136	10	8	30	30	14	12	125	161	19	13	140	46	8.3	8.0	37	40	H
Lake Nabugabo	8	00°21.283′S	31°52.789′E	214	4.5	25	25	20	20	8	8	100	90	2	8	25	29	94	18	80	19	3.2	2.2	8	11	M
Lake Victoria (Bunjako)	9	00°04.340′S	32°07.520′E	60.1	5	23	25	82	108	7	9	120	120	3	5	57	51	11	8	25	16	0.6	0.1	10	8	E
Lake Victoria (Murchison)	10	00°16.911′N	32°38.398′E	18	4	26	26	100	100	6		80	80	5	8	122	105	28	25	150	199	1.0	0.4	13	23	E
Lake Victoria (Napoleon)	11	00°24.177′N	33°14.756′E	66.1	18	27	26	94	98		9	100	110	3	7	70	75	19	5	45	14	0.3	0.1	11	10	E
Pond (Jinja)	12	00°29.227′N	33°12.068′E	0.001	1	31	26	1134	1095		9	11	20	25	12	222	213	24	18	60	46	18.2	21.8	33	22	H

M, mesotrophic; E, eutrophic; H, hypertrophic; SRP, soluble reactive phosphorus; SRSi, soluble reactive silica; NH_4-N, ammonia nitrogen; NO_3-N, nitrate nitrogen.

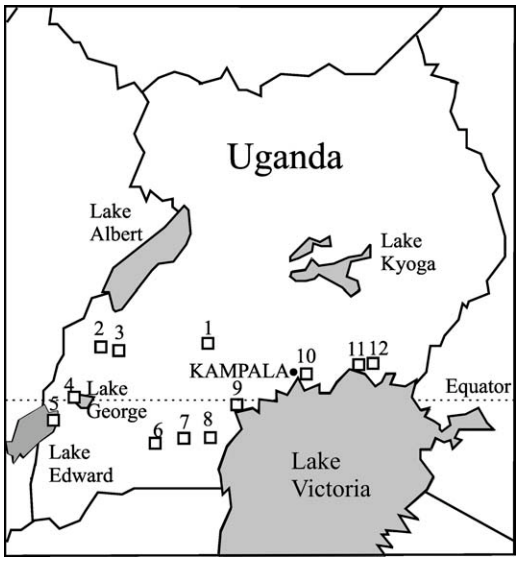

Fig. 1. Location of the study area and sampling sites in Uganda. Numbering of the sampling sites as in Table I.

Phytoplankton Composition and Abundance

Chlorophyll *a* analysis was based on hot ethanol extraction (International Organisation for Standardisation, 1992). Algae were counted from Lugol fixed samples by using the inverted microscope, as described (Wetzel and Likens, 2000). Cyanobacteria were identified according to the morphological keys published by Talling (1987), Komárek and Kling (1991), and Komárek and Anagnostidis (1999). At least 400 specimens of the dominant phytoplankton genera were counted at 400× magnification. Most of the genera were counted as single cells [*Anabaena*, *Chroococcus*, *Merismopedia*, *Microcystis*; the pennate diatom *Nitzschia* and unidentified centric diatoms, green algae (including desmids) and cryptomonads]. Filamentous cyanobacteria were counted as filaments (*Planktolyngbya*, *Pseudanabaena*). *Aphanocapsa* was counted as colonies: Cells from 20 of these colonies were counted to determine the average number of cells per colony. *Microcystis* was counted as single cells and could be discriminated from other cyanobacteria by morphological characters, such as cell size [2.5–3.6 ± 0.2–4.9] and the formation of small colonies. To compare the reproducibility of the *Microcystis* counting method between two different labs (Kampala *vs*. Mondsee), a number of depth-integrated and plankton net samples (nos. 4, 5, 7, and 11) were repeatedly counted in September 2004 (Kampala) and in November 2006 (Mondsee). On average, the *Microcystis* cell numbers as determined in November 2006 accounted for 76 ± 40% (1 SD, $n = 13$) of the cell numbers as determined in September 2004. This implies that *Microcystis* cells could be reliably identified among other phytoplankton under an inverted microscope. The biovolume was calculated based on the measured dimensions of cells/filaments (Wetzel and Likens, 2000).

Microcystin Analysis

The extraction of MC from filters was performed as described (Kurmayer et al., 2003). One hundred microliters of the extracts were injected into high performance liquid chromatography coupled to diode array detection (HPLC-DAD), and MCs were identified by their retention time and characteristic absorption spectra according to Lawton et al. (1994) and Fastner et al. (1999). MCs were quantified at 240 nm, and the concentrations of all the MC variants were determined as concentration equivalents of [D-MeAsp, D-Mdha]-MC-LR (Cyanobiotech GmbH, Berlin, Germany). HPLC peaks identified as MC were collected manually and were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as described by Kurmayer et al. (2004) and identified by the postsource decay (PSD) fragment structure analysis as described by Erhard et al. (1997) and Fastner et al. (1999). The constitution of the new MC variants was assigned by ESI-MS and ESI-MS^2 experiments that were performed on a Q-TOF Ultima mass spectrometer (Waters, Milford, MA) equipped with a nanospray source and operated in the positive ionization mode under the control of MassLynx 4.1. The sample was loaded into a PicoTip Emitter (New Objective, Woburn, MA). Single MS measurements were followed by MS^2 experiments on the selected precursor ions. Fragmentation was operated by manually increasing the collision energy (4–60) to provide optimum fragment ion coverage over the mass range. External calibration was performed with glu-fibinopeptide-B (standard error 50 ppm), and an additional postcalibration was applied when needed. Assignment of fragments by collision-induced dissociation (CID) enhanced PSD was carried out following a published procedure (Diehnelt et al., 2006).

Genetic Analysis of the Field Samples

For DNA extraction, 25–200 mL of the depth integrated samples and 25–60 mL of the plankton net samples were filtered using Whatman GF/C filters. The filters were dried in the oven at 50°C overnight. DNA was extracted by the phenol–chloroform method as described (Kurmayer et al., 2003). The DNA was diluted to 2–20 ng μL^{-1} for PCR analyses.

A part of the *mcy*E gene region was amplified by PCR using the HEPF/HEPR primers (Jungblut and Neilan, 2006). These primers bind to the conserved regions of the aminotransferase domain and, therefore, amplify the *mcy*E genes of all the MC-producing cyanobacteria (472 bp). Subsequently, the genus-specific primers *mcy*E-F2/F8 (specific for *Microcystis*; 370 bp), *mcy*E-F2/12R (specific for *Anabaena*;

370 bp), and *mcy*E-F2/plaR3 (specific for *Planktothrix*; 370 bp) were used to detect the respective MC-producing species (Rantala et al., 2006). These primers bind to the adenylation domain of the *mcy*E gene, which is responsible for the activation of the strictly conserved glutamic acid residue during MC synthesis (Tillett et al., 2000).

For three sites (nos. 4, 7, and 11) sampled in 2008, the PCR products that were obtained by using the HEPF/HEPR primer pair were cloned using the pDrive Cloning Vector system (Qiagen, VWR, Austria) according to the manufacturer's instructions. For the construction of a clone library, clones were picked for each of the samples and analyzed by using restriction fragment length polymorphism (RFLP) using two restriction enzymes (*BsuR*I and *Tru1*I) following standard protocols. Using the NEBcutter program (Vincze et al., 2003), the restriction of *mcy*E of the genus *Microcystis* (472 bp, PCC7806, AF183408) resulted in three fragments (restriction type A: 176/160, 92, 18/16/8 bp) that were visualized using ethidium-bromide staining and electrophoresis in 2.0% agarose in 0.5× TBE-buffer. In contrast, the same restriction analysis for *mcy*E (472 bp) of the genus *Anabaena* (strain *Anabaena* 90, AY212249) resulted in two fragments only [352, 110, 8 bp (invisible)], denoted as the restriction type B. While the *mcy*E gene is generally more conserved and useful to detect all MC-producing cyanobacteria, this gene region cannot reveal the genetic diversity within a certain genus on a subpopulation level (Rantala et al., 2006; Jungblut and Neilan, 2006). Consequently, the tox4F/tox4R primers (Kurmayer et al., 2002) amplifying the first adenylation domain of the *mcy*B gene in *Microcystis* (*mcy*BA1, 1313 bp) were used to estimate the genetic diversity within the MC-producing *Microcystis* subpopulation. The tox4F/tox4R PCR products were cloned and the clones were screened by restriction using *BsuR*I. Following the NEBcutter program, the *BsuR*I digestion of *mcy*BA1 (1313 bp) of the *Microcystis* strain PCC7806 [AF183408, representing *mcy*B1(B), Mikalsen et al., 2003] resulted in two fragments (restriction type I: 973, 340 bp). In contrast, the recombination type *mcy*B1(C) (e.g., strain HUB524, Z28338) revealed one much longer and one much shorter fragment (restriction type II: 1236, 77 bp).

From the same sites, twelve *Microcystis* strains were isolated using standard plating procedures as has been described (Okello, 2004). Six strains were found to contain *mcy*B (strains nos. 1B5, 2D6, 18A8, 20A2, 6C5, and 20A5; access nos. EU014158–EU014163), and this *mcy*B genotype was not digested by *BsuR*I (restriction type III). Both *mcy*E and *mcy*B environmental sequences that were obtained from various environmental clones identified by RFLP were submitted to the DDBJ/EMBL/GenBank database (access nos. FJ429838–FJ429844).

To analyze all the field samples for the occurrence of potentially MC-producing *Microcystis*, a PCR test that allows for the detection of lowest *Microcystis* cell densities was required. To check for the quality of the extracted DNA and the presence of potential PCR inhibitors, a gene region part of a housekeeping gene was amplified in parallel to the PCR amplifying the *mcy*B gene. Primers specified to amplify the intergenic spacer region of the phycocyanin operon (PC-IGS) of *Microcystis* sp. were designed by aligning the sequences of PC-IGS (576 bp) from the twelve freshly isolated *Microcystis* strains (strains nos. R14, 1B5, 2D6, 18A8, R50, 2A3, 20A2, 21A1, 17B7, 6C5, 19G6, and 20A5; access nos. EU014146–EU014157). In addition, 128 *Microcystis* sequences of PC-IGS from the DDBJ/EMBL/GenBank (June 10, 2007) were included in a multiple sequence alignment (ClustalW2) to design the primer pair: MaPCnewfwd, 5′-GGAGCTTCCGTAGCTGC-3′ (57.6°C), and MaPCnewrev, 5′-TGCAATAAGTTTCCTACGGT-3′ (53.5°C), yielding a total amplification fragment of 192 bp. Pilot experiments showed that these primers were specific to PC-IGS of *Microcystis*. The six freshly isolated *Microcystis* strains that were found to contain *mcy*B (1312 bp) were aligned with eleven sequences from entries in the DDBJ/EMBL/GenBank (June 10, 2007) representing the two recombination types that were reported within *mcy*B of *Microcystis* sp. [*mcy*B1 (B), *mcy*B1(C), Mikalsen et al., 2003]. The following primers were designed: Ma*mcy*Bnewfwd, 5′-GGATATCCTCTCA GATTCGG-3′ (57.3°C), and Ma*mcy*Bnewrev, 5′-CTGATG TATAAATAACATAGGCTAAA-3′ (55.3°C), yielding a total amplification fragment of 188 bp for both recombination types (Mikalsen et al., 2003). For both PC-IGS and the *mcy*B primers, the lower limit of detection was equivalent to two cells of *Microcystis* strain 18A8.

In general, PCR amplifications from field samples were carried out in 20 μL volumes containing 2 μL PCR buffer (Qiagen, Austria), 1.2 μL $MgCl_2$ (25 mM), 0.6 μL deoxynucleotide triphosphates (10 μM each, MBI Fermentas, Germany), 1 μL of each primer (10 pmol), 0.1 μL Taq DNA polymerase (Qiagen), 13.6 μL sterile Millipore water, and 0.5 μL of 2–20 ng μL^{-1} DNA. The PCR cycling conditions for the two separate regions of the *mcy*E gene were performed as described (Jungblut and Neilan, 2006; Rantala et al., 2006). For the PCR amplifying *Microcystis*-specific PC-IGS and *mcy*B, the thermal cycling protocol included an initial denaturation step at 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. PCR products (4 μL of the reaction mix) were visualized by ethidium–bromide staining and electrophoresis in 2.0% agarose in 0.5× TBE-buffer.

RESULTS

Trophic Characterization of the Sampling Sites

In the swamp sample (no. 1), the turbidity was high (secchi depth < 10 cm; Table I) and no chlorophyll *a* was detected. The three Crater lakes (nos. 2, 3, and 6) and L. Nabugabo

(no. 8) showed low concentrations of chlorophyll *a* (5–13 μg L^{-1}) resulting in high transparency (>1 m) suggesting a mesotrophic status. In two of the Crater Lakes (nos. 2 and 3), however, high TP concentrations were recorded, co-occurring with high SRP and NH_4-N concentrations. Therefore, they were classified as eutrophic lakes. All three sites (nos. 9–11) from L. Victoria were classified as eutrophic. The highest chlorophyll *a* values were recorded in L. George (no. 4), L. Edward (no. 5), and L. Mburo (no. 7), and in Jinja pond (no. 12), which indicates hypertrophic conditions. Correspondingly, the secchi depths were the lowest (<0.5 m) and the pH was the highest under hypertrophic conditions.

Phytoplankton Species Composition and Abundance

The total biovolume estimated via the microscopical counting correlated significantly with the chlorophyll *a* concentration (R^2 = 0.76, n = 37). Five phytoplankton classes were observed: Cyanobacteria, green algae, desmids, diatoms, and cryptomonads [Fig. 2(A)]. Cyanobacteria occurred in all the water bodies. The other phytoplankton classes were generally of minor importance. The Crater Lakes (nos. 2 and 3) and mesotrophic L. Nabugabo (no. 8) had the lowest biovolume of cyanobacteria and mostly contained diatoms, i.e., centric diatoms and *Nitzschia* (nos. 2 and 3) or green algae/desmids, i.e., *Chlorella*, *Cosmarium*, and *Staurastrum* (no. 8). Cyanobacterial dominance was only observed under eutrophic and hypertrophic conditions (nos. 4, 5, 7, and 9–12), including the genera *Anabaena*, *Aphanocapsa*, *Chroococcus*, *Merismopedia*, *Microcystis*, *Planktolyngbya*, and *Pseudanabaena* [Fig. 2(B)]. *Anabaena* was present in the Crater lakes (nos. 2 and 3) and in the deep eutrophic L. Victoria (nos. 9–11), while *Aphanocapsa* occurred in both the hypertrophic shallow lakes (nos. 4, 5, and 7) and the deep eutrophic L. Victoria (nos. 9–11). *Microcystis* was most abundant in the hypertrophic shallow lakes (nos. 4, 5, and 7). In summary, the dominance of cyanobacteria was positively related with the trophic situation.

Microcystin Analyses of the Field Samples

MCs were detected in plankton net samples only, i.e., in five plankton net samples in 2004 (no. 4, May; no. 5, May and June; no. 7, May and June) and in all five plankton net

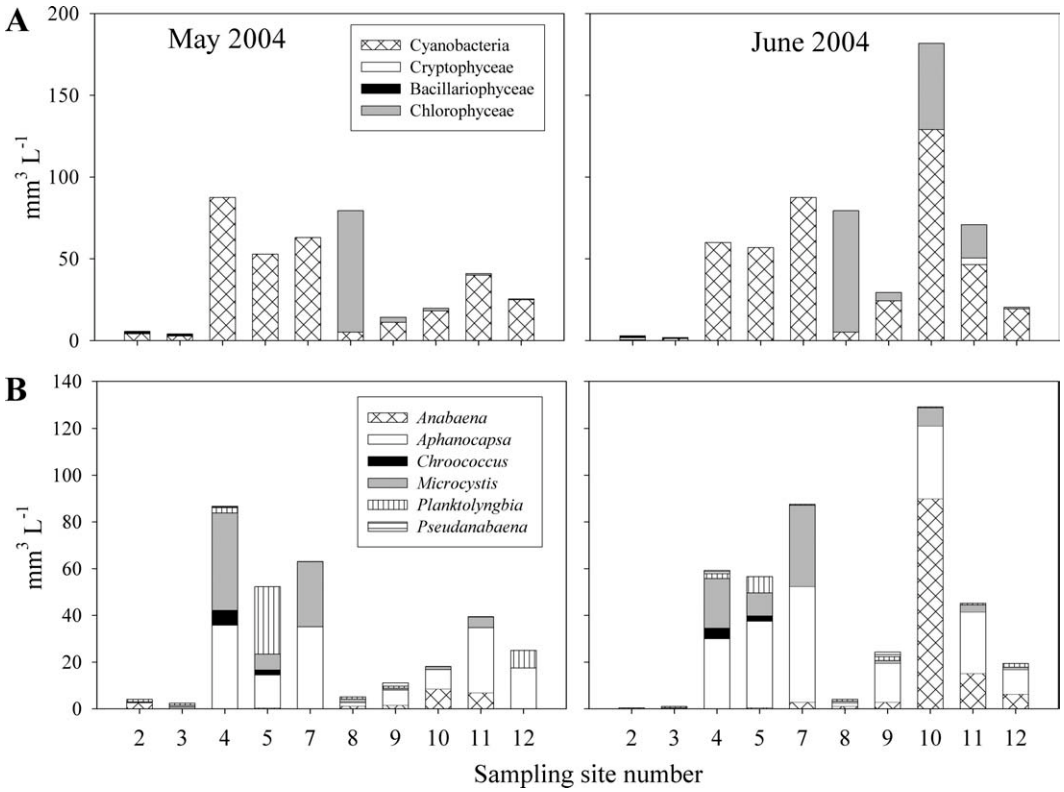

Fig. 2. (A) Phytoplankton composition and (B) cyanobacterial composition in various Ugandan freshwater habitats during May and June 2004. Numbering of the sampling sites as in Table I. No phytoplankton was detected at sites (1) and (6). Only taxa contributing >5% of the total phytoplankton biovolume (upper panel) or cyanobacterial biovolume (lower panel) are shown.

samples in 2008. MC 1 [molar weight 1038 ($M+H^+$)] occurred in all samples containing MC and eluted at 15.6 min and was identified by PSD analysis of the fractionated peak as [D-MeAsp^3, Mdha^7]-MC-RR. In addition, the MCs extracted from the plankton net samples (L. Edward, L. George, L. Mburo, June 2004, 2008) eluted at 21.7 min [MC 2, molar weight 1031 ($M+H^+$)] and 23.2 min [MC 3, molar weight 1045 ($M+H^+$)]. MCs 2 and 3 were also identified in aqueous methanolic extracts of the *Microcystis* strains 2D6, 6C5, and 18A8 isolated from L. Edward and L. Mburo. The original UV spectrum as well as the first-order derivative of the peak apex showed a close match with [MeAsp^3, Mdha^7]-MC-YR, which is available from the spectrum library. However, MC 2 and MC 3 differed in the retention time from [MeAsp^3, Mdha^7]-MC-YR. The sequence and constitution of MC 2 and MC 3 were investigated by LC-MS^2. CID-enhanced PSD produced 34 and 33 fragments for MC 2 and MC 3, respectively, detected with a mass accuracy of <50 ppm (Tables II and III). The amino acid at position 7 was assigned as either Mdha or Dhb. The distinction between these isomers is difficult by MS, as D labeling experiments on MC 2 and MC 3 were inconclusive. The assignment of MC 2 as [Asp^3]-MC-RY was based on fragments such as $[Ala\text{-}Arg\text{-}Asp+H]^+$ (343.1826), $[Ala\text{-}Arg+H]^+$ (228.1474), $[Mdha \text{ or } Dhb\text{-}Ala\text{-}Arg+H]^+$ (311.1879), $[Tyr\text{-}Adda\text{-}Glu+H]^+$ (606.3105), $[Tyr\text{-}Adda+H]^+$ (477.2565), and many others (Table II). The assignment of MC 3 as [MeAsp^3]-MC-RY was based on fragments such as $[Ala\text{-}Arg\text{-}MeAsp+H]^+$ (357.2023), $[Ala\text{-}Arg\text{-}NH_3+H]^+$ (211.1247), $[Mdha \text{ or } Dhb\text{-}Ala\text{-}Arg+H]^+$ (311.1938), $[MeAsp\text{-}Tyr+H]^+$ (293.1255), $[Tyr\text{-}Adda+H]^+$ (477.2628), $[Arg\text{-}MeAsp\text{-}Tyr+H]^+$ (449.2364), and many others (Table III). These two MC variants have not yet been described in the literature, to the best of our knowledge, and thus constitute new MCs.

There was a linear relationship between the *Microcystis* cell concentrations and the total MC concentrations: $y = 0.03x + 3.54$ ($R^2 = 0.38$, $n = 31$, $P < 0.001$, Fig. 3), where

TABLE II. Composition of ions in the MS^2 spectrum of the $[M+H]^+$ ion of [Asp^3]-MC-RY

Composition and Sequence	Calculated *m/z*	Measured *m/z*	Error (ppm)
M (+ H^+)	1031.5197	1031.5197	0.0
M (− CO) (+ H^+)	1003.5248	1003.5244	−0.4
Glu-(Mdha or Dhb)-Ala-Arg-Asp (+ H^+)	555.2522	555.2643	21.8
Glu-(Mdha or Dhb)-Ala-Arg (+ H^+)	440.2252	440.2386	30.4
Glu-(Mdha or Dhb)-Ala-Arg (− COOH) (+ H^+)	395.2276	395.2124	−38.5
Glu-(Mdha or Dhb) (+ H^+)	213.0870	213.0879	4.2
(Mdha or Dhb)-Ala-Arg-Asp (+ H^+)	426.2096	426.2177	19.0
(Mdha or Dhb)-Ala-Arg-Asp (− NH_3) (+ H^+)	409.1831	409.1936	25.7
(Mdha or Dhb)-Ala-Arg (+ H^+)	311.1826	311.1879	17.0
(Mdha or Dhb)-Ala-Arg (− CO) (+ H^+)	283.1877	283.1771	−37.4
(Mdha or Dhb)-Ala-Arg (− NH_3) (+ H^+)	294.1561	294.1605	15.0
(Mdha or Dhb)-Ala (+ H^+)	155.0815	155.0824	5.8
(Mdha or Dhb)-Ala (− CO) (+ H^+)	127.0866	127.0899	26.0
Ala-Arg-Asp-Tyr-Adda (+ H^+)	819.4400	819.4226	−21.2
Ala-Arg-Asp (+ H^+)	343.1724	343.1826	29.7
Ala-Arg (+ H^+)	228.1455	228.1474	8.3
Arg-Asp-Tyr (+ H^+)	435.1987	435.2142	35.6
Arg-Asp-Tyr (− COOH) (+ H^+)	390.2010	390.1989	−5.4
Arg-Asp (+ H^+)	272.1353	272.1411	21.3
Arg (+ H^+)	157.1084	157.1098	8.9
Asp-Tyr (+ H^+)	279.0975	279.1034	21.1
Asp-Tyr (− CO) (+ H^+)	251.1026	251.1096	27.9
Tyr-Adda-Glu-(Mdha or Dhb)-Ala-Arg (+ H^+)	916.4927	916.4935	0.9
Tyr-Adda-Glu-(Mdha or Dhb)-Ala-Arg (− CO) (+ H^+)	888.4978	888.5056	8.8
Tyr-Adda-Glu-(Mdha or Dhb) (+ H^+)	689.3545	689.3567	3.2
Tyr-Adda-Glu (+ H^+)	606.3174	606.3105	−11.4
Tyr-Adda (+ H^+)	477.2748	477.2565	−38.3
Adda-Glu-(Mdha or Dhb)-Ala-Arg-Asp (− NH_3) (+ H^+)	851.4298	851.4353	6.5
Adda-Glu-(Mdha or Dhb)-Ala-Arg-Asp (− NH_3) (− 134 Adda) (+ H^+)	717.3565	717.3604	5.4
Adda-Glu-(Mdha or Dhb) (− NH_3) (+ H^+)	509.2647	509.2621	−5.1
Adda-Glu-(Mdha or Dhb) (− NH_3) (− 134 Adda) (+ H^+)	375.1915	375.1996	21.6
Adda-Glu-(Mdha or Dhb) (− CO) (− NH_3) (− 134 Adda) (+ H^+)	347.1966	347.2035	19.9
Adda (− NH_3) (− 134 Adda) (+ H^+)	163.1118	163.1127	5.5
134Adda (+ H^+)	135.0804	135.0824	14.8

TABLE III. Composition of ions in the MS^2 spectrum of the $[M+H]^+$ ion of [$MeAsp^3$]-MC-RY

Composition and Sequence	Calculated *m/z*	Measured *m/z*	Error (ppm)
M (+ H^+)	1045.5353	1045.5353	0.0
M (− CO) (+ H^+)	1017.5404	1017.5602	19.5
Glu-(Mdha or Dhb)-Ala-Arg-MeAsp (+ H^+)	569.2678	569.2905	39.9
Glu-(Mdha or Dhb)-Ala-Arg (− NH_3) (+ H^+) or (Mdha or Dhb)-Ala-Arg-MeAsp (− NH_3) (+ H^+)	423.1987	423.2173	44.0
Glu-(Mdha or Dhb)-Ala-Arg (− COOH) (+ H^+)	395.2276	395.2204	−18.2
Glu-Mdha + H	213.0870	213.0916	21.6
(Mdha or Dhb)-Ala-Arg-MeAsp-Tyr-Adda (+ H^+) or Tyr-Adda-Glu-(Mdha or Dhb)-Ala-Arg (+ H^+)	916.4927	916.5134	22.6
(Mdha or Dhb)-Ala-Arg-MeAsp-Tyr (− NH_3) (+ H^+)	586.2620	586.2884	45.0
(Mdha or Dhb)-Ala-Arg-MeAsp (+ H^+) or Glu-(Mdha or Dhb)-Ala-Arg (+ H^+)	440.2252	440.2413	36.6
(Mdha or Dhb)-Ala-Arg-MeAsp (− CO) (+ H^+) or Glu-(Mdha or Dhb)-Ala-Arg (− CO) (+ H^+)	412.2303	412.2466	39.5
(Mdha or Dhb)-Ala-Arg (+ H^+)	311.1826	311.1938	36.0
(Mdha or Dhb)-Ala-Arg (− NH_3) (+ H^+)	294.1561	294.1656	32.3
(Mdha or Dhb)-Ala-Arg (− CO) (+ H^+)	283.1877	283.1843	−12.0
(Mdha or Dhb)-Ala (+ H^+)	155.0815	155.0850	22.6
(Mdha or Dhb)-Ala (− CO) (+ H^+)	127.0866	127.0922	44.1
Ala-Arg-MeAsp-Tyr-Adda (+ H^+)	833.4556	833.4562	0.7
Ala-Arg-MeAsp (+ H^+)	357.1880	357.2023	40.0
Ala-Arg (− NH_3) (+ H^+)	211.1190	211.1247	27.0
Arg-MeAsp-Tyr (+ H^+)	449.2143	449.2364	49.2
Arg-MeAsp-Tyr (− COOH) (+ H^+)	404.2167	404.2209	10.4
Arg-MeAsp (+ H^+)	286.1509	286.1632	43.0
Arg (+ H^+)	157.1084	157.1132	30.6
MeAsp-Tyr (+ H^+)	293.1131	293.1255	42.3
Tyr-Adda-Glu-(Mdha or Dhb)-Ala-Arg (+ H^+)	916.4927	916.5134	22.6
Tyr-Adda-Glu-(Mdha or Dhb)-Ala-Arg (− CO) (+ H^+)	888.4978	888.5134	17.6
Tyr-Adda (+ H^+)	477.2748	477.2628	−25.1
Adda-Glu-(Mdha or Dhb)-Ala-Arg-MeAsp (− NH_3) (+ H^+)	865.4454	865.4628	20.1
Adda-Glu-(Mdha or Dhb)-Ala-Arg-MeAsp (− NH_3) (− 134 Adda) (+ H^+)	731.3722	731.3859	18.7
Adda-Glu-(Mdha or Dhb) (− NH_3) (+ H^+)	509.2647	509.2813	32.6
Adda-Glu-(Mdha or Dhb) (− NH_3) (− 134 Adda) (+ H^+)	375.1915	375.2071	41.6
Adda-Glu-(Mdha or Dhb) (− CO) (− NH_3) (− 134 Adda) (+ H^+)	347.1966	347.2095	37.2
Adda (− NH_3) (− 134 Adda) (+ H^+)	163.1118	163.1154	22.1
134Adda (+ H^+)	135.0804	135.0852	35.5

y is the MC concentration in ng mL^{-1} and *x* is the cell concentration mL^{-1}. In contrast, the MC concentrations were found to vary independently from the *Anabaena* cell concentrations.

Identification of Microcystin Producers and Genetic Diversity

With the exception of a plankton net sample from L. Mburo (June 2004), all the samples that contained detectable MCs revealed PCR products for *mcy*E by using HEPF/HEPR primers. To identify the MC-producing genus, further PCR analyses were performed using the *Microcystis* (*mcy*E-F2/F8)-, *Anabaena* (*mcy*E-F2/12R)-, and *Planktothrix* (*mcy*E-F2/plaR3)-specific primers. In all of the cases, the *mcy*E products that are indicative of *Microcystis* were obtained from the plankton net samples (Table IV), while no PCR products that are indicative of *mcy*E of *Anabaena* were obtained.

Three samples (lakes George, Mburo, and Victoria-Napoleon, nos. 4, 7, and 11) revealed abundant *Microcystis* but also showed a frequent occurrence of *Anabaena* [Fig. 2(B)]. Using the HEPF/HEPR primers, the *mcy*E gene was amplified and the clones were analyzed by RFLP. In the vast majority of the clones ($n = 165$), only restriction type A, which is indicative of *Microcystis*, was found (Table V). No restriction type B, which is indicative of *mcy*E of *Anabaena*, was obtained. Only in L. Mburo, three clones were not digested (restriction type C). Those sequences could not be assigned and consequently restriction type C was

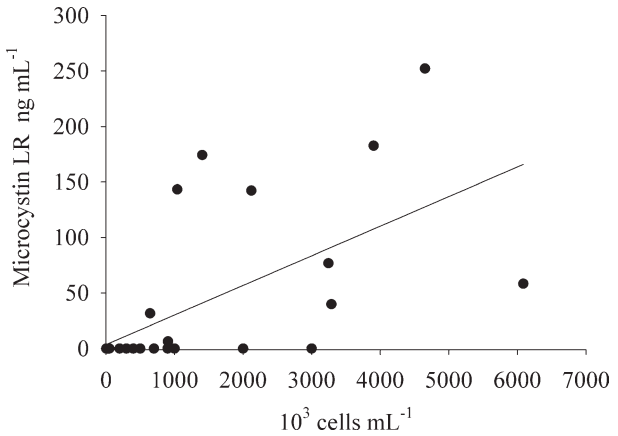

Fig. 3. Relationship between the MC concentrations (in ng mL^{-1} of MC-LR equivalents) and the concentration of cells mL^{-1} of *Microcystis* during May and June 2004 and April 2008. For details on the regression curve, see the text.

considered an unspecific PCR product. Eleven clones of restriction type A were sequenced (337 bp) and revealed a 96.1–96.7% similarity to the *M. aeruginosa* strain PCC7806, AF183408.

To characterize the genetic diversity within MC-producing *Microcystis*, the first adenylation domain of the *mcy*B gene was amplified by PCR, cloned, and then the clones were screened by RFLP. Restriction type I, which is indicative of *mcy*B1(B) and restriction type III (undigested), occurred, while restriction type II, which is indicative of *mcy*B1(C), was not detected. Three environmental clones that were assigned to restriction type I had the highest sequence similarity to the strain PCC7806 (1190 bp, 97.2–98.1% similarity). In contrast, clones that were assigned to restriction type III had the highest sequence similarity to the strains 18A8, 20A2, 20A5, and 2D6 (1190 bp, 97.4–98.4%). Restriction type I differed by 24.7–25.7% of the nucleotides (1190 bp) from restriction type III.

Distribution of Microcystin-Producing *Microcystis*

Using the PC-IGS primers that were designed in the present study, the distribution of *Microcystis* was studied in all the field samples that have been analyzed for phytoplankton composition under the microscope (Table IV). PCR products were obtained from all the samples containing *Microcystis* as inferred from the microscope. *Vice versa*, in the swamp (no. 1) and in Nyabikere Crater Lake (no. 2), no PC-IGS of *Microcystis* was detected, which was in accordance with the microscopical analysis. Except for the depth-integrated sample from the Nkuruba Crater Lake, all the samples containing PC-IGS, indicative of *Microcystis*, were found to contain *mcy*B.

DISCUSSION

Environmental Parameters and Phytoplankton Composition

We recorded high concentrations of nutrients, but low concentrations of chlorophyll *a* and phytoplankton in the two Crater lakes (nos. 2 and 3). Correspondingly, Kizito et al. (1993) reported low chlorophyll *a*, but high nutrient concentrations for Lake Nkuruba (no. 3). The highest NH_4-N concentration that was observed in the Crater lakes may indicate significant vertical stratification, since the nitrification of ammonia to nitrate requires oxygen. During a 2-year study, Chapman et al. (1998) reported that the average anoxia (0 mg L^{-1} O_2) in Lake Nkuruba (no. 3) was down to 9 m. In this study, it is possible that a part of the anoxygenic water column with the highest ammonium concentrations was sampled resulting in a higher average ammonium concentration due to the vertical integration down to 15 m.

Despite its shallowness, L. Nabugabo (no. 8) also had the lowest phytoplankton density, which was dominated by green algae and desmids. The soils of the catchment area of Lake Nabugabo have a very low salt content, possibly resulting in low calcium carbonate levels and the lowest conductivity (Beadle, 1981). A belt of mosses (*Sphagnum*, *Miscanthidium violaceum*) that is surrounding the lake has been suggested to indicate more acidic conditions (Kateyo, 2006). The general low ionic content in combination with a low nutrient concentration might be responsible for the dominance of green algae and desmids.

The cyanobacterial dominance in L. Victoria (nos. 9–11) as well as in Jinja Pond was linked to a high concentration of nutrients. Kling et al. (2001) reported that the eutrophic condition in L. Victoria supported a high algal biomass that has risen by a factor of 4–5 since the 1960s. Hecky (1993), Lipiatou et al. (1996), and Verschuren et al. (2001) reported a shift in dominance from diatoms and green algae to cyanobacteria. Mugidde et al. (2003) suggested that because of their ability to fix nitrogen cyanobacteria may particularly increase in L. Victoria in response to phosphorus loading and increasing nitrogen limitation.

In all the hypertrophic shallow lakes (nos. 4, 5, and 7), *Microcystis* was abundant. These three lakes situated within the national park had a high pH ranging from 8 to 10. Cyanobacteria are better competitors due to their efficient carbon dioxide-concentrating mechanism at a higher pH (Shapiro, 1984). In addition, these shallow waters are polymictic, while at calm conditions a high insolation will penetrate the whole water column. In general, these physical conditions favor the genus *Microcystis* sp. at the expense of other genera, and very often *Microcystis* sp. is able to dominate phytoplankton for long times (Reynolds et al., 2002). The dominance of *Microcystis* sp. probably remained unaltered in Lake George for decades (Ganf, 1974).

TABLE IV. Field samples of twelve Ugandan freshwater sites analyzed for cyanobacterial biovolume, *Microcystis* cell numbers, by PCR for the presence (+) or absence (−) of microcystin (MC) producers (HEP), *mcy*E, PC-IGS, *mcy*B of *Microcystis* and for MCs by HPLC

Sample ID	Site No.	Filtered Volume (mL)			Cyanobacterial Biovolume ($mm^3\ L^{-1}$)			*Microcystis* (cells mL^{-1})			HEP-PCR Product			*Microcystis* *mcy*E-F2/F8[a] PCR Product			PC-IGS PCR Product			*mcy*B PCR Product			fg MC-LR Equivalent $Cell^{-1}$		
		M4	J4	A8	M4	J4	A8	M4	J4	A8	M4	J4	A8	M4	J4	A8	M4	J4	A8	M4	J4	A8	M4	J4	A8
Depth integrated samples																									
Kiranzi swamp	1	250	250					−	−		−	−		−	−		−	−		−	−		−	−	
Nyabikere Crater Lake	2	400	700		4	0.4		−	−		−	−		−	−		−	−		−	−		−	−	
Nkuruba Crater Lake	3	500	500		3	1		5×10^3	5×10^3		−	−		−	−		+	+		−	−		−	−	
Lake George	4	25	50		88	60		4×10^5	7×10^5		+	+		+	+		+	+		+	+		−	−	
Lake Edward (Katwe)	5	150	150		53	57		2×10^5	2×10^5		+	+		+	+		+	+		+	+		−	−	
Nkugute Crater Lake	6	700	500																						
Lake Mburo	7	100	200		63	88		9×10^5	9×10^5		+	+		+	+		+	+		+	+		−	−	
Lake Nabugabo	8	250	250		5	5		5×10^4	2×10^4		−	−		−	−		+	+		+	+		−	−	
Lake Victoria (Bunjako Bay)	9	800	500		11	24		2×10^4	4×10^4		−	−		−	−		+	+		+	+		−	−	
Lake Victoria (Murchison Bay)	10	500	500		18	129		4×10^4	2×10^5		+	+		+	+		+	+		+	+		−	−	
Lake Victoria (Napoleon Gulf)	11	500	500		40	46		3×10^4	3×10^4		+	+		+	+		+	+		+	+		−	−	
Jinja Pond	12		200			19			3×10^4			−			−			−			−			−	
Plankton net samples																									
Kiranzi swamp	1	40	30		Not applicable			−	−		−	−		−	−		−	−		−	−		−	−	
Nyabikere Crater Lake	2	50	50		Not applicable			−	−		−	−		−	−		−	−		−	−		−	−	
Nkuruba Crater Lake	3	50	50		Not applicable			−	−		−	−		−	−		−	−		−	−		−	−	
Lake George	4	25	50	60	Not applicable			3×10^6	3×10^6	6×10^6	+	−	+	+	−	+	+	+	+	+	+	+	8.1	−	9.6
Lake Edward (Katwe)	5	35	40	60	Not applicable			1×10^6	4×10^6	2×10^6	+	+	+	+	+	+	+	+	+	+	+	+	92.9	31.2	67.1
Lake Mburo	7	35	50	60	Not applicable			3×10^6	3×10^6	4×10^6	+	−	+	+	−	+	+	+	+	+	+	+	5.2	15.8	52.4
Lake Nabugabo	8	40	40		Not applicable			3×10^5	5×10^5		−	−		−	−		+	+		+	+		−	−	
Lake Victoria (Bunjako Bay)	9	50	50		Not applicable			2×10^5	2×10^5		−	−		−	−		+	+		+	+		−	−	
Lake Victoria (Murchison Bay)	10	50	25	60	Not applicable			4×10^5	4×10^6	1×10^7	+	+	+	+	+	+	+	+	+	+	+	+	−	−	1.3
Lake Victoria (Napoleon Gulf)	11	50	50	60	Not applicable			1×10^6	7×10^5	6×10^5	+	+	+	+	+	+	+	+	+	+	+	+	−	−	49.1
Jinja Pond	12		50		Not applicable																				

M4, May 2004; J4, June 2004; A8, April 2008.

Only the plankton net samples from the site nos. 4, 5, 7, 10, and 11 were analyzed in April 2008.

[a] Using *mcy*E-F2/12R for *Anabaena* and plaR3 for *Planktothrix*, no PCR products were obtained.

TABLE V. Number of restriction types as observed in clone libraries obtained from HEP-PCR products (*mcy*E, restriction types A–C) and tox4F/4R PCR products (*mcy*B, restriction types I–III)

Sites/Restriction Type	Site No.	Date	HEPF/HEPR-PCR (*mcy*E, 470 bp)					Tox4f/4r-PCR (*mcy*B, 1330 bp)				
			No of Clones	A (*Microcystis*)	B (*Anabaena*)	C Undigested	Not Assigned	No. of Clones	I (PCC7806)	II (HUB524)	III (Strain 18A8)[a]	Not Assigned
L. George	4	26 April 2008	20	20	0	0	0	20	0	0	20	0
L. Mburo	7	27 April 2008	75	71	0	3	1	24	8	0	15	1
L. Victoria (Napoleon)	11	26 April 2008	70	70	0	0	0	35	11	0	20	4

For each restriction type, the respective genus (A, B) or *Microcystis* strain (I–III) is indicated in parentheses.

[a]In addition, twelve *Microcystis* strains were isolated from the same sites (Okello, 2004). Six strains were found to contain *mcy*B (strain nos.: 1B5, 2D6, 18A8, 20A2, 6C5, 20A5; access nos. EU014158–EU014163).

[b]Sequencing revealed a 382 bp (FJ429839) with a 96% similarity to *Microcystis* strain PCC7806.

Microcystin Analyses

In the present study, MCs were found in plankton net samples that were obtained from five sites (nos. 4, 5, 7, 10, and 11). In contrast, no MCs were found in aqueous methanolic extracts obtained from depth-integrated samples. In general, the high silt content that was clogging the filters during the filtration process resulted in low filtration volumes (50–200 mL), increasing the lower limit of detection for MC by HPLC significantly above 1 μg L^{-1}. It is expected that MC concentrations would have been in the measurable range, if more phytoplankton biomass would have been extracted. Indeed in the phytoplankton net samples with higher *Microcystis* cell numbers ranging from 2×10^5 to 1×10^7 cells mL^{-1}, MCs were recorded more frequently. Nevertheless, in plankton net samples, the cellular MC content was found to be within the range that was also observed in other field studies previously (1.3–93 fg MC-LR equivalent $cell^{-1}$; Kurmayer et al., 2003). It is, therefore, concluded that the net samples were useful for analyzing the mean cellular MC content. These results are in agreement with other studies that document, by means of plankton net samples, that whole water conditions can be adequately represented (Rogalus and Watzin, 2008).

Genetic Diversity and Microcystin Net Production

In most of the samples, the genera *Anabaena*, *Aphanocapsa*, *Chroococcus*, *Merismopedia*, *Microcystis*, *Planktolyngbya*, and *Pseudanabaena* were identified under the microscope. Among those, *Anabaena*, *Chroococcus*, *Microcystis*, and *Pseudanabaena* have been reported to produce MCs (Jungblut and Neilan, 2006; Sivonen and Börner, 2008). We used the conserved HEPF/HEPR primer pair to amplify all the potential MC-producing cyanobacteria. Surprisingly, only PCR products that are indicative of *mcy*E of *Microcystis* were obtained. Correspondingly, the independent application of the genus-specific *mcy*E primers revealed the occurrence of MC-producing *Microcystis* only. Consequently, *Anabaena* and other taxa, although rather abundant, did not have the potential to produce MC. In other studies, the populations of *Microcystis* from all five continents typically have been found to contain *mcy* genotypes as well as MCs (Sivonen and Börner, 2008). In contrast, other taxa show a more irregular pattern of MC production, for example, MC-producing *Anabaena* have been reported from Europe, Canada, and North Africa, but not from Australia (Sivonen and Jones, 1999; Sivonen and Börner, 2008).

Using the more sensitive primers, all the *Microcystis* populations were found to be able to produce MCs. This result is in agreement with other studies showing that *Microcystis* populations always contain the *mcy* genotype. For example, in a European survey including nine water

bodies in seven countries, *mcy*A and *mcy*B genes as a part of the *Microcystis* population were always detected (Via-Ordorika et al., 2004). Within *mcy*BA1, the highest genetic diversity was found (24.7–25.7%), which has also been reported for populations sampled in Europe, e.g., Lake Wannsee (Kurmayer et al., 2002), and in Scandinavia (Mikalsen et al., 2003). It is generally accepted that this highest genetic diversity resulted from frequent recombination processes involving the exchange of shorter fragments (<1000 bp) of DNA. Besides for the *mcy*BA1 restriction type I showing the highest similarity to the *Microcystis* strain PCC7806, a new *mcy*BA1 restriction type III that is indicative of a *mcy*BA1 genotype, which was so far unknown from the strains of the northern temperate hemisphere (Mikalsen et al., 2003), was found. In contrast, restriction II *mcy*B (C) was not detected. Notably, from the same *mcy*BA1 restriction type, two new MCs, [Asp^3]-MC-RY and [$MeAsp^3$]-MC-RY, were isolated. The genetic variation that is causing the biosynthesis of MC-RY remains to be elucidated.

Although the linear relationship between the *Microcystis* cell numbers and the total MC concentration was highly significant, as much as 62% of the variation remained unexplained (Fig. 3). Other studies documenting a significant linear relationship between the *Microcystis* cell concentration and the total MC concentration also report a relatively high proportion of unexplained variation (Kotak et al., 2000; Ozawa et al., 2005; Hotto et al., 2008). It is emphasized that so far no effort has been made to identify the contribution of systematic errors in cell counting and/or analysis of MC concentrations to the unexplained part of variation that is observed. However, in a consecutive field study sampling the same water bodies for 1 year, consistent lake-specific differences in MC cell quotas were observed, e.g., MC cell quotas obtained from Lake George were on average twenty-fold lower when compared with MC cell quotas obtained from Lake Mburo. We are currently investigating this phenomenon; however, lake-specific differences in MC cell quotas could explain the relatively large part of unexplained variation observed.

CONCLUSION

The trophic conditions and water depth were of profound importance for the occurrence of MC-producing cyanobacteria. Seven of the sampling sites were nutrient rich. However, only the shallow sites had favorable environmental conditions that enabled MC-producing cyanobacteria to flourish. From the extensive genetic analysis, it is concluded that *Microcystis* sp. constitute the major MC-producing taxon in Ugandan freshwaters. Besides MC-RR, the new MC-RY variants constitute abundant structural variants in the phytoplankton dominated by *Microcystis* sp. in Uganda. However, the annual MC production rate in tropical freshwater systems will exceed that in comparable systems in the temperate region due to the generally higher primary production rate.

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