Article

Cyanotoxin production in seven Ethiopian Rift Valley lakes

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Abstract

We hypothesized that unusual deaths and illnesses in wild and domestic animals in lake areas of the Rift Valley south of Addis Ababa were caused by toxic cyanobacteria. In the first cyanotoxic analyses conducted in samples from Ethiopia, we found lakes Chamo, Abaya, Awassa, Chitu, Langano, Ziway, and Koka all had concentrations of microcystins (MC) ranging from trace to hazardous, whereas only traces less than limits of detection (LOD) of cylindrospermopsin (CYN) were found. In the December 2006 dry season we sampled the lakes for analyses of MC, CYN, species structures, and calculations of cyanobacteria biomass. We used the Utermöhl technique to analyse cyanobacterial biomass and monitored MC toxins using HPLC-DAD, LC-ESI-MS-MRM, and ELISA-test and CYN with HPLC-DAD and ELISA. The various toxicity tests coincided well. In 4 of the lakes (Chamo, Langano, Ziway, and Koka), the inter-lake range of total MC concentration was 1.3–48 µg L^{-1}; in 3 (Abaya, Awassa, and Chitu), we found only traces of MC. *Microcystis aeruginosa* was the dominant species, with *Microcystis panniformis*, *Anabaena spiroides*, and *Cylindrospermopsis* spp. as subdominants. The MC concentration, especially in Lake Koka, exceeded levels for serious health hazards for humans, cattle, and wildlife.

Key words: CyanoHAB, cylindrospermosin, ELISA, Ethiopian Rift Valley lakes, HPLC, mass spectrometry, microcystins, *Microcystis*

Introduction

Cyanobacterial blooms occur in brackish and freshwaters worldwide (Codd et al. 2005a, Carmichael 2008). Although the densest blooms are usually associated with nutrient enrichment, some species also mass-develop in nutrient-poor lakes at higher latitudes (Willén and Willén 1999). In addition to and more serious than aesthetic problems, cyanobacterial blooms of some species produce toxic metabolites. The cytotoxin cylindrospermopsin (CYN) is produced by *Cylindrospermopsis* and some filamentous cyanobacteria, including species of the genera *Anabaena* and *Aphanizomenon* (Banker et al. 1997, Falconer and Humpage 2006, Spoof et al. 2006). Microcystins (MC) are common hepatotoxins produced by genera including *Anabaena*, *Planktothrix*, and *Microcystis* (Chorus and Bartram 1999). In this study we focus on CYN and MC, which have both been associated with a number of critical health hazards (Carmichael et al. 2001, Griffiths and Saker 2003).

Toxic cyanobacterial blooms are reported most frequently from the temperate zone, especially North America and Europe (Carmichael 2008), but reports also come from Australia, South Africa, and Asia (Ueno et al. 1996, van Halderen et al. 1996, Falconer 2001). A very early report counted "many thousands" of intoxicated cattle in Africa, especially South Africa, over several decades (Steyn 1945). The taxon *Microcystis toxica* dominated in

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the area, and a feeding experiment indicated its toxicity (Stephens 1948). However, the description of this species is superficial and its validity is difficult to confirm.

The few reports from tropical areas are probably due more to limited access to limnological and technical experience than to insufficient toxic bloom formations. Several studies in some lakes in Kenya and Uganda, including toxicity tests of separate taxa, have revealed cyanotoxins, mainly from *Microcystis aeruginosa*, *Anabaena* spp., and *Arthrospira fusiformis* (Krienitz et al. 2002, 2003, Ballot et al. 2003, Mwaura et al. 2004, Haande et al. 2007). *Arthrospira* was first recorded as a producer of microcystins and anatoxins in strains from Lake Sonachi (Ballot et al. 2005). Other toxin-producing strains known in tropical and subtropical regions, such as *Microcystis panniformis* (Bittencourt-Oliveira et al. 2005) and *Cylindrospermopsis raciborskii* (Berger et al. 2006, Haande et al. 2008), were not reported as toxic in African lakes, but further investigations are likely to show otherwise. A recent, as yet unpublished, year-round study in the Kenyan part of Lake Victoria, however, reveals that considerable MC concentrations of 90.5 μg L^{-1} dominated by *Microcystis* spp. have been recorded at inshore sites in the Nyanza Gulf. At offshore sites concentrations were considerably lower, ranging 0–8 μg L^{-1} (Sitoki 2010).

In December 2006 we sampled 7 lakes in the Ethiopian Rift Valley (from south to north: Chamo, Abaya, Awassa, Chitu, Langano, Ziway, and Koka; Fig. 1) for phytoplankton and cyanotoxin analyses to add to health-related information on their water quality. Few studies have focused on species structure and biomass in these lakes. In lakes Ziway, Awassa, and Chamo, seasonal patterns were investigated (Girma Tilahun 2006, Girma Tilahun and Ahlgren 2010), and especially profound studies were made in L. Awassa, which also included depth distributional patterns of species biomass and diversity (Elizbeth Kebede and Amha Belay 1994). All lakes except L. Chitu were sampled for phytoplankton during a period of short rains in March–May 1991 (Elizabeth Kebede and Willén 1998). In lakes Awassa and Ziway, cyanobacteria dominated all months, while L. Chamo was characterized by a more diverse flora of diatoms, flagellates, and green algae in addition to cyanobacteria.

Local knowledge suggests toxic cyanobacteria as a likely cause of animal and fish mortalities at some of the lakes. At L. Chamo, for example, the deaths of 75 zebras after drinking at the east shore in 1978 coincided with an unusually high fish kill (Amha Belay and Wood 1982). Unfortunately, no analyses of cyanotoxins followed this disastrous case, nor have similar analyses been performed anywhere in Ethiopia (Codd et al. 2005b). Cattle illnesses near L. Koka have been reported several times from the 1980s to as recently as March 2010. Local people have also complained about stomach problems after drinking untreated water from L. Koka and many other lakes.

To ascertain whether these problems could be caused by cyanotoxins we sampled 7 lakes where potentially toxic cyanobacterial species are commonly found. We hypothesized that we would find at least measurable concentrations of MC in L. Koka and of CYN in L. Chamo; however, because we had no support for the analyses, we were limited to using only one sample from each lake. Despite the limited data, our findings may provide valuable justification to support applications from our Ethiopian colleagues for further research in this field.

Materials and methods

Study area

The 7 study lakes (from south to north: Chamo, Abaya, Awassa, Chitu, Langano, Ziway, and Koka) vary greatly in altitude, size, physical and chemical characteristics (Fig. 1; Table 1), and history, as described in several papers (e.g., Elizabeth Kebede et al. 1994, Elizabeth Kebede and Willén 1998). All lakes except L. Koka were formed through tectonic or volcano-tectonic processes

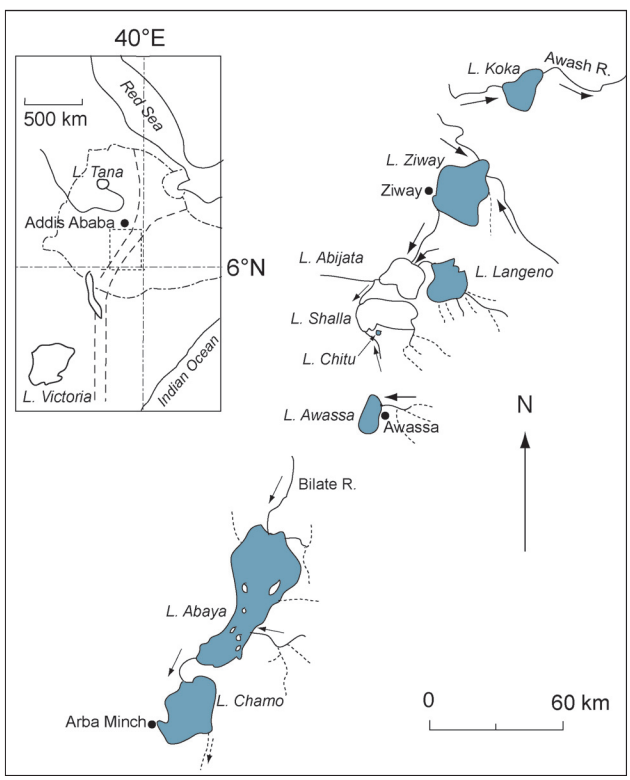

Fig. 1. Location of the 7 studied lakes in the Ethiopian Rift Valley (shaded) and major cities (filled circles). The dashed line in the map insert indicates the limit of the Rift Valley.

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Table 1. Morphometric and chemical data of the 7 Ethiopian Rift Valley lakes from Girma Tilahun (2006), Elizabeth Kebede et al. (1994), Elizabeth Kebede and Willén (1998), and Girma Tilahun and Ahlgren (2010). Coordinates from Google Earth.

Parameter	Chamo	Abaya	Awassa	Chitu	Langano	Ziway	Koka
Coordinates N	5°42′–5°58′	5°58′–6°34′	6°58′–7°07′	7°23′–7°24′	7°30′–7°42′	7°51′–8°06′	8°18′–8°28′
Coordinates E	37°27′–37°38′	37°36′–38°03′	38°23′–38°28′	38°24′–38°25′	38°40′–38°49′	38°43′–38°56′	38°59′–39°09′
Altitude (m)	1233	1285	1680	1600	1582	1636	1660
Catchment areas (km^2)	2210	17300	1250	—	1600	7025	—
Surface area (km^2)	551	1162	88	0.8	241	434	200
Max. depth (m)	20	13	22	21	48	7	—
Mean depth (m)	13	7.1	11	—	17	2.5	—
Alkalinity (meq L^{-1})	16.9	9.4	7.7	573	12.5	4.95	2.6
Conductivity (K_{25}, µS cm^{-1})	1910	925	830	49100	1770	410	286
Salinity (g L^{-1})	1.7	0.9	0.8	45	2.4	0.4	0.2
Total N (mg L^{-1})	1.60	0.193*	1.44	—	0.01*	1.32	0.01*
Total P (µg L^{-1})	182	237	34.1	2190	99	68.5	224
Chlorophyll *a* (µg L^{-1})	30	5.0	19	145	5.9	39	16

* NO_3- + NO_2- + NH_4-N; —, no data available

associated with the formation of the Great Rift System in Africa. Most lakes have closed basins or are part of closed drainage basins. The Rift Valley is a region of rainfall deficit, with higher rates of evaporation than annual rainfall. Rainy seasons usually occur between March and May (short rains) and from July to September (main rains). In the 2 southernmost lakes, Chamo and Abaya, the main rains usually start in late August. The seasonal rainfalls sometimes cause great fluctuations in size, salinity, alkalinity, and other chemical variables in the individual lakes. December, which was the sampling month in this study, is usually the driest period of the year (Girma Tilahun 2006).

Sampling procedure

We collected duplicate quantitative samples in the pelagic zone well outside the littoral zone using a van Dorn sampler and integrated samples from the upper metre to perform cyanotoxin (MC and CYN) analyses and to quantify cyanobacterial species. We also collected net samples (10 and 25 µm mesh size) to facilitate species identification. We preserved samples for phytoplankton counts with Lugol's solution supplemented with acetic acid and preserved net samples with formaldehyde solution. We kept the remaining quantitative samples cold on glass-fibre filters in the laboratory (≤4 h after sampling) for later filtration for MC and CYN analyses. The volume of filtered samples was 100–200 mL, depending on the plankton density. We kept the filters with the samples in a freezer (–20 °C) until analysis.

Cyanobacterial toxin analyses

Meriluoto and Codd (2005) and Spoof et al. (2003, 2006) describe the theoretical background and standard operating procedures of practical cyanotoxin analyses. Using ultra-sonication we extracted cyanobacterial material from glass fibre filters (GF/C) in either 75% methanol (for MC) or water (for CYN) and identified and quantified MC and CYN using high-performance liquid chromatography (HPLC), followed by UV absorbance diode-array detection (DAD) or mass spectrometry (MS). For verification, we used enzyme-linked immunosorbent assays, Envirologix Quantiplate Microcystin ELISA Kit (limit of detection [LOD] = 0.15 µg L^{-1}), and Abraxis Cylindrospermopsin ELISA Kit (LOD = 0.04 L^{-1}).

Correlation between log-transformed data of HPLC versus ELISA gave an R-square of 0.991 and p-value of 0.0001 (Tukey-Kramer HSD). Some data in HPLC and ELISA contained zero values; therefore, digit 1 was added to each observation (x + 1) before log-transformation.

Identification and biomass evaluation of cyanobacteria

The samples preserved in Lugol's were settled in counting chambers and counted in an inverted microscope (Nikon Diaphot 200) using the European standard Utermöhl technique (CEN 2006). The plankton density or the turbidity of the water was usually so high that sedimentation was possible only in 2 mL settling chambers, and the organisms were counted over the whole chamber bottom or parts of it (diagonals). Because many species are small, we used 40× objective magnification to assure correct identification, and photomagnified images with calibrated mesh-scale equipment connected to the microscope to measure species sizes.

In addition to the floristic keys in the Süsswasserflora von Mitteleuropa (Komárek and Anagnostidis 1999, 2005), we used the following floristic works to determine the tropical species: Hindák (1975), Komárek and Kling (1991), Watanabe (1995), Komárková-Legnerová and Tavera (1996), Komárková (1998), Komárek and Cronberg (2001), Komárek et al. (2002), Cronberg and Komárek (2004), Komárek (2005), Komárek and Zapomelová (2007), and Nguyen et al. (2007a).

Results

Cyanotoxins

We detected and quantified MC by HPLC-DAD in 4 of the lakes: Chamo, Langano, Ziway, and Koka (Table 2). All samples except that from L. Chitu contained MC on the LC-ESI-MS-MS-MRM analyses. Several variants were identified, the main microcystins being MC-RR, MC-YR, and MC-LR. In the ELISA assay MC were detected in all samples, but only in trace amounts in lakes Abaya, Awassa, and Chitu (Table 2).

No CYN was detected by chromatographic methods, but small amounts of CYN were detected by ELISA in all concentrated extracts. In the lake waters, however, all concentrations were below detection levels (LOD = 0.04 μg L^{-1}). The trace amounts we found might then be false positives, caused by matrix effects in the concentrated samples.

Dominant phytoplankton species

In the Koka reservoir, where the highest total concentration of MC was recorded, *Microcystis aeruginosa* Kütz. dominated, comprising up to 80% of the total cyanobacterial biomass, with *Anabaena spiroides* Komárek as a subdominant (18%) (Table 3).

Lake Chamo was characterized by *Cylindrospermopsis raciborskii* (Wolosz.) Subba (50%) and *A. cf carmichaelii* Cronberg and Komárek. A small-celled *Microcystis* (cell dia 2.5–3.4 μm) attributed to *panniformis* (Komárek, Komárk.-Legn. Sant'Anna, Azevedo, Senna) due to its flake-like character, was also present in the sample. A first glance of the fresh net sample in the low-magnification stereo-microscope at a nearby laboratory revealed dense colonies of this *Microcystis*. During the time between sampling and analysis, the colonies fell apart into single cells that made it difficult to attribute species level with certainty, and they may also have decomposed to some degree. Another common cyanophyte in L. Chamo was *C. curvispora* M. Watanabe (Table 3). Lake Langano contained a dominance of *M. aeruginosa* (Kütz) Kütz., *M. botrys* Teiling, and *M. flos-aquae* (Wittr.) Kirchn. ex

Table 2. Compilation of all results of microcystin analyses in 7 Ethiopian lakes. HPLC and ELISA refer to microcystin concentrations. The 2 values in the ELISA results derive from duplicates.

Lakes	Main MC by HPLC and LC-MS-MS (μg L^{-1})				Trace amounts of MCs detected by LC-MS-MS	HPLC-DAD total (μg L^{-1})	ELISA (μg L^{-1})
	MC-RR	MC-YR	MC-dmLR	MC-LR			
Chamo	2.9			1.0	dmRR, dmLR YR, LY, LF	3.9	6.1
Abaya					RR, LR, dmRR LF, YR,	0	trace
Awassa					RR, LR	0	trace
Chitu						0	
Langano	0.2	0.6		0.7	dmLR, dmRR, LA	1.5	1.3
Ziway	0.3	0.4		0.6	dmLR, dmRR	1.3	1.3, 1.3
Koka	2.1	9.7	4.2	28.6	dmRR, LA, LF,	45	48, 54

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Table 3. Relation between microcystin and total cyanobacterial biomass in 7 Ethiopian lakes, December 2006. Cell-numbers are classified. C/dw = 40–60% and C/ww = 10% (Vollenweider 1960), leading to C/ww/C/dw = dw/ww = 10/50 = 0.2.

Lake/reservoir	Dominating taxa	Total cyano-bacterial biomass ww(mg L^{-1}) Cell number (mL^{-1})	Toxin conc. (µg mg cyano ww^{-1})	Toxin conc. (µg g^{-1} dw)
Chamo	*Cylindrospermopsis raciborskii, C. curvispora, Anabaena cf. carmichaelii, Microcystis aeruginosa, M. panniformis, M. botrys*	1.25 20 000–100 000	3.99	20 000
Abaya	Very turbid water with dominance of picoplankton <2 µm	—	0	
Awassa	*Cylindrospermopsis spp., Planktolyngbya tallingii, Raphidiopsis mediterranea, Microcystis. aeruginosa*	7.56	0	
Chitu	*Arthrospira fusiformis, Anabaenopsis abijatae*	*	0	
Langano	*Microcystis aeruginosa, M. botrys, M. flos-aquae*	0.17 10 000–20 000	8.41	42 000
Ziway	*Microcystis aeruginosa*	2.09 20 000–100 000	0.62	3100
Koka	*Microcystis aeruginosa, M. flos-aquae, M. panniformis, Anabaena spiroides*	6.43 > 100 000	7.44	37 000

*Sample destroyed due to inefficient preservation. Species determinations made from formaldehyde preserved netsamples.

Forti, while the cyanobacterial-community in L. Ziway was represented almost solely by *M. aeruginosa*. Lake Awassa, where no measurable amounts of cyanotoxins were recorded, had the most diverse cyano-flora of the investigated lakes, characterized by several species of the genera *Cylindrospermopsis*, *Raphidiopsis*, *Planktolyngbya*, *Microcystis*, and *Chroococcus*. The other 2 lakes had no signs of cyanotoxins: L. Abaya had such silty water that detecting any algal species was impossible, and L. Chitu, a saline flamingo lake, was totally dominated by *Arthrospira fusiformis* (Voronichin) Komárek and Lund and *Anabaenopsis abijatae* Kebede and Willén.

Discussion

The different analyses of MC by HPLC and ELISA coincided well (Table 2); the results can therefore be considered accurate and highly reliable. The highest concentration of total MC, 45–51 µg L^{-1}, was detected in L. Koka. This level is much higher than that reported from other lakes in Africa, where most have concentrations <3 µg L^{-1} (Table 4). One exception may be in the Nyanza Gulf of Lake Victoria, mentioned earlier (data not yet published). The only tropical waters shown in published work to have concentration levels as high as L. Koka are 2 water bodies in Vietnam (Nguyen et al. 2007b).

In lakes Chamo, Langano, and Ziway the microcystin concentrations varied between 1 and 6 µg L^{-1}, which are close to concentrations in some other tropical lakes and reservoirs in Kenya (Table 4). Three other lakes, Abaya, Awassa, and Chitu, had concentrations below the detection limit. Toxin content in dried cyanobacterial mass (dw) reached extremely high values in 2 of the lakes, between 20 000 and 42 000 µg g^{-1} dw (Table 3), while one lake (Ziway) had levels similar to those of some other tropical lakes (Table 4). Considering the difficulty of estimating the volume of cyanophytes in the present work, particularly with many filamentous species and *Microcystis* morphotypes, and the conventional use of the factor 0.2 (Vollenweider 1969; Table 3) for calculation of dry weight from wet weight values, the reported estimates of the toxin content (Table 3) should be treated with caution.

Table 4. Cyanotoxin concentrations and concomittant dominant cyanobacteria in some African lakes and other tropical areas.

Lake and year	Dominant cyano-phytes	Analytical methods	Cyano-toxins	Toxin content (µg g^{-1} dw)	Toxin conc. extra-cellular (µg L^{-1})	Toxin conc. intra-cellular (µg L^{-1})	References
L. Baringo Kenya 2001–2002 (n = 4)	*Microcystis aeruginosa*	HPLC-PDA, MALDI-TOF MS	Microcystins (LR,RR,YR)	310–19 800	—	0.08–3.3	Ballot et al. (2003)
L. Bogoria Kenya 2001–2003 (n = 9)	*Arthrospira fusiformis* (>97%)	HPLC-PDA, MALDI-TOF-MS ELISA	Microcystins (LR, RR, YR, LF, LA)	16–155 9–1164	<1	—	Ballot et al. (2004); Krienitz et al. (2005)
L. Nakuru Kenya 2001–2002 (n = 11)	*Arthrospira fusiformis, Anabaenopsis abijatae, A. arnoldii*	HPLC-PDA, MALDI-TOF-MS ELISA	Microcystin (LR, RR, YR, LF, LA)	130–4593 149–4593	<1	—	Ballot et al. (2004); Krienitz et al. (2005)
L. Sonachi Kenya 2001–2002 (n = 5)	*Arthrospira fusiformis*	HPLC-PDA, MALDI-TOF-MS	Microcystin (RR)	1.6–12.0	<1	—	Ballot et al. (2005)
L. Simbi Kenya 2001–2002 (n = 2)	*Arthrospira fusiformis Anabaenopsis abijatae*	HPLC-PDA, MALDI-TOF-MS	Microcystin (LR, RR, LA, YR)	19.7–39.0	<1	—	Ballot et al. (2005)
L. Victoria Nyanza Gulf Kenya 2001 (n = 8)	*Anabaena flos-aquae, A. discoidea, Microcystis aeruginosa*	HPLC-PDA, MALDI-TOF-MS	Microcystins (RR, LR, LA, LF)	39 41	<1.0	—	Krienitz et al. (2002)
Hot springs at L. Bogoria 2001–2003 (n = 18)	*Phormidium terebiformis, Spirulina subsalsa*	HPLC-PDA MALDI-TOF-MS	Microcystins	220–845 0–836	—	—	Krienitz et al. (2003); Krienitz et al. (2005)
Oxidation ponds[1)] Kenya 2001–2006 (n = 8)	Coccoid greens, *Arthrospira fusiformi, Microcystis* sp.	HPLC-PDA MALDI-TOF-MS	Microcystins (LR, RR)	0–280 (50000)	—	0–1.7	Kotut et al. (2010)

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Table 4. *Continued*

Lake and year	Dominant cyano-phytes	Analytical methods	Cyano-toxins	Toxin content (μg g^{-1} dw)	Toxin conc. extra-cellular (μg L^{-1})	Toxin conc. intra-cellular (μg L^{-1})	References
L. Chivero[2)] Zimbabwe 2003–2004 (n = 13)	*Microcystis aeruginosa, M. wesenbergii, M. novacekii,*	ELISA	Microcystins	—	—	0.1–1.6	Mhlanga et al. (2006)
Headwater reservoirs[3)] Kenya 2000–2001 (n = 9)	*Anabaena circinalis, A. crassa, Microcystis aeruginosa, M. flos-aquae, M. viridis*	ELISA	Microcystin	—	—	0–2.85	Mwaura et al. (2004)
Other tropical	areas:						
L. PhewaTal Nepal 1997–2000 (n = 8)	*Aphanizomenon* sp., *Microcystis* sp., *Anabaena* sp.	ELISA	Microcystins (LR, RR, YR)	3–277	—	—	Jones and Jones (2002); Guring et al. (2006)
L Begnas Tal Nepal 1997–2000 (n = 8)	*Aphanizomenon* sp., *Microcystis* sp., *Anabaena* sp.	ELISA	Microcystins	262–3300	—	—	Jones and Jones (2002)
Various water bodies[4)] Vietnam 2004 (n ≈ 60)	*Microcystis* spp. *Arthrospira massartii, Planktothrix zahidii*	ELISA	Microcystins	—	—	0 – 76	Nguyen et al. (2007b)
Barra Bonita Reservoir Brazil 2002 (n = 1)	*Microcystis aeruginosa*	ELISA	Microcystins	311 46.0–80.2	—	—	Sotero-Santos et al. (2006); Okumura et al. (2007)
Ibitinga Reservoir Brazil 2002 (n = 1)	*Microcystis* spp.	ELISA	Microcystins	32.6–35.8 265	—	—	Okumura et al. (2007)

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Table 4. *Continued*

Lake and year	Dominant cyano-phytes	Analytical methods	Cyano-toxins	Toxin content (μg g^{-1} dw)	Toxin conc. extra-cellular (μg L^{-1})	Toxin conc. intra-cellular (μg L^{-1})	References
Monjolinho reservoir Brazil 2004 (n = 3)	*Anabaena circinalis*, *A. spiroides*	ELISA	Microcystins	138–223	—	28–45	Sotero-Santos et al. (2008)
Utinga Reservoir[2)] Brazil 1999 (n = 8)	*Aphanizomenon*, *Microcystis viridis; Nostoc* sp.; *Planktothix* sp.; *Radiocystis fernandoi*	ELISA	Microcystins	2470–4220	—	0–1.25	Vieira et al. (2005)

[1)] Oxidation ponds used by wildlife.
[2)] Source of drinking water for the cities Harare (Zimbabwe) and Belém-PA (Brazil)
[3)] Muruaki, Kahuru, Murungari
[4)] Huong river. (0), Hoamy Reservoir (0), small water bodies such as L. Mung (0.05), Nhuy river (3–19), Dapda site (1–76), Xuano pond (48) and Anluu (1.3) (i.e., 2 high values, 76 and 48 μg L^{-1}, the rest ≤20 μg g L^{-1}.

Kotut et al. (2010) also presented high toxic content (551 000 μg g^{-1} dw) of cyanobacterial biomass in an oxidation pond of a Nakuru town sewage treatment plant. However, at that sampling occasion chlorophytes predominated and cyanophytes were very rare (0.002 μg L^{-1}), causing an unlikely high ratio between toxin concentration and biomass. We believe measures of algal toxins are more usefully expressed as concentrations (μg L^{-1}) than as calculated content per dry weight. Concentrations facilitate comparisons between lakes. Errors caused by routine recalculations are less likely, and people and animals are usually exposed to the wet form, with the obvious exception of phytoplankton harvested and dried for consumption. Toxins are expressed as concentrations in 7 of the 17 papers cited in Table 4.

Several prerequisites have to be fulfilled for cyanotoxin concentrations to reach harmful levels. Aside from adequate light, temperature, and nutrient conditions, the species involved must have the genetic basis for toxin production. *Microcystis aeruginosa*, which was a prevalent cyanophyte in almost all the lakes with confirmed toxin contents in Ethiopia, is a well-known and widespread producer of MC (Wilson et al. 2005). From water bodies in Kenya and Uganda, however, a test of 24 different *M. aeruginosa* strains revealed just 4 produced MC (Haande et al. 2007). Other *Microcystis* species with genes for microcystin production are *M. botrys*, *M. ichtyoblabe*, *M. panniformis*, and *M. viridis* (Via-Ordorika et al. 2004, Bittencourt-Oliveira et al. 2005). The genus *Cylindrospermopsis*, which was present especially in lakes Awassa and Chamo, contains toxin-producing strains in the species *C. raciborskii* (Schembri et al. 2001). However, the ELISA test revealed CYN in only trace amounts; therefore, when *C. raciborskii* is present, possible toxicity must be checked during other times of the year. An example of intraannual variation in a Zimbabwian lake was given by Mhlanga et al. (2006), who showed that only 1 of 13 tested months reached measurable toxin concentrations (1.6 μg L^{-1}), while all other months had values <0.5 μg L^{-1}.

The alkaline, saline L. Chitu, however, had a plankton flora that was not revealed as toxin-producing in this study. One of the dominants, *Arthrospira fusiformis*, is used in many countries as a nutrient-rich diet supplement under the name of *Spirulina*; however, this species has been recorded as toxin-producing in 2 cases in African lakes, which is certainly a matter of concern (Krienitz et al. 2005, Lugomela et al. 2006).

It is extremely important to repeat toxin tests during different times of the year and to record the dominant species connected to toxin production. Note that different genotypes with different toxin-producing abilities may coexist in the same lake. A deeper knowledge of species biomasses in relation to toxin levels may be enough to

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follow the development and warn people close to the lake when a potentially harmful bloom appears. Many countries have developed guidelines and regulations, especially concerning concentrations of MC-LR or LR concentration equivalents, and in most cases follow the WHO provisional value of 1 μg L^{-1} for drinking water quality.

Some countries have also made recommendations for CYN where the variations are larger, ranging from 1 to 15 μg L^{-1}. Australia has developed trigger values for drinking water of livestock with recommendations to keep an MC-LR toxicity equivalent of ≤2.3 μg L^{-1} (WHO 2003). To rate health risks in recreational uses of water, WHO uses a combination of number of cyanobacterial cells and MC concentration:

- Low risk: ≤20 000 cyanobacterial cells/mL^{-1} and approximate MC-LR-levels of 2–10 μg/L^{-1}.
- Moderate risk: 20 000–100 000 cells/mL^{-1} and approximately 20 μg/L^{-1} of MC-LR. The health risk increases for people with specific health conditions, for example chronic hepatitis B.
- High risk: scum formations of cyanobacteria with >100 000 cells/mL^{-1}. The MC concentration in this category may vary considerably and may also reach mg levels. Such high concentrations cause liver injury in children and are hazardousness for all ages.

The cell count of >100 000 mL^{-1} in L. Koka represents a high level of risk, especially considering that the sample was taken from outside the littoral scum formation, from the water most people and cattle were using. People using this water for their own or their cattle's consumption definitely need adequate and timely information about health risks.

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References

Amha Belay, Wood RB. 1982. Limnological aspects of an algal bloom on L. Chamo in Gamo Goffa administration region of Ethiopia in 1978. SINET: Ethiopian J Sci 5:1−19.

Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf JS, Codd G, Pflugmacher S. 2004. Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya – Lakes Bogoria, Nakuru and Elementeita. J Plankton Res. 26:925−935.

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

Ballot A, Pflumacher S, Wiegand, Kotut K, Krienitz L. 2003. Cyanobacterial toxins in Lake Baringo, Kenya. Limnologica. 33:2−9.

Banker R, Carmeli S, Hadas O, Teltsch B, Porat R, Sukenik A. 1997. Identification of cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophyceae) isolated from Lake Kinneret, Israel. J Phycol. 33:313−316.

Berger C, Ngansoumanaba BA, Gugger M, Bouvy M, Rusconi F, Couté A, Troussellier M, Bernard C. 2006. Seasonal dynamics and toxicity of *Cylindrospermopsis raciborskii* in Lake Guiers (Senegal, West Africa). FEMS Microbiol Ecol. 57:355−366.

Bittencourt-Oliveira MC, Kujbida P, Cardozo KHM, Carvalho VM, Moura AN, Colepicolo P, Pinto E. 2005. A novel rhythm of microcystin biosynthesis is described in the cyanobacterium *Microcystis panniformis* Komárek et al. Biochem Biophys Res Comm. 326:687−694.

Carmichael W. 2008. A world overview. One-hundred-twenty-seven years of research on toxic cyanobacteria. Where do we go from here? In: Hudnell HK, editor. Cyanobacterial harmful algal blooms: state of the science and research needs. Advances in experimental medicine and biology. New York: Springer-Verlag. 619:105−125.

Carmichael WW, Azevedo SMFO, An JS, Molica RJR, Jochimsen EM, Lau S, Rinehart KL, Shaw GR, Eaglesham GK. 2001. Human fatalities from cyanobacteria. Chemical and biological evidence for cyanotoxins. Environ Health Persp. 109:663−668.

Chorus I, Bartram J. editors. 1999. World Health Organization. Toxic cyanobacteria in water. London & New York: E & FN Spon.

Codd GA, Azevedo SMFO, Bagchi SN, Burch MD, Carmichael WW, Harding WR, Kava K, Utkilen HC. 2005a. CYANONET a global network for cyanobacterial bloom and toxic risk management. Paris (France): UNESCO. IHP-VI. Working series SC-2005/WS/55 Technical Document in Hydrology No. 76.

Codd GA, Morrison LF, Metcalf JS. 2005b. Cyanobacterial toxins: risk management for health protection. Toxicol Appl Pharmacol. 203:264−272.

Cronberg G, Komárek J. 2004. Some nostocalean cyanoprokaryotes from lentic habitats of eastern and southern Africa. Nova Hedwigia. 78(1-2):71−106.

[CEN] European Committee for Standardization. 2006. Water quality – guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). Brussels (Belgium): European Committee for Standardization. EN 15204:2006.

Elizabeth Kebede, Amha Belay. 1994. Species composition and phytoplankton biomass in a tropical African lake (Lake Awassa, Ethiopia). Hydrobiologia 288:13−32.

Elizabeth Kebede, Willén E. 1998. Phytoplankton in a salinity-alkalinity series of lakes in the Ethiopian Rift Valley. Arch Hydrobiol Suppl. 124: Algological Studies. 89:63−96.

Elizabeth Kebede, Zinabu Gebre-Marian, Ahlgren I. 1994. The Ethiopian Rift Valley lakes: chemical characteristics of a salinity-alkalinity series. Hydrobiologia. 288:1−12.

Falconer I. 2001. Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. Phycologia. 40:228–233.

Falconer I, Humpage A. 2006. Cyanobacterial (blue-green algal) toxins in water supplies: Cylindrospermopsins. Environ Toxicol. 21:299−304.

Girma Tilahun 2006. Temporal dynamics of the species composition, abundance and size-fractionated biomass and primary production of phytoplankton in Lakes Ziway, Awassa and Chamo (Ethiopia) [dissertation]. [Addis Ababa (Ethiopia)]: Addis Ababa University.

Girma Tilahun, Ahlgren G. 2010. Seasonal variations in phytoplankton biomass and primary production in the Ethiopian Rift Valley lakes Ziway, Awassa and Chamo. Limnologica. 40:330−342.

Griffiths DJ, Saker ML. 2003. The Palm Island mystery disease 20 years on: a review of research on the cyanotoxin cylindrospermopsin. Environ Toxicol. 18:78−93.

Gurung TB, Dhakal RP, Bista JD. 2006. Phytoplankton primary production, chlorophyll-*a* and nutrient concentrations in the water column of mountainous Lake Phewa, Nepal. Lakes Reserv Res Manage. 11:141−148.

Haande S, Ballot A, Rohrlack T, Fastner J, Wiedner C, Edvardsen B. 2007. Diversity of *Microcystis aeruginosa* isolates (Chroococcales, Cyanobacteria) from East-African water bodies. Arch Microbiol. 188:15−25.

Haande S, Rohrlack T, Ballot A, Røberg K, Skulberg R, Beck M, Wiedner C. 2008. Genetic characterization of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) isolates from Africa and Europe. Harmful Algae. 7:692−701.

Hindák F. 1975. Einige neue und interessante Plankonblaualgen aus der Westslowakei [Some new and interesting cyanobacteria from western Slovakia]. Arch Hydrobiol Suppl 46: Algological Studies 13:330−353. German.

Jones SB, Jones JR. 2002. Seasonal variation in cyanobacterial toxin production in two Nepalese lakes. Verh Internat Verein Limnol. 28:1017−1022.

Komárek J. 2005. Phenotype diversity of the heterocytous cyanoprokaryotic genus *Anabaenopsis*. Czech Phycology. 5:1–35.

Komárek J, Anagnostidis K. 1999. Cyanoprokaryota 1: Chroococcales. In: Ettl H, Gärtner G, Heynig H, Mollenhauer D. editors. Süsswasserflora von Mitteleuropa 19/1. Jena (Germany): Gustav Fischer Verlag.

Komárek J, Anagnostidis K. 2005. Cyanoprokaryota 2: Oscillatoriales. In: Büdel B, Gärtner G, Krienitz L, Schagerl M. editors. Süsswasserflora von Mitteleuropa 19.2. Heidelberg (Germany): Elsevier.

Komárek J, Cronberg G. 2001. Some chroococcalean and oscillatorialean cyanoprokaryotes from southern African lakes, ponds and pools. Nova Hedwigia. 73(1–2):129−160.

Komárek J, Kling H. 1991. Variation in six planktonic cyanophyte genera in Lake Victoria (East Africa). Arch Hydrobiol Suppl 88: Algological Studies. 61:21−45.

Komárek J, Komárková-Legnerová J, Sant' Anna CL, Azevedo de Paiva MT, Senna P. 2002. Two common *Microcystis* species (Chroococcales, Cyanobacteria) from tropical America, including *M. panniformis* sp. nov. *Cryptogamie*. Algologie. 23(2):159−177.

Komárek J, Zapomelová EA. 2007. Planktic morphospecies of the cyanobacterial genus *Anabaena* = subg. *Dolichospermum* – 1. part: coiled types. Fottea. 7:1−31.

Komárková J. 1998. The tropical genus *Cylindrospermopsis* (Cyanophytes, Cyanobacteria). In: Azevedo MTP, Santos SD, Pinto LSC, Mehta M, Fujii MT, Yokoyama NS, Senna PAC, Guimarães SMPB, editors. Proceedings of the 4th Latin American Congress of Phycology. Phycology Society of Latin America and the Caribbean, St. Paul. p. 324−340.

Komárková-Legnerová J, Tavera R. 1996. Cyanoprokaryota (Cyanobacteria) in the phytoplankton of Lake Catemaco (Veracruz, Mexico). Arch Hydrobiol Suppl. 117: Algological Studies. 83:403−422.

Kotut K, Ballot A, Wiegand C, Krienitz L. 2010. Toxic cyanobacteria at Nakuru sewage oxidation ponds – a potential threat to wildlife. Limnologica. 40:47−53.

Krienitz L, Ballot A, Wiegand C, Kotut K, Codd GA, Pflugmacher S. 2002. Cyanotoxin-producing bloom of *Anbaena flos-aquae, Anabaena discoidea* and *Microcystis aeruginosa* (Cyanobacteria) in Nyanza Gulf of Lake Victoria, Kenya. J Appl Bot. 76:179−183.

Krienitz L, Ballot A, Kotut K, Wiegand C, Pütz S, Metcalf JS, Codd GA, Pflugmacher S. 2003. Contribution of the hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS Microbiol Ecol. 43:141−148.

Krienitz L, Ballot A, Casper P, Codd GA, Kotut K, Metcalf JS, Morrison LF, Pflugmacher S, Wiegand C. 2005. Contribution of toxic cyanobacteria to the massive deaths of Lesser Flamingos at saline-alkaline lakes of Kenya. Verh Internat Verein Limnol. 29:783−786.

Lugomela C, Pratap H, Mgaya Y. 2006. Cyanobacteria blooms – a possible cause of mass mortality of Lesser Flamingos in Lake Manyara and Lake Big Momela, Tanzania. Harmful Algae. 5:534−541.

Meriluoto J, Codd GA, editors. 2005. Cyanobacterial monitoring and cyanotoxin analyses. ISBN 951-765-259-3. Turku (Finland): Åbo Akademi University Press.

Mhlanga L, Day J, Cronberg G, Chimbari M, Siziba N, Annadotter H. 2006. Cyanobacteria and cyanotoxins in the source water from Lake Chivero, Harare, Zimbabwe, and the presence of cyanotoxins in drinking water. Afr J Aquat Sci. 31:165−173.

Mwaura F, Koyo A, Zech B. 2004. Cyanobacterial blooms and the presence of cyanotoxins in small high altitude tropical headwater reservoirs in Kenya. J Water Health. 2:49−57.

Nguyen LTT, Cronberg G, Annadotter H, Larsen J, Moestrup Ø. 2007a. Planktic cyanobacteria from freshwater localities in Thuathien-Hue province, Vietnam. I. Morphology and distribution. Nova Hedwigia. 85:1−34.

Nguyen LTT, Cronberg G, Annadotter H, Larsen J. 2007b. Cyanobacteria from freshwater localities in Thuathien-Hue province, Vietnam. II. Algal biomass and microcystin production. Nova Hedwigia. 85:35−49.

Okumura DT, Sotero-Santos RB, Takenaka RA, Rocha O. 2007. Evaluation of cyanobacteria toxicity in tropical reservoirs using crude extracts bioassay with cladocerans. Ecotoxicology. 16:263−270**.**

Schembri M, Neilan B, Saint C. 2001. Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. Environ Toxicol. 16: 413−421.

 DOI: 10.5268/IW-1.2.391

Sitoku LM. 2010. Phytoplankton and eutrophication in Nyanza Gulf of Lake Victoria with special reference to toxic cyanobacteria and planctonic diatoms [dissertation]. [Innsbruck (Austria)]: University of Innsbruck.

Sotero-Santos RB, Carvalho EG, Dellamano-Oliveira MJ, Rocha O. 2008. Occurrence and toxicity of an *Anabaena* bloom in a tropical reservoir (southeast Brazil). Harmful Algae. 7:590−598.

Sotero-Santos RB, De Souza E, Silva, CR, Verani NF, Nonaka KO, Rocha O. 2006. Toxicity of a cyanobacteria bloom in Barra Bonita Reservoir (Middle Tieté River, São Paulo, Brazil). Exotox Environ Safe. 64:163−170.

Spoof L, Berg KA, Rapala J, Lahti K, Lepistö L, Metcalf JS, Codd GA, Meriluoto J. 2006. First observation of cylindrospermopsin in *Anabaena lapponica* isolated from the boreal environment (Finland). Environ Toxicol. 21:552−560.

Spoof L, Vesterkvist P, Lindholm T, Meriluoto J. 2003. Screening for cyanobacterial hepatotoxins, microcystins and nodularin in environmental water samples by reversed-phase liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr A. 1020:105−119.

Stephens EL. 1948. Microcystis toxica sp. nov. a poisonous alga from the Transvaal and Orange Free State. Hydrobiologia. 1:14.

Steyn DG. 1945. Poisoning of animals and human beings by algae. S Afr J Sci. 41:243−244.

Ueno Y, Nagata S, Tsutsumi T, Hasegawa A, Watanabe MF, Park H-D, Chen C-G, Yu S-Z. 1996. Detection of microcystins, a bluegreen algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis. 17:1317−1321.

van Halderen A, Harding WR, Wessels JC, Schneider DJ, Heine EW, van Der Merwe J, Fourie JM. 1996. Cyanobacterial (blue-green algae) poisoning of livestock in the western Cape Province of South Africa. J S Afr Vet Assoc. 66:260−264.

Via-Ordorika L, Fastner J, Kurmayer R, Hisbergues M, Dittman E, Komárek J, Erhard M, Chorus I. 2004. Distribution of microcystin-producing and non-producing *Microcystis* sp. in European freshwater bodies: detection of microcystins and microcystin genes in individual colonies. System Appl Microbiol. 27:592-602.

Vieira JMSA, Azevedo MTP, Azevedo SMFO, Honda RY, Correa BB. 2005. Toxic cyanobacteria and microcystin concentrations in a public water supply reservoir in the Brazilian Amazonia region. Toxicon. 45:901−909.

Vollenweider RA. 1969. A manual on methods for measuring primary production in aquatic environments. IBP handbook no. 12. Oxford (UK): Blackwell Scientific.

Watanabe W. 1995. Studies on planktonic blue-green algae 5. A new species of *Cylindrospermopsis* (Nostocaceae) from Japan. Bull Natl Sci Mus Ser B. 21:45−48.

Willén T, Willén E. 1999. Byssus flos-aquae L. Arch Hydrobiol Suppl 129: Algological Studies. 94:377−382.

Wilson A, Sarnelle O, Neilan B, Salmon T, Gehringer M, Hey M. 2005. Genetic variation of the bloom-forming cyanobacterium *Microcystis aeruginosa* within and among lakes: implications for harmful algal blooms. Appl Environ Microbiol. 71:6126−6133.

[WHO] World Health Organization. 2003. Water sanitation and health. Guidelines for safe recreational waters. Vol 1. Coastal and fresh waters. Chapter 8: Algae and cyanobacteria in fresh water. Geneva (Switzerland): WHO Publishing. p. 136−158. [cited 10 Feb 2011]. Available from http://www.who.int/water_sanitation_health/bathing/srwe1-chap8.pdf.