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Cyanobacterial toxins in Lake Baringo, Kenya

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Abstract

This paper presents data on the first identification, characterization and quantification of hepatotoxic microcystins and neurotoxic anatoxin-a in water samples of Lake Baringo, Kenya. The shallow turbid Lake Baringo was investigated five times between June 2001 and May 2002. The phytoplankton community was mainly dominated by the cyanobacterium *Microcystis aeruginosa*. Due to the high turbidity the phytoplankton biomass was low, ranging between 1.5 and 8.2 mg L^{-1}. High mean total phosphorus concentration (1.0 mg L^{-1}) and mean total nitrogen concentration (2.8 mg L^{-1}) typical for hypertrophic lakes were found. Using HPLC technique the hepatotoxins microcystin-LR, -RR and -YR and the neurotoxin anatoxin-a were detected in the water samples. The microcystin concentrations varied from 310 to 19800 µg microcystin-LR equivalents g^{-1} DW and the anatoxin-a concentration ranged from 270 to 1260 µg g^{-1} DW. To our knowledge this is the first evidence of cyanobacterial toxins in Lake Baringo.

Key words: Cyanobacteria – *Microcystis* – microcystin – anatoxin-a – Lake Baringo, Kenya

Introduction

The first investigation of Lake Baringo has been conducted at the end of the 19th century by J.W. GREGORY (BEADLE 1932). Since then several studies on the physico-chemistry, phytoplankton, primary production, flora and fauna have been carried out (JENKIN 1929; BEADLE 1932; VACELET et al. 1991; PATTERSON & WILSON 1995; ODOUR 2000).

The turbid lakewater is characterised by a greenish colour caused by the dominant cyanobacterium *Microcystis aeruginosa* (KÜTZING) KÜTZING. This was already mentioned by JENKIN (1929). The genus *Microcystis* has a broad geographical distribution and is often a major cause of freshwater cyanobacterial blooms (CHORUS 2001). It can produce toxic secondary metabolites, the microcystins (hepatotoxins) (SIVONEN 1996; CARMICHAEL 1997; CODD et al. 1999; JÄHNICHEN et al. 2001). The neurotoxin anatoxin-a has also been reported to be produced in some *Microcystis* strains, isolated from Japanese lakes and ponds (PARK 1993). Especially during cyanobacterial blooms, these toxins represent a growing problem for fisheries, agriculture and public health. According to CHORUS (2001), studies in several European and Asian countries revealed microcystin production in 60 to 90% of all samples investigated, in up to 25% of the samples neurotoxins could be found. So far only few records of the occurrence of cyanobacterial

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toxins have been documented for the African continent, mainly from South Africa and Morocco (WICKS & THIEL 1990; SCOTT 1991; VAN HALDEREN et al. 1995; OUDRA et al. 2001, 2002). More recently cyanotoxins have been determined in Kenya in Lake Victoria and in hot springs at the shore of Lake Bogoria (KRIENITZ et al. 2002, 2003). Although it is well known that *Microcystis aeruginosa* is an abundant species in Lake Baringo and that the lake is an important source for the livelihood of the local population, to our knowledge cyanobacterial toxins have not been investigated so far.

This paper reports on the first identification and quantification of microcystins and anatoxin-a in phytoplankton samples from Lake Baringo, Kenya. In addition physico-chemical and biological data will also be discussed.

Materials and Methods

Site description

Lake Baringo (N 00°36′, E 36°01′) is a shallow freshwater lake, located at 998 m above sea level in the Kenyan part of the Eastern Rift Valley in an arid region (Fig. 1). The annual rainfall is between 500 and 1000 mm in the catchment area and is highly variable (ODOUR 2000). The surface area is between 130 and 160 km^2 with a maximum depth of 8 m (SCHLÜTER 1993; ODOUR 2000). The catchment area is 6820 km^2 with several rivers (Endau, Molo, Ol Arabel and Mukutan River) feeding the lake (ODOUR 2000). On Kokwa Island alkaline hot springs discharge into the lake (BEADLE 1932). Lake Baringo has no surface outlet. Due to its lower conductivity in comparison to the other alkaline Rift Valley lakes it is suspected that the lake has a subterranean outlet (BEADLE 1932; PATTERSON & WILSON 1995). Lake Baringo supports a small fish industry used by the local people, mainly based on the four species of fish *Barbus intermedius* RÜPPEL, *Clarias mossambicus* PETERS, *Labeo cylindricus* PETERS and *Oreochromis niloticus* L. (PATTERSON & WILSON 1995). The annual yield is between 100 and 400 t (ODOUR 2000). At present a fishing ban is in force due to continuous low lake water levels and overfishing. Another important economical factor is tourism.

Measurements and sampling

The present study was carried out in the period from June 2001 to May 2002. The sampling point was near the jetty of the Lake Baringo Club (N 00°36.786′, E 36°01.395′). The physico-chemical parameters pH, conductivity and salinity were measured directly in the lake with a WTW Multiline P4 (Wissenschaftlich Technische Werkstätten Weilheim, Germany). Water transparency was measured with a Secchi disc (∅ 20 cm). Samples for the determination of total nitrogen (TN) and total phosphorus (TP) were taken a few centimeters below the water surface. Laboratory analysis was carried out within three hours from the time of collection using Nanocolor tube tests (two replicates each) and a field photometer Nanocolor 300 D (Macherey-Nagel GmbH Düren, Germany). Detection limit was 0.5 mg N L^{-1} and 0.01 mg P L^{-1}, respectively. For quantitative phytoplankton analysis, 125 ml water samples were taken out of a sample from the surface and fixed with Lugol's solution. For qualitative phytoplankton analysis 5 liters of the lake water were concentrated with a plankton net (20 µm mesh size) and fixed with formaldehyde (end concentration 1%). For cyanotoxin analysis, up to one liter of lakewater taken from the surface was filtered through glass fibre filters (0.45 µm pore size) (Whatman GF/C, Whatman International Ltd Maidstone, England) using a vacuum pump. The filters were air dried and packed in aluminium foil and stored in the dark at room temperature. The residual water was passed through a Sep-Pak cartridge Plus tC18 (Waters Corporation, Milford USA) to fix and enrich the water soluble toxins.

Fig. 1. Western shore of Lake Baringo near the village Kampi ya Samaki.

Microscopy

Phytoplankton taxa were counted in sedimentation chambers (Hydro-Bios Apparatebau GmbH Kiel, Germany) using a compound microscope Eclipse TS 100 (Nikon Corporation, Tokyo, Japan) according to the method of UTERMÖHL (1958). Phytoplankton biomass was calculated by geometrical approximations using the computerized counting program Opticount (HEPPERLE 2000). The specific density of phytoplankton cells was calculated as 1 g cm^{-3}. For calculation of cyanotoxin concentrations the dry weight (DW) of all cyanobacteria was used. The dry weight was calculated as 10% from the wet weight biomass (RUTTNER 1938). The mass of the filtered material could not be used for calculation because of the high amount of suspended inorganic material in the samples.

Cyanotoxin analysis

Filtered samples were extracted by adding 10 ml of 70% v/v aqueous methanol, followed by ultrasonication for 15 min and constant shaking for 24 h on an orbital shaker. Filter material and algal debris were removed by centrifugation for 5 min at 5000 rpm. The supernatant was evaporated to dryness at 30 °C under constant nitrogen flow. The residuals including the toxins were resolved in 1 mL 70% methanol (FASTNER et al. 1998). 50 µL of this elution were used for analysis by high performance liquid chromatography with photodiode array detection (HPLC-PDA) and matrix laser desorption/ionization time flight mass spectroscopy (MALDI-TOF) (PFLUGMACHER et al. 2001). The enriched Sep-Pak cartridges Plus tC18 were eluted with 90% methanol. The eluates were blown to dryness with nitrogen and resolved in 500 µL 100% methanol for HPLC analysis. The detection limit for cell-bound microcystins was 1 µg g^{-1} of dry algal material and for dissolved microcystins in the range of 1 µg L^{-1} on HPLC-PDA. Standards for calculation were microcystin-LR (MC-LR) (gravimetric standard), and dhb-microcystin-LR provided by G.A. CODD (University of Dundee); microcystin-LA (MC-LA) and anatoxin-a were obtained from Sigma-Aldrich Chemie GmbH Taufkirchen, Germany; microcystin-RR (MC-RR), microcystin-LF (MC-LF), microcystin-LW (MC-LW) from Alexis Corporation Biochemicals Grünberg, Germany; and microcystin-YR (MC-YR) from Calbiochem Novabiochem GmbH Bad Soden, Germany.

Results

Physico-chemical data

The levels of the physico-chemical parameters investigated during the study are provided in Table 1.

The physical conditions of Lake Baringo were characterised by high water temperature and a low transparency throughout the year. Compared to the physical factors the chemical parameters showed a higher variability. The inflow of hot springs on Kokwa Islands into Lake Baringo may explain the pH with values around 9.

Table 1. The physico-chemical conditions of Lake Baringo during the period June 2001 to May 2002.

Parameters	June 2001	Nov. 2001	Jan. 2002	May 2002
Water temperature (°C)	26.3	26.1	23.7	24.9
Secchi depth (m)	< 0.1	< 0.1	< 0.1	< 0.1
pH	9.0	8.8	9.1	9.1
Conductivity (mS cm^{-1})	1.66	1.39	1.51	1.67
Salinity (‰)	0.7	0.5	0.6	0.7
TN (mg L^{-1})	8.0	1.8	1.0	0.5
TP (mg L^{-1})	1.3	1.0	0.6	1.0

TN and TP values are means of two replicates with a standard deviation at all sampling dates < 0.01.

Table 2. Biomass of the main phytoplankton groups in Lake Baringo at different dates during the period June 2001 to May 2002.

Phytoplankton groups	Sampling date				
	June 2001	Nov. 2001	Jan. 2002	Mar. 2002	May 2002
	wet weight (mg L^{-1})				
Cyanophyceae	5.45 ± 0.05	1.64 ± 0.14	0.67 ± 0.01	0.22 ± 0.00	2.53 ± 0.05
Bacillariophyceae	0.26 ± 0.03	0.19 ± 0.07	0.04 ± 0.05	0.46 ± 0.14	0.03 ± 0.00
Chlorophyceae	1.84 ± 0.14	0.52 ± 0.02	0.34 ± 0.02	0.80 ± 0.26	0.16 ± 0.07
Euglenophyceae	0.62 ± 0.03	0.08 ± 0.00	0.56 ± 0.34	0.03 ± 0.02	0.00 ± 0.00
Cryptophyceae	n.d.*	0.04 ± 0.02	0.07 ± 0.06	0.03 ± 0.02	0.05 ± 0.05
Total biomass	8.17 ± 0.09	2.47 ± 0.07	1.68 ± 0.26	1.54 ± 0.36	2.77 ± 0.07

Biomass data are means ± standard deviation, based on two countings.
*n.d. = not detected.

The conductivity and salinity indicate the subsalinity of the lake (HAMMER 1986). The high TN and TP values reflect the hypertrophic condition of Lake Baringo.

Phytoplankton commmunity

The phytoplankton community was dominated by colonies and single cells of the cyanobacterium *Microcystis aeruginosa*. Cell diameters of 3 µm and 5 µm could be distinguished. The mucous covers of the colonies were often colonized by the cyanobacterium *Pseudanabaena* sp. and the diatom *Nitzschia* sp. in various amounts. Hyperscums could occasionally be found at the shore of the lake. Other dominant organisms were *Phacotus lenticularis* (EHRENBERG) STEIN and *Thorakomonas* sp. (Chlorophyceae). In the sample of November 2001, the cyanobacteria *Anabaena* sp. and *Phormidium* sp. were present in small numbers. Table 2 shows the wet weight of the biomass (mg L^{-1}) of the main phytoplankton groups at the different sampling dates.

In Table 3 the biomass (wet weight in mg L^{-1}) of the different cyanobacterial species at the different sampling dates is presented.

Cyanotoxins

Cyanobacterial hepatotoxins (microcystins) and a neurotoxin (anatoxin-a) were detected by HPLC-PDA in all algal samples of Lake Baringo and the masses confirmed by MALDI-TOF. Fig. 2 shows HPLC chromatograms of cyanobacterial toxin standards and microcystin variants detected in Lake Baringo.

Three different cell-bound microcystin structural variants (microcystin-LR, -RR and -YR) were present (Table 4). In all samples extracellular microcystins and anatoxin-a were below the detection limit of 1 µg L^{-1}.

Total microcystin concentrations varied between 310 and 19800 µg microcystin-LR equivalents g^{-1} DW. Anatoxin-a concentration ranged from 270 to 1260 µg g^{-1} DW (Table 4).

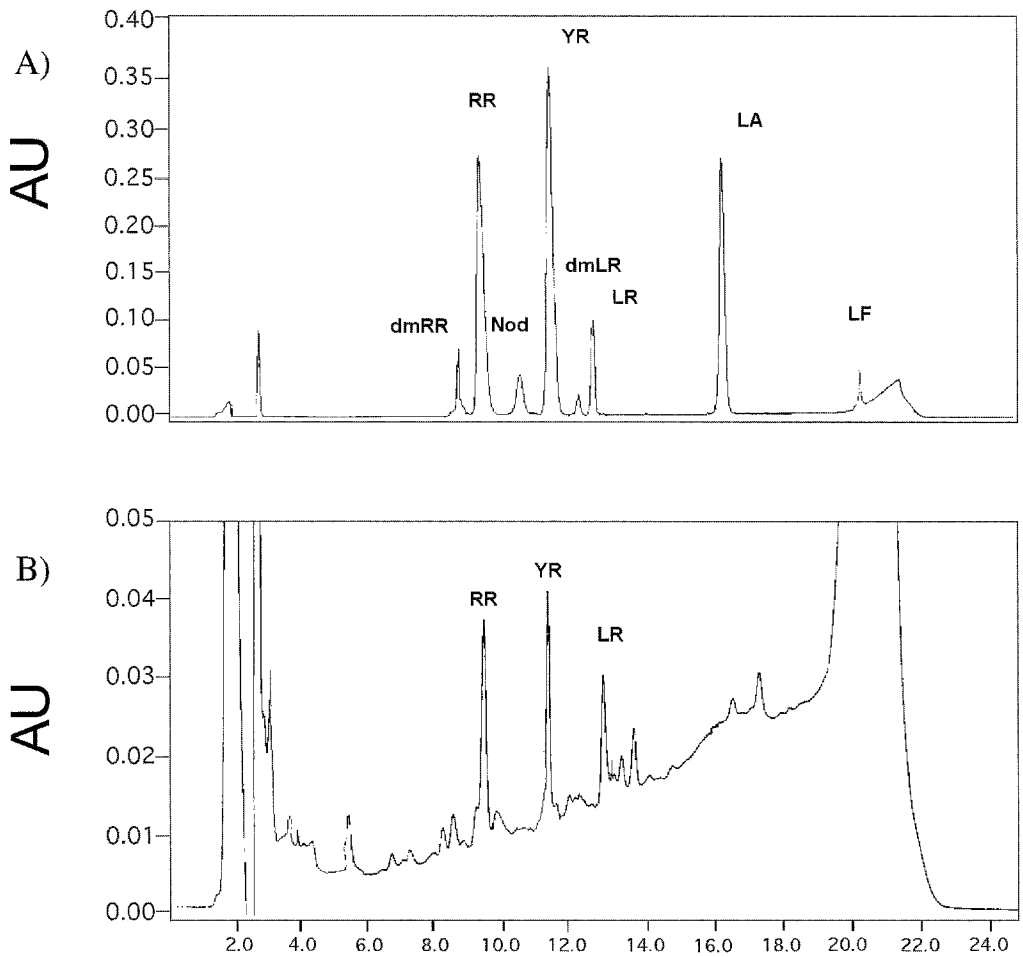

Fig. 2. HPLC chromatograms of A) cyanobacterial toxins as standards, B) a filter sample of Lake Baringo with MC-RR, MC-YR and MC-LR detectable. All toxins are confirmed by MALDI-TOF spectrometry. AU = Absorption unit.

Table 3. Biomass of the different cyanobacterial taxa in Lake Baringo at different dates during the period June 2001 to May 2002.

Taxa	Sampling date				
	June 2001	Nov. 2001	Jan. 2002	Mar. 2002	May 2002
	wet weight (mg L^{-1})				
Microcystis aeruginosa	5.44	1.56	0.66	0.22	2.47
Pseudanabaena sp.	0.01	0.01	0.01	n.d.	0.06
Anabaena sp.	n.d.*	0.01	n.d.	n.d.	n.d.
Phormidium sp.	n.d.	0.06	n.d.	n.d.	n.d.

*n.d. = not detected.

Table 4. Cyanobacterial dry weight related concentrations of cell-bound microcystins and anatoxin-a in Lake Baringo.

Cyanotoxin	Sampling date			
	June 2001	Nov. 2001	Jan. 2002	May 2002
µg MC-LR g^{-1} DW	1507	3457	5883	160
µg MC-RR g^{-1} DW	1961	7646	5283	150
µg MC-YR g^{-1} DW	1995	8719	n.d.*	n.d.
µg anatoxin-a g^{-1} DW	294	1256	696	273

*n.d. = not detected.

Table 5. Lake water related concentrations of cell-bound microcystins and anatoxin-a in Lake Baringo.

Cyanotoxin	Sampling date			
	June 2001	Nov. 2001	Jan. 2002	May 2002
µg cell-bound MC equ L^{-1} lake water	2.98	3.25	0.75	0.08
µg cell-bound anatoxin-a L^{-1} lake water	0.16	0.21	0.05	0.07

In Table 5 the total microcystin concentrations are presented as cell-bound total microcystins per liter lake water.

Discussion

In our study the cyanobacterium *Microcystis aeruginosa* was the dominating phytoplankton species in Lake Baringo. This was also mentioned by JENKIN (1929), BEADLE (1932), PATTERSON & WILSON (1995) and ODOUR (2000).

According to KOMÁREK & ANAGNOSTIDIS (1999) the colonies with different cell size of 3 and 5 µm would be classified as different *Microcystis* species. However investigations on different *Microcystis* strains which were classified as *Microcystis aeruginosa, M. ichthyoblabe* KÜTZING, *M. novacekii* (KOMÁREK) COMPÈRE, *M. viridis* and *M. wesenbergii* (KOMÁREK) KOMÁREK in KONDRATEVA have shown that morphological diversity does not reflect genotypic diversity. DNA-DNA hybridization between these strains has revealed values higher than 70%, which is considered as sufficient to classify these strains as the same species *Microcystis aeruginosa* (OTSUKA et al. 2001).

Several factors may explain the dominance of *Microcystis aeruginosa* in Lake Baringo.

The lake water is characterised by a low transparency and a resulting small euphotic zone, caused by suspended clay material. This is confirmed by Secchi depth less than 0.1 m at all measuring dates. *Microcystis aeruginosa* is able to develop gas vacuoles in the cells and can regulate buoyancy (KROMKAMP et al. 1988; ZOHARY & ROBERTS 1990). This ability to control the vertical location is an important factor in very turbid water like in Lake Baringo (PATTERSON & WILSON 1995). Other factors are temperature and nutrient loading which have been considered important environmental factors that control the dominance of cyanobacteria (PAERL 1996). According to RESSOM et al. (1994) growth rates of bloom forming cyanobaceria like *Microcystis* are optimal at around 25 °C. The water temperature in Lake Baringo (23.7 to 26.3 °C) was within this optimal range.

The mean total phosphorus value of 1.0 mg L^{-1} and mean total nitrogen value of 2.8 mg L^{-1} indicate a high nutrient loading of the lake during the investigation period. According to OECD (1982) Lake Baringo would be classified as a hypertrophic lake. In the South African Hartebeesport Reservoir similar amounts of TP and TN resulted in enhanced growth of *Microcystis* sp. with mean biomasses between 20 mg L^{-1} and 50 mg L^{-1} sometimes forming hyperscums (ZOHARY & ROBARTS 1990). Due to the high turbidity in Lake Baringo the biomass of cyanobacteria between 0.2 mg L^{-1} and 5.5 mg L^{-1} is much lower. However, hyperscums could also occasionally be found at the shore of Lake Baringo.

Our investigation revealed that *Microcystis aeruginosa* in Lake Baringo produces microcystins. Microcystin-LR, microcystin-RR and microcystin-YR were found in variable amounts. These types of microcystins are the dominant microcystins in blooms of *Microcystis* sp. (SIVONEN & JONES 1999; CHORUS 2001). Worldwide more than 60 structural variants of microcystins have been isolated (CODD et al. 1999; SIVONEN & JONES 1999). According to BELL & CODD (1994) in 75% of cyanobacterial blooms toxins can be found. In several investigations from Denmark, Germany, Czech Repub-

lic and Korea 80 to 90% of the samples containing *Microcystis* also contained microcystins (CHORUS 2001). We found concentrations of microcystin-LR equivalents up to 19800 µg g^{-1} DW. They are in the upper range of worldwide detected concentrations. Only in a few studies total concentrations of microcystins over 10000 µg g^{-1} DW have been reported, in the Bautzen Reservoir (Germany) with microcystin equivalents up to 14700 µg g^{-1} DW and in waters of northeastern Wisconsin with microcystin equivalents up to 12800 µg g^{-1} DW (JUNGMANN et al. 1996; MCDERMOTT et al. 1995). In a German investigation program including 406 net samples, 76% of the investigated samples contained microcystin concentrations less than 1000 µg g^{-1} DW and only 24% more than 1000 µg g^{-1} DW with a upper limit of 6000 µg g^{-1} DW (FASTNER et al. 2001).

According to WICKS & THIEL (1990) the toxicity of *M. aeruginosa* is influenced by pH and water temperature. They have shown in the South African Hartebeesport Dam that the toxicity of *M. aeruginosa* increased with increasing pH. Higher pH values between 8.8 and 9.1 were also observed in Lake Baringo. WICKS & THIEL (1990) assumed that the maximum toxicity of *M. aeruginosa* cultures is achieved by temperatures between 18 and 25 °C. In the Hartebeesport Dam the highest toxin concentration in *Microcystis* was found at temperatures up to 27 °C. The water temperature in Lake Baringo is within this optimal range. However, ORR & JONES (1998) and JÄHNICHEN et al. (2001) have shown that abiotic factors like pH and temperature probably have only an indirect influence on the toxicity of *M. aeruginosa.* The microcystin production is controlled by the growth phase of a particular microcystin producer, the species composition and the availability of dissolved inorganic carbon (JÄHNICHEN et al. 2001). The maximum of microcystins found in Lake Baringo therefore is probably caused by an increase in the growth rate of *M. aeruginosa.*

Another source for the production of microcystins could be the cyanobacterium *Pseudanabaena* sp., an epiphytic organism on some of the *Microcystis* colonies in Lake Baringo. According to CHANG (1985) *Pseudanabaena* is nontoxic and is growing only on nontoxic *Microcystis* strains. However, OUDRA et al. (2001) have reported microcystin production in isolated *Pseudanabaena* strains from water bodies in Morocco. The microcystin concentration in these strains was much lower as in isolated and cultivated *Microcystis* strains from the same water bodies.

We have also found the neurotoxin anatoxin-a in the samples from Lake Baringo. The exact origin of anatoxin-a remains unclear. *Microcystis aeruginosa* is possibly a source for the detected anatoxin-a. PARK et al. (1993) have detected anatoxin-a in *Microcystis* strains from Japanese lakes and ponds. The concentration measured in Lake Barato, Japan (357 µg g^{-1} DW) is in the same order as the findings in Lake Baringo between 270 and 1260 µg g^{-1} DW.

Anabaena sp. and *Phormidium* sp. were found in the sample of November 2001 in small numbers. Both genera are known as producers of microcystins and anatoxin-a (MEZ et al. 1997; CODD et al. 1999). Due to their low biomass less than 0.1 mg L^{-1} they can be seen only as a contributing source to the production of microcystins and anatoxin-a.

Calculated as microcystin concentration per liter lakewater, the concentrations of microcystins-LR equivalents in Lake Baringo range from 0.1 to 3.3 µg MC equ L^{-1} lakewater (Table 5). These amounts exceed at two of four sampling dates the consumption safe level of 1.0 µg MC equ L^{-1} (WHO 1998) and the safe level of HEALTH CANADA of 1.5 µg MC equ L^{-1} (HEALTH CANADA 1998; WHO 1998; FALCONER et al. 1999). Both types of cyanotoxins, the hepatotoxin microcystin and the neurotoxin anatoxin-a can have serious ecological impacts on aquatic food webs of lakes and cause serious health problems (CARMICHAEL & FALCONER 1993; CHRISTOFFERSEN 1996; CARMICHAEL 1997; CODD et al. 1999; CHORUS 2001). The impact of the cyanobacterial toxins on the food web of Lake Baringo, domestic animals and on public health in the Baringo area so far is not investigated. The lake water is used by several small communities as drinking water for domestic animals and the local people. We observed that the water is either used without treatment or treated with aluminium sulfate to settle down the clay material. To minimize harmful effects on consumers we recommend that the water of Lake Baringo should not be used without pretreatment to remove the toxic cyanobacteria. Inexpensive and simple techniques for treatment of the lake water are:

- bank filtration
- filtration of lake water over cheese cloth (dense woven fabric).

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