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Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya

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Abstract

The phytoplankton communities and the production of cyanobacterial toxins were investigated in two alkaline Kenyan crater lakes, Lake Sonachi and Lake Simbi. Lake Sonachi was mainly dominated by the cyanobacterium *Arthrospira fusiformis*, Lake Simbi by *A. fusiformis* and *Anabaenopsis abijatae*. The phytoplankton biomasses measured were high, reaching up to 3159 mg l^{-1} in L. Sonachi and up to 348 mg l^{-1} in L. Simbi. Using HPLC techniques, one structural variant of the hepatotoxin microcystin (microcystin-RR) was found in L. Sonachi and four variants (microcystin-LR, -RR, -LA and -YR) were identified in L. Simbi. The neurotoxin anatoxin-a was found in both lakes. To our knowledge this is the first evidence of cyanobacterial toxins in L. Sonachi and L. Simbi. Total microcystin concentrations varied from 1.6 to 12.0 μg microcystin-LR equivalents g^{-1} DW in L. Sonachi and from 19.7 to 39.0 μg microcystin-LR equivalents g^{-1} DW in L. Simbi. Anatoxin-a concentrations ranged from 0.5 to 2.0 μg g^{-1} DW in L. Sonachi and from 0 to 1.4 μg g^{-1} DW in L. Simbi. In a monocyanobacterial strain of *A. fusiformis*, isolated from L. Sonachi, microcystin-YR and anatoxin-a were produced. The concentrations found were 2.2 μg microcystin g^{-1} DW and 0.3 μg anatoxin-a g^{-1} DW. This is the first study showing *A. fusiformis* as producer of microcystins and anatoxin-a. Since *A. fusiformis* occurs in mass developments in both lakes, a health risk for wildlife can be expected.

Keywords: Anatoxin-a; *Arthrospira fusiformis*; *Anabaenopsis abijatae*; Lake Simbi; Lake Sonachi; Microcystin

1. Introduction

Lake Sonachi and Lake Simbi are crater lakes of volcanic origin. They belong to the alkaline and saline lakes of Kenya. At Lake Sonachi, studies on hydrological evolution (Damnati and Taieb, 1996), meromixis (MacIntyre and Melack, 1982) and sedimentation regime (Verschuren, 1999) have been carried out. Various studies on phytoplankton community, photosynthetic production, nutritional status, nutrient-phytoplankton relationships and macroinvertebrates have been conducted in L. Sonachi (Beadle, 1932; Melack, 1976, 1982; Peters and MacIntyre, 1976; Melack et al., 1982; Njuguna,

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1988; Clark et al., 1989; Verschuren et al., 1999). Fewer studies have been carried out in Lake Simbi. These have focused on physical chemical conditions and the phytoplankton and zooplankton communities (Melack, 1979; Tuite, 1981; Finlay et al., 1987).

Like many other soda lakes of Africa, both lakes are dominated by mass growth of the filamentous cyanobacterium *Arthrospira fusiformis* (Vorochinin) Komárek (syn. *Spirulina fusiformis* Voronichin) (Melack, 1979; Tuite, 1981; Finlay et al., 1987). Worldwide, cyanobacterial blooms and mats are increasingly recognized as sources of potent toxins (cyanotoxins) (Bell and Codd, 1994; Sivonen, 1996; Carmichael, 1997; Codd et al., 1999; Chorus, 2001). Recent studies by Ballot et al. (2002) and Krienitz et al. (2003) have shown that microcystins (cyanobacterial hepatotoxins) and anatoxin-a (cyanobacterial neurotoxin) are present in cyanobacterial samples from the Kenyan alkaline Lakes Bogoria and Nakuru and in cyanobacterial mats of the hot springs at the shore of L. Bogoria. Lake Bogoria is mainly dominated by *A. fusiformis*, L. Nakuru by *A. fusiformis* and *Anabaenopsis* div. sp.

Arthrospira/Spirulina has a history of human consumption in the region of Lake Chad and in Mexico and is widely used in mass cultures as a source for food, feed, and specific chemicals in a number of subtropical and tropical countries (Richmond and Vonshak, 1978; Ciferri, 1983; Vonshak, 1987; Vonshak, 1997; Abdulqader et al., 2000). An analysis of commercial *Arthrospira/Spirulina*-based human dietary-supplements for microcystins using a combined HPLC-ELISA method gave positive results (Gilroy et al., 2000). However, it is not clear whether the toxins were from *Spirulina* or from cells, filaments or cell fragments of other cyanobacterial species in the tablets and capsules.

This paper reports on the investigation of the phytoplankton communities and the identification and quantification of microcystins and anatoxin-a in phytoplankton samples and cultured strains of *A. fusiformis* and *Anabaenopsis abijatae* Kebede et Willén from lakes Sonachi and Simbi, Kenya. In addition, selected physico-chemical and biological data are discussed.

2. Materials and methods

2.1. Site description

The alkaline, saline crater lake Sonachi is located in the Kenyan part of the Eastern Rift Valley in the vicinity of Lake Naivasha. Lake Simbi is situated 1 km south of the Nyanza Gulf of Lake Victoria. Both lakes have no inflowing rivers and no surface outlet. Table 1 summarizes some geomorphologic features of both lakes (Njuguna, 1988; Damnati and Taieb, 1996; Verschuren, 1999).

2.2. Measurements and sampling

The present study was carried out from June 2001 to June 2002. The sampling point at L. Sonachi was at the western shore near the Crater Lake Tented Camp (00°47.333′S, 36°15.150′E). At L. Simbi the samples were taken at the eastern shore (00°22.297′S, 34°37.904′E).

Water temperature, pH, conductivity (corrected to 25 °C) and salinity were measured directly in the lakes with a WTW Multiline P4 (Wissenschaftlich Technische Werkstätten Weilheim, Germany). Water transparency was measured with a Secchi disc (20 cm in diameter). Samples for the determination of total nitrogen (TN), total phosphorus (TP) and total alkalinity were taken a few centimeters below the water surface. Laboratory analyses were 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). The detection limits were 0.5 mg N l^{-1} and 0.01 mg P l^{-1}. Total alkalinity was determined titri-

Table 1
Geographic position, altitude, surface area, depth, catchment area and rainfall of Lake Sonachi and Lake Simbi (Melack, 1979; Njuguna, 1988; Damnati and Taieb, 1996; Verschuren, 1999)

Characteristics	Lake Sonachi	Lake Simbi
Geographic position	00°47′S 36°15′E	00°22′S 34°38′E
Altitude above sea level (m)	1884	1142
Surface area (km^2)	0.18	0.29
Depth (m)	5.3	23
Catchment area (km^2)	∼1	Unknown
Annual rainfall (mm)	∼680	Unknown

metrically using bromocresol green-methyl red indicator and 0.1 M HCl according to APHA (1995).

For quantitative phytoplankton analysis, 125 ml water sub-samples were taken from samples from the surface and fixed with formaldehyde. Qualitative phytoplankton samples were obtained through the filtration of 5 l of lake water using a plankton net (20 μm mesh size) and fixing with formaldehyde (final concentration 1% v/v).

For cyanotoxin analysis, up to 1 l of lake water 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, packed in aluminum foil and stored in the dark at room temperature. The filtrate was passed through a Sep-Pak Plus tC18 cartridge (Waters Corporation, Milford USA) to recover the dissolved extracellular fraction.

A strain of *A. fusiformis* from each lake and *A. abijatae* from L. Simbi was isolated and cultivated in Bourrelly medium (Hegewald et al., 1994), modified with an addition of 0.3 g l^{-1} Na_2CO_3 and 15 g l^{-1} NaCl. For cyanotoxin analysis a volume representing approximately 500 mg dry weight of cyanobacterial biomass was filtered through glass fibre filters.

2.3. 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 Utermöhl (1958). Phytoplankton biomass was calculated by geometrical approximations using the computerized counting program Opticount (Hepperle, 2000). The specific density of phytoplankton cells was assumed as 1 g cm^{-3} (wet weight). For calculation of cyanotoxin concentrations the dry weight (DW) of all cyanobacteria was used. For dry weight estimations, 10% of the wet weight biomass was calculated according to Ruttner (1938). The alternative approach of determining the weight of filtered and dried samples could not be used in the case of the lake samples due to the high amount of suspended inorganic matter in the samples. In the case of the cultivated *Arthrospira* and *Anabaenopsis* strains the measured dry weight of the filtered samples could be used for the toxin calculation.

2.4. Analysis of cyanotoxins

2.4.1. Microcystin

Filtered samples were extracted by adding 10 ml of 70% (v/v) aqueous methanol, followed by ultrasonication for 15 min (Ultra Turrax T 25, Janke & Kunkel GmbH & Co. KG-IKA-Labortechnik, Staufen, Germany) and constant shaking for 24 h on an orbital shaker. Filter material and phytoplankton 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 redissolved in 1 ml 70% (v/v) methanol (Fastner et al., 1998). 50 μl of this solution were used for analysis by high performance liquid chromatography with photodiode array detection (HPLC-PDA; Waters Corp. Milford USA) and matrix laser desorption/ionization time flight mass spectroscopy (MALDI-TOF) (Pflugmacher et al., 2001). The enriched Sep-Pak Plus tC18 cartridges were eluted with 90% (v/v) methanol. The eluates were blown to dryness with nitrogen and the residues dissolved in 500 μl 100% methanol for HPLC-PDA analysis. The detection limit for cell-bound microcystins was 1 μg g^{-1} of dry cyanobacterial 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), 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.

2.4.2. Anatoxin-a

Analysis of anatoxin-a was performed using HPLC-PDA (Waters Corp. Milford, USA) with diode array detection at λ 227 nm on a reverse phase column μBondapak C18 (Waters Corp. Milford, USA) with a mobile phase of acetonitrile and water (10:90 v/v) with 0.05% trifluoro acetic acid (TFA) over 15 min according to Harada et al. (1989). Injection volume was 25 μl. Standard for calculation was anatoxin-a from Alexis Corp. (Grünberg, Germany). 50 μl of the same solution was used for MALDI-TOF analysis.

3. Results

3.1. Physico-chemical data

Both lakes were characterised by low water transparencies. The Secchi depths in L. Sonachi never exceeded 0.15 and 0.5 m in L. Simbi on all measuring dates. The water temperatures were at all sampling dates above 20 °C.

Values of pH between 10 and 10.4 were measured at all sampling dates in both lakes. Conductivity, salinity and alkalinity in both lakes were in similar ranges (Fig. 1). Total phosphorus concentrations measured were: 0.9–6.1 mg l^{-1} in L. Sonachi and 4.3–5.5 mg l^{-1} in L. Simbi. Total nitrogen concentrations ranged from 2.3 to 5.2 mg l^{-1} in L. Sonachi, and from 1.0 to 2.7 mg l^{-1} in L. Simbi (Fig. 1).

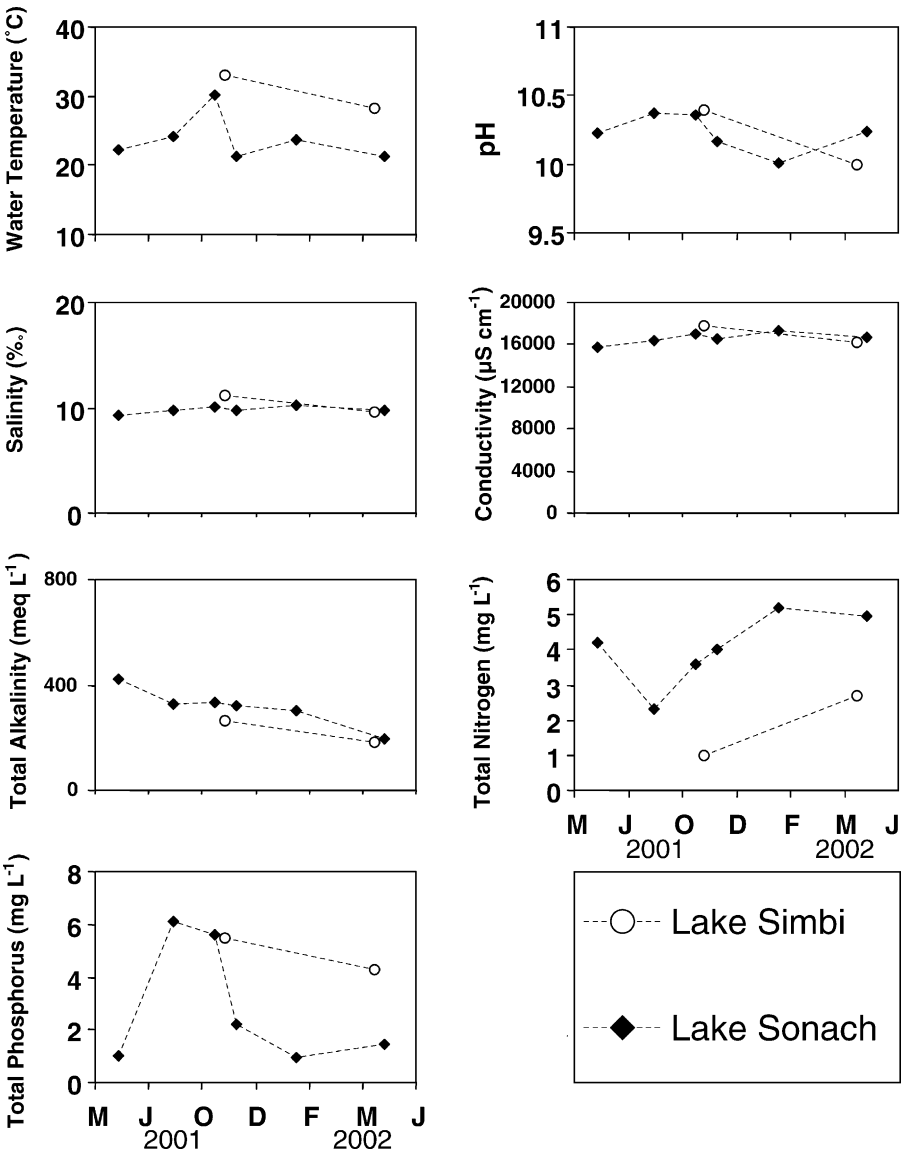

Fig. 1. Physico-chemical conditions in Lake Sonachi and Lake Simbi from June 2001 to June 2002.

Table 2
Biomass (wet weight in $mg\,l^{-1}$) of the main phytoplankton groups in Lake Sonachi and Lake Simbi from June 2001 to June 2002

Phytoplankton groups	Lake Sonachi							Lake Simbi	
	June 2001	August	October	November	February 2002	March	June	November 2001	May 2002
Wet weight ($mg\,l^{-1}$)									
Cyanophyceae	577.8	563.7	312.8	1434.9	1107.9	3158.4	799.9	347.0	61.1
Bacillariophyceae	[a]–	0.1	–	–	–	0.5	–	–	0.01
Chlorophyceae	–	–	–	–	0.003	–	0.004	1.2	–
Total biomass	577.8	563.8	312.8	1434.9	1107.9	3158.9	799.9	348.2	61.1

[a] (–): Not detected.

3.2. Phytoplankton community

The phytoplankton community in L. Sonachi and L. Simbi was dominated by cyanobacteria. The diatoms *Nitzschia* sp. and *Navicula* sp. and coccoid Chlorophyceae were present in the samples, but the biomass was very low (Table 2).

In L. Sonachi, *A. fusiformis* was the dominant cyanobacterial species accounting for more than 94% of the cyanobacterial biomass in all samples (Fig. 2A). In most of the samples *Anabaenopsis arnoldii* Aptekarj and coccoid cyanobacteria (*Synechococcus* div. sp.) were present at a low biomass. Other cyanobacteria found in small amounts were *Oscillatoria* sp. and *Spirulina subtilissima* Kütz. In L. Simbi, the sample collected in November 2001 was dominated by *A. fusiformis* and *A. abijatae* (Fig. 2B). In the sample collected in May 2002, the biomass of *Arthrospira* reached more than 99% of the total phytoplankton biomass. Only few cells of *A. abijatae* could be observed then. Other cyanobacteria found in small amounts were *Phormidium* sp., *Pseudanabaena* sp. and *Oscillatoria* sp. (Table 3).

3.3. Cyanotoxins

Cyanobacterial hepatotoxins (microcystins) and neurotoxins (anatoxin-a) were detected by HPLC-PDA in all algal samples and in the cultivated strain of *A. fusiformis* from L. Simbi. The masses were confirmed

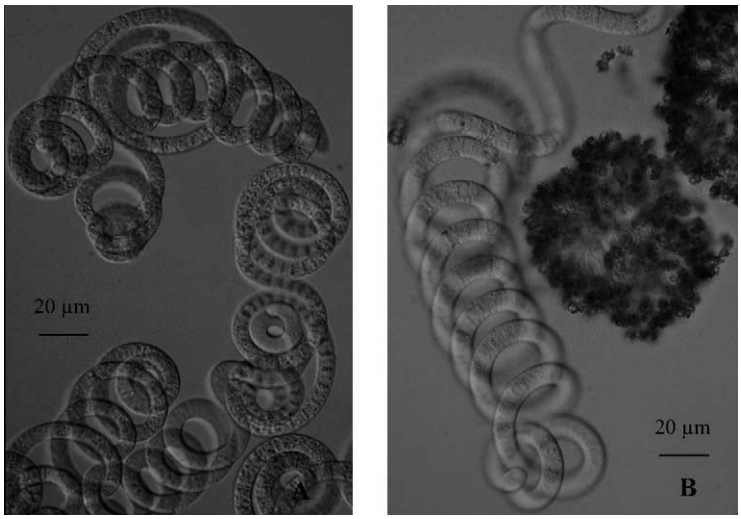

Fig. 2. (A) *A. fusiformis* from Lake Sonachi; (B) *A. fusiformis* and *A. abijatae* from Lake Simbi.

Table 3
Cyanobacterial community and species biomass (wet weight in mg l^{-1}) in Lake Sonachi and Lake Simbi from June 2001 to June 2002

Cyanobacterial species	Lake Sonachi							Lake Simbi	
	June 2001	August	October	November	February 2002	March	June	November 2001	May 2002
Wet weight (mg l^{-1})									
Arthrospira fusiformis	547.0	557.2	310.5	1428.1	1104.2	3153.7	793.0	224.2	60.9
A. arnoldii	8.7	4.8	1.8	3.3	0.4	–	–	–	–
A. abijatae	[a]–	–	–	–	–	–	–	119.3	0.01
Oscillatoria sp.	–	0.3	–	0.1	0.3	0.2	–	0.6	0.02
Phormidium sp.	–	–	–	–	–	–	–	0.03	–
S. subtilissima	–	–	0.04	–	–	0.2	0.1	–	–
Pseudanabaena sp.	–	–	–	–	–	–	–	1.5	–
Synechocystis sp.	–	–	–	–	0.01	–	–	0.3	–
Synechococcus sp.	22.1	1.3	0.5	3.4	3.1	4.4	6.8	1.0	0.2

[a] (–): Not detected.

by MALDI-TOF. One microcystin structural variant (microcystin-RR) was identified in L. Sonachi and four variants (microcystin-LR, -RR, -LA and -YR) were identified in L. Simbi (Fig. 3).

Total microcystin concentrations ranged from 1.6 to 12.0 μg microcystin-LR equivalents g^{-1} DW in L. Sonachi. In the two samples from L. Simbi, 19.7 and 39.0 μg microcystin-LR equivalents g^{-1} DW were measured (Fig. 4). Anatoxin-a concentrations ranged from 0.5 to 2.0 μg g^{-1} DW in L. Sonachi. An anatoxin-a concentration of 1.4 μg g^{-1} DW was measured in one sample of L. Simbi (Fig. 5). In all samples from the two lakes extracellular microcystins and anatoxin-a were below the detection limit of 1 μg l^{-1}.

Microcystin and anatoxin-a were also detected in the cultivated strain of *A. fusiformis* AB2002/02 from L. Sonachi. The measured concentrations were 2.2 μg microcystin-YR g^{-1} DW and 0.3 μg g^{-1} DW anatoxin-a (Table 4). In the cultivated strains of *Arthrospira* and *Anabaenopsis* from L. Simbi, cyanotoxins could not be detected (Table 4).

4. Discussion

The alkaline lakes of Eastern Africa are often densely inhabited by cyanobacteria. Previous studies on these lakes have described *A. fusiformis* as the dominant cyanobacterial species (Milbrink, 1977, Melack, 1979, Vareschi, 1982, Kebede and Ahlgren, 1996). In our samples from L. Sonachi, *A. fusiformis* also was the dominant species accounting for more than 94% of the cyanobacterial biomass. Palaeolimnological investigations of the fossil pigment stratigraphy of L. Sonachi by Verschuren et al. (1999) have shown that in the past dense blooms of filamentous cyanobacteria alternated with dominant growth of non-filamentous cyanobacteria and other non-cyanobacterial phytoplankton organisms. Under conditions of stable meromixis between ca. 1915–1945, the algal community was characterized by dense blooms of filamentous cyanobacteria. This condition was described by Beadle (1932), who observed vast numbers of *A. fusiformis* in L. Sonachi 1931. The decline of filamentous cyanobacteria followed disruption of meromixis in the mid 1940s and continued until the 1970s. Melack et al. (1982) and Njuguna (1988) found *A. fusiformis* only in small amounts during investigations in the early 1980s. During this period the phytoplankton community of Lake Sonachi was dominated by five species. The dominant species was the coccoid cyanobacterium *Synechocystis bacillaris* Butcher which reached 30–70% of the biomass. Other abundant species were *Lyngbya limnetica* Lemm., *Monallantus salina* Bourrelly, *Spirulina laxissima* G.W. West and *Synechocystis aquatilis* Sauv.

A. arnoldii, which we found in small numbers in the phytoplankton samples, is not reported by former studies on Lake Sonachi. *A. arnoldii* has been described in the phytoplankton communities of Lakes Nakuru and Elmenteita (Vareschi, 1978; Melack, 1988).

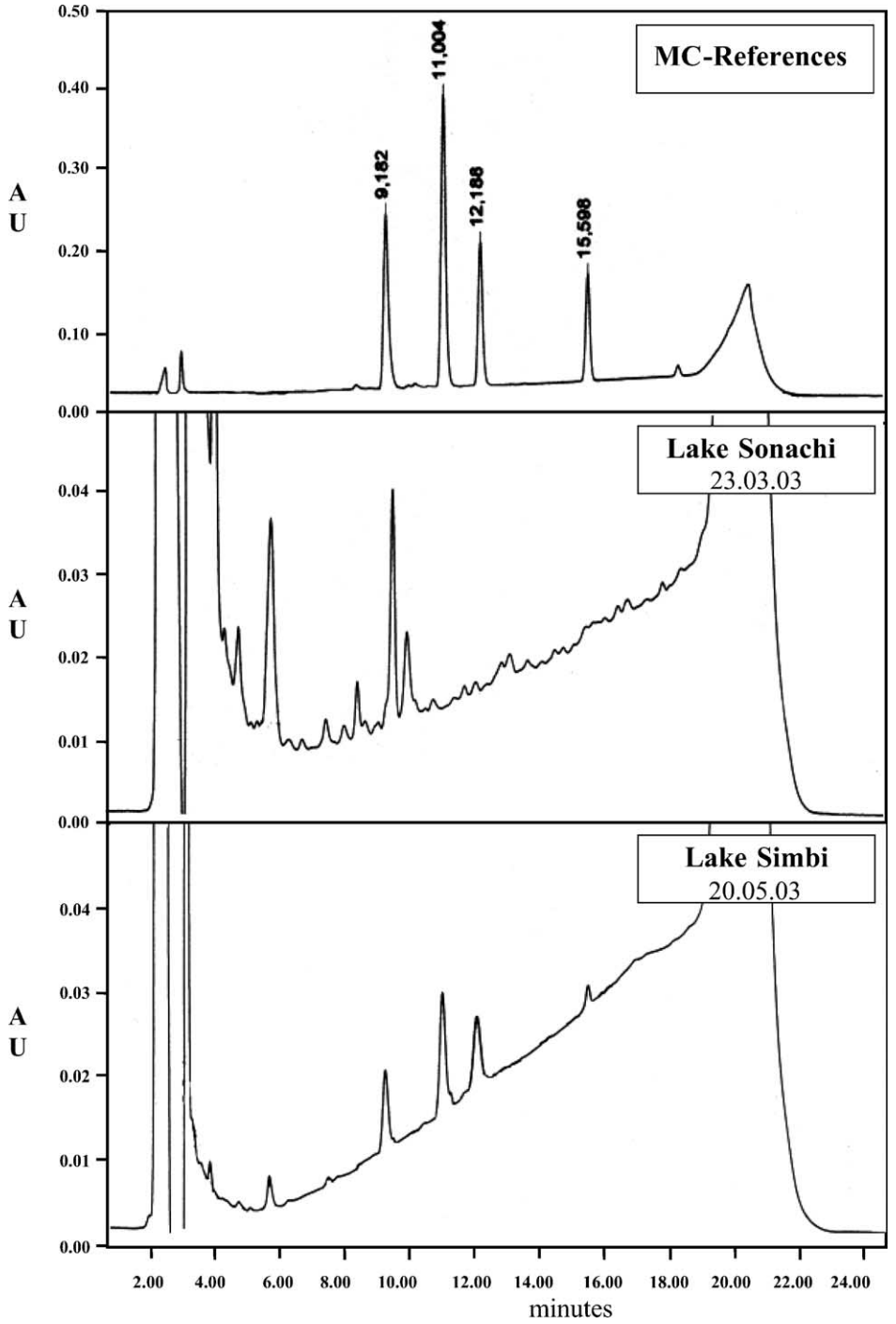

Fig. 3. Determination of cyanobacterial toxins from cyanobacteria samples of Lake Sonachi and Lake Simbi by HPLC-PDA. The comparison and detection of the toxin were done with reference to purified commercially available toxin retention times (MC-RR 9.1 min, MC-YR 11.0 min, MC-LR 12.1 min and MC-LA 15.5 min), PDA spectra. [AU–absorption units].

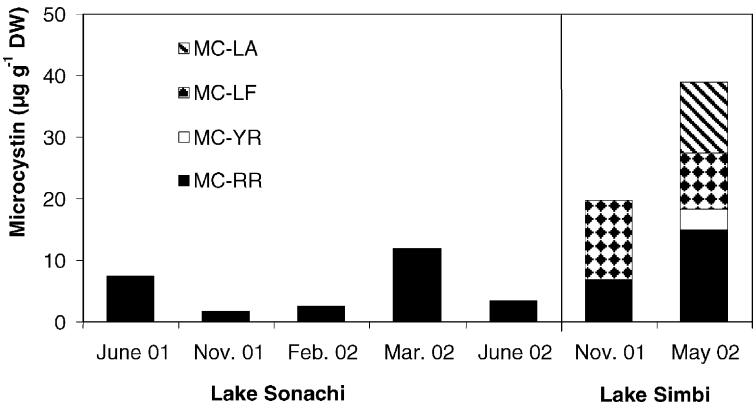

Fig. 4. Microcystins in Lake Sonachi and Lake Simbi in the samples from June 2001 to June 2002.

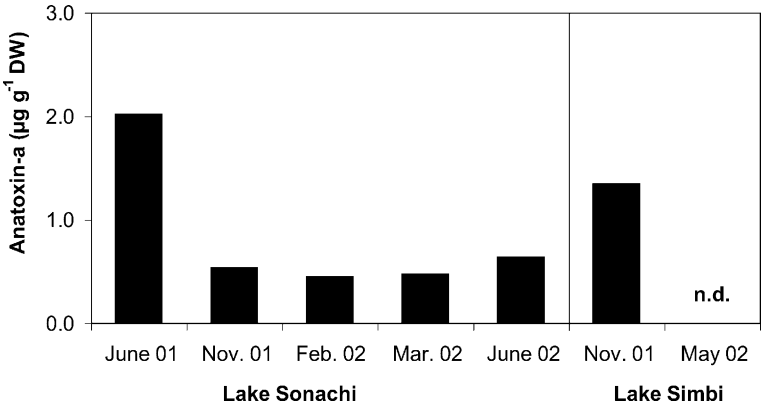

Fig. 5. Anatoxin-a in Lake Sonachi and Lake Simbi in the samples from June 2001 to June 2002. n.d.: Not detected.

A. abijatae, which dominated one of our phytoplankton samples from L. Simbi, was not mentioned in earlier studies on L. Simbi. It was first described from the Ethiopian Lake Abijata (Kebede and Willén, 1996). Previous studies by Melack (1979) and Finlay et al. (1987) on L. Simbi have described almost monocyanobacterial blooms of *A. fusiformis*.

Several factors influence the dominance of cyanobacteria in phytoplankton communities. As in other alkaline lakes of Eastern Africa, both lakes are characterized by high conductivity, alkalinity and salinity due to chemical weathering of the surrounding volcanic rocks and the lack of an outflow. The conductivity, alkalinity and salinity values are similar

Table 4
Cyanobacterial toxins in cultured strains of cyanobacteria from Lakes Sonachi and Simbi

Cyanobacterial toxin	Lake Sonachi *A. fusiformis* AB2002/02	Lake Simbi *A. fusiformis* AB2002/11	Lake Simbi *A. abijatae* AB2002/09
Microcystin ($\mu g\,g^{-1}$ DW)	2.2 (MC-YR)	n.d.[a]	n.d.
Anatoxin-a ($\mu g\,g^{-1}$ DW)	0.3	n.d.	n.d.

[a] n.d.: Not detected.

in both lakes. Vareschi (1982) and Kebede (1997) have demonstrated that *Arthrospira* can tolerate a wide range of salinity. Kebede (1997) tested growth of isolated strains of *A. fusiformis* from Lake Chitu, Ethiopia in laboratory experiments over a range of salinity from 13 to 88 g l^{-1}. With increasing salinity the specific growth rate declined, but growth did not stop.

At the end of the 1970s and the beginning of the 1980s, lower conductivities between 4600 and 8700 μS cm^{-1} were measured at the surface of L. Sonachi (Peters and MacIntyre, 1976; Melack, 1982; Njuguna, 1988). The phytoplankton community at this time was dominated by coccoid cyanobacteria. Finlay et al. (1987) have recorded a conductivity of 17500 μS cm^{-1} at the surface of L. Simbi. This is close to our measurements of 16200 and 17800 μS cm^{-1}.

Beside salinity, other factors possibly influence the dominance of cyanobacteria. The waters of both lakes are characterised by a low transparency (all Secchi depths, <0.5 m) resulting in a narrow euphotic zone. The low transparency results from a combination of a high population density of the phytoplankton community and the presence of suspended inorganic material from sediment disturbance. Vareschi and Jacobs (1985) describe self-shading as probably the most important factor limiting net production and density of phytoplankton organisms. The Secchi disc visibilities in L. Sonachi in the present investigation of less than 0.15 m were lower than those values of 0.23 and 0.76 m, reported by Melack et al. (1982) and Njuguna (1988). The higher values from the previous studies can be explained by different phytoplankton community composition. At that time, the phytoplankton community comprised mainly coccoid cyanobacteria and chrysophytes. The phytoplankton biomasses recorded by Njuguna (1988) (0.3–1.3 mg l^{-1}) were considerably lower than the amounts (313–3159 mg l^{-1}) recorded in the present investigation. Finlay et al. (1987) have reported a Secchi depth of 0.23 m at L. Simbi, which is similar to the present findings.

A. fusiformis, *A. abijatae* and *A. arnoldii* possess gas vacuoles, which enable them to form free-floating colonies (Jeeji-Bai et al., 1977; Hindák, 1985; Komárek and Lund, 1990; Kebede and Willén, 1996; Tomaselli, 1997). The ability to float and to control vertical location is an important factor in very turbid water (Walsby, 1994). Temperature and nutrient loading also have been considered as important environmental factors that influence cyanobacterial dominance (Paerl, 1996). The growth physiology and growth rates of bloom-forming cyanobacterial genera are optimal around 25 °C or higher (Robarts and Zohary, 1987). From laboratory cultivation of *Arthrospira* it is known that the optimal temperature is in the range of 35–38 °C (Vonshak, 1997). We measured water temperatures between 21.3 and 30.1 °C in L. Sonachi. In L. Simbi temperatures of 28.3 and 33.1 °C were recorded. These temperatures are near the optimal range for *Arthrospira*. Similar temperatures from L. Sonachi and L. Simbi were reported by Melack (1976) and Finlay et al. (1987).

The high mean TP concentrations of 2.9 mg l^{-1} (L. Sonachi) and 4.9 mg l^{-1} (L. Simbi) and the high mean TN concentrations of 4.0 (L. Sonachi) and 1.9 mg l^{-1} (L. Simbi) indicate the high nutrient loading in both lakes. According to OECD (1982), they would be classified as hypertrophic lakes. Njuguna (1988) has reported a considerably lower mean TP concentration (0.1 mg l^{-1}) and higher mean TN concentration (10.9 mg l^{-1}) from L. Sonachi. Finlay et al. (1987) have described similar TP concentrations of 6.7 mg l^{-1} from surface samples of L. Simbi. The high nutrient concentrations of the lakes can also be explained by the lack of an outflow and the resulting accumulation of nutrients.

Our investigation revealed that the cyanobacterial communities in L. Sonachi and L. Simbi produce microcystins and anatoxin-a. To our knowledge, this is the first evidence of microcystins and anatoxin-a in L. Sonachi and L. Simbi. Freshwater, brackish and marine habitats worldwide are known for the production of cyanobacterial toxins associated with blooms of planktonic and benthic cyanobacteria (Sivonen, 1996; Codd et al., 1999; Tyagi et al., 1999).

One type of microcystin (MC-RR) was found in variable amounts in L. Sonachi and four types in L. Simbi (MC-LR, -RR, -LA and -YR). Worldwide, more than 60 structural variants of microcystins have been isolated (Codd et al., 1999; Sivonen and Jones, 1999). The microcystin-LR equivalents up to 12 μg g^{-1} DW in Lake Sonachi and up to 39 μg g^{-1} DW in Lake Simbi are in the lower range of values reported worldwide. Fastner et al. (2001) have described microcystin concentrations, measured in field sam-

ples, ranging between 0.02 and 14700 $\mu g\,g^{-1}$ DW. In the Kenyan L. Baringo microcystin concentrations of up to 19800 $\mu g\,g^{-1}$ DW were recorded (Ballot et al., 2003). The occurrence of microcystins has mainly been reported from blooms of *Microcystis*, *Anabaena*, *Oscillatoria* and *Planktothrix* (Sivonen, 1996; Codd et al., 1999; Tyagi et al., 1999; Fastner et al., 2001).

The anatoxin-a concentrations detected in L. Sonachi (up to 2.0 $\mu g\,g^{-1}$ DW) and L. Simbi (1.4 $\mu g\,g^{-1}$ DW) are similar to concentrations detected in Japanese freshwater lakes (Park et al., 1993). Higher amounts of up to 4400 $\mu g\,g^{-1}$ DW have been recorded from Finland (Sivonen et al., 1989). Anatoxin-a is mainly reported from blooms of *Anabaena*, *Oscillatora*, *Phormidium*, and on rarer associations with *Cylindrospermum* and *Aphanizomenon* (Sivonen, 1996; Codd et al., 1999).

Several potential sources of microcystin and anatoxin-a in Lakes Sonachi and Simbi exist. In one cultured strain of *A. fusiformis* from L. Sonachi we detected microcystin-YR and anatoxin-a. This finding is important as members of the genus *Arthrospira/Spirulina* are regarded as non-toxic (Ciferri, 1983; Jassby, 1988). Several strains of this taxon are widely used in mass culture as a source for food, feed, and specific chemicals in subtropical and tropical countries (Richmond and Vonshak, 1978; Vonshak, 1987; Vonshak, 1997). Toxicity studies with *Spirulina maxima* on mice have shown that a toxic effect of this species could be excluded (Salazar et al., 1998). However, Iwasa et al. (2002) have related liver injury of a 52-year-old Japanese to the consumption of *Spirulina*, thus indicating a possible *Spirulina* associated hepatotoxicity. Analysis of commercially available *Spirulina*-based human dietary-supplements for microcystins by a combined HPLC-ELISA method gave positive results of up to 2.12 $\mu g\,g^{-1}$ DW (Gilroy et al., 2000). It is not clear whether the *Spirulina* tablets and capsules contained cyanobacterial products including cells, filaments or cell fragments of other species than *Spirulina*.

A. abijatae and *A. arnoldii* may also be sources of microcystins. Lanaras and Cook (1994) have reported microcystin in bloom material dominated by *Anabaenopsis milleri* Voronichin from Lake Porto Lagos, Greece, raising the possibility that members of the genus *Anabaenopsis* may produce microcystins. In the cultivated strain of *A. abijatae* from L. Simbi, cyanotoxins could not be detected.

Small quantities of *S. subtilissima* and *Oscillatoria* sp. were observed in the phytoplankton community of L. Sonachi. In L. Simbi, small amounts of *Phormidium* sp., *Oscillatoria* sp. and *Pseudanabaena* sp. were also observed. Microcystins, anatoxin-a and the related neurotoxin homoanatoxin-a have been identified in cyanobacterial mat samples consisting of *Oscillatoria* sp. and *Phormidium* sp. or in benthic *Oscillatora* sp. and *Phormidium* sp. (Edwards et al., 1992; Skulberg et al., 1992; Mez et al., 1997). Oudra et al. (2001) have reported microcystin production in isolated and cultured *Pseudanabaena* strains from Moroccan water bodies.

The hepatotoxic microcystin and the neurotoxic anatoxin-a can have ecological impacts on aquatic food webs of lakes and cause serious health problems (Carmichael and Falconer, 1993; Christoffersen, 1996; Carmichael, 1997; Codd et al., 1999; Chorus, 2001). The highly alkaline waters of L. Sonachi and L. Simbi are not used for human activities. However, they are sometimes visited by small flocks of flamingo, which feed on the cyanobacteria (Finlay et al., 1987; personal observation).

5. Conclusion

The data show that strains of *A. fusiformis* can be a source for the production of the cyanobacterial toxins microcystin and anatoxin-a in alkaline lakes. Since *A. fusiformis* often occurs in mass developments, a health risk for wildlife cannot be excluded.

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