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**Cyanotoxin-producing Bloom of *Anabaena flos-aquae*, *Anabaena discoidea*
and *Microcystis aeruginosa* (Cyanobacteria) in Nyanza Gulf of Lake
Victoria, Kenya**

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Summary

A bloom of *Anabaena flos-aquae*, *Anabaena discoidea* and *Microcystis aeruginosa*, was characterized in Nyanza Gulf of Lake Victoria, near Kisumu, Kenya. According to classical literature data, *A. discoidea* was considered to be endemic to the Great Lakes of Africa. Its morphological characteristics are described and discussed in relation to *A. flos-aquae*. The two samples investigated contained the cyanobacterial toxins, microcystin-LR, -RR, -LA and -LF.

Introduction

Phytoplankton investigations on Lake Victoria have been conducted since the end of the 19th century (Schmidle, 1898). Since then the results of more than 20 studies on the phytoplankton flora have been published. A review of these investigations was given by Talling (1987). The occurrence of cyanobacteria blooms in the lake was already evident at the beginning of the 20th century (Ostenfeld, 1908). Mass populations of cyanobacteria near the shores of Lake Victoria, are currently becoming an increasingly common phenomenon (Ochumba and Kibaara, 1989; Lung'Ayia et al., 2000). We studied a bloom dominated by *Anabaena flos-aquae* and *Anabaena discoidea*. The latter taxon was rarely mentioned in classical studies (Schmidle, 1902; Ostenfeld, 1908; Wołoszynska, 1914) and has not been reported at all in modern phytoplankton studies (Talling, 1987; Lung'Ayia et al., 2000).

Several cyanobacterial species can produce toxic secondary metabolites, the cyanotoxins (Carmichael, 1997; Codd et al., 1999). At present, only a few records on the occurrence of cyanotoxins have been documented for the African continent, principally from South Africa, where associated animal poisonings have been reported for over 50 years (see Wicks and Thiel, 1990; Scott, 1991; Harding and Paxton, 2001). Cyanotoxins have been reported more recently from Morocco (Oudra et al., 2002). Gastroenteritis among school children in Harare, Zimbabwe, was associated with exposure to cyanobacteria in the municipal drinking water

supply in the 1960s (Zilberg, 1966), although cyanotoxins were not specifically investigated at that time. Here, we describe the identification and quantification of microcystins (potent hepatotoxins) in cyanobacterial bloom material, dominated by *Anabaena* spp., recently collected from Lake Victoria.

Materials and Methods

Sampling date and site

Two samples were collected on 05 November 2001 at the shore of the Nyanza Gulf of Lake Victoria near the jetty of Dunga fish landing bay (Fig. 1), four km south of Kisumu City Center. The physico-chemical properties of the surface water at the site were as follows: temperature 29.9°C, pH 8.12, conductivity 186 $\mu\text{S cm}^{-1}$, alkalinity 2.02 meq L^{-1} , $\text{NH}_4\text{-N}$ 0.77 mg L^{-1} , $\text{NO}_3\text{-N}$ 0.9 mg L^{-1} , total-N 21.5 mg L^{-1} , $\text{PO}_4\text{-P}$ 0.82 mg L^{-1} , total P 1.9 mg L^{-1} , dissolved oxygen 8.0 mg L^{-1} (saturation 120 %).

Sampling design

Sample 1, for microscopic and cyanotoxin analyses was collected from the surface (50 % of the sample) and a depth of 20 cm (the other 50 % of the sample). Sample 2, only for cyanotoxin analyses was collected exclusively from the surface water. For quantitative phytoplankton analysis, sample 1 was fixed with Lugol's solution. For qualitative phytoplankton analysis, approximately 5 liters were concentrated by passing through a plankton net with a mesh size of 25 μm and fixed with formaldehyde (final concentration 1 %). For cyanotoxin analysis in particulate material, one liter of each sample was filtered using a vacuum pump through glass fibre filters (Whatman GF/C, Whatman International Ltd Maidstone England). Monitoring of dissolved cyanobacterial toxins in lake water samples, normally 1 L of water was enriched on C18 Sep-Pak Cartridges (Waters, Milford, MA, USA). The filters with the cyanobacterial mass and the C18 cartridges were air-dried and stored in the dark at room temperature.

Microscopy

The phytoplankton species were identified and photographically documented under an Eclipse E600 light microscope (Nikon Corporation, Tokyo, Japan). Phytoplankton taxa were counted in sedimentation chambers (Hydro-Bios Apparatebau GmbH Kiel, Germany) using an Eclipse TS100 compound microscope (Nikon Corporation, Tokyo, Japan). Phytoplankton biomass was estimated by geometrical approximations using a computer based plankton count program Opticount (Hepperle, 2000)

Cyanotoxin analysis

Filtered samples were extracted by adding 10 ml of 70 % v/v aqueous methanol, followed by ultrasonication for 15 minutes and constant shaking for 24 h on an orbital shaker. Filter material and cell debris were removed by centrifugation for 5 min at 5000 rpm and the supernatants evaporated to dryness at 30°C under constant nitrogen flow. The dried residues were redissolved in 1 ml 70 % methanol (Fastner et al., 1998). 50 µl subsamples were used for analysis by high performance liquid chromatography with photodiode array detection (HPLC-PDA) and matrix assisted laser desorption/ionization time flight mass spectrometry (MALDI-TOF) analysis (Pflugmacher et al., 2001). Elution of the content of the C18 cartridges was done with 90 % methanol, which was blown to dryness with nitrogen and resolved in 250 µL 90 % methanol before analyzed by HPLC. Purified reference toxins used were: microcystin-LR (gravimetric standard) and dhb-microcystin-LR from the Dundee laboratory; microcystin-LA and anatoxin-a from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany); microcystins -RR, -LF and -LW from Alexis Corporation Biochemicals (Grünberg, Germany); and microcystin-YR from Calbiochem Novabiochem GmbH (Bad Soden, Germany).

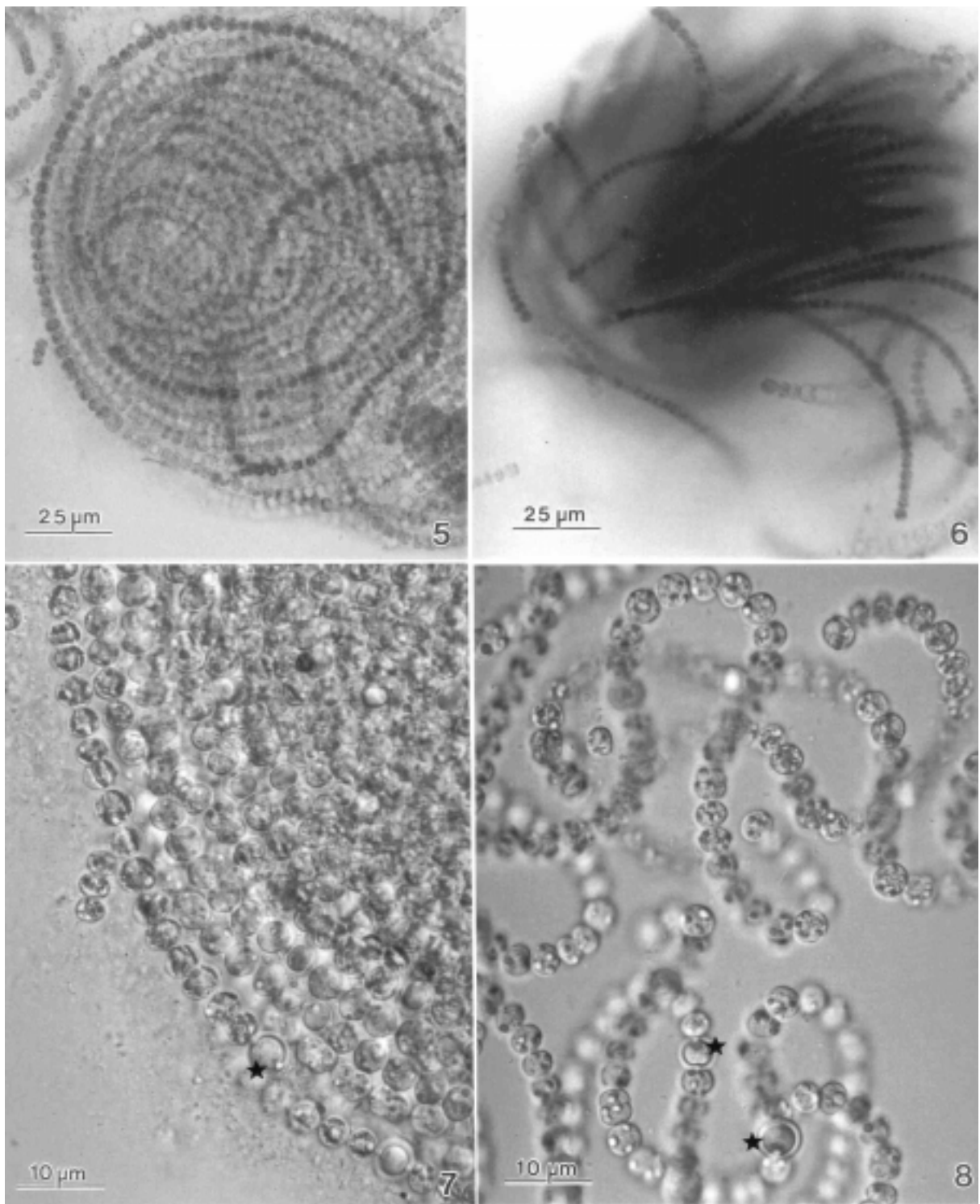


Fig. 1. Sampling site: Jetty of the fisherman's village Dunga, near Kisumu, Kenya.

Fig. 2. Yellow colored cyanobacterial bloom induced by colonies of *Anabaena*.

Fig. 3. Colony of *Anabaena flos-aquae* with irregularly spirally twisted trichomes.

Fig. 4. Filament of *Anabaena discoidea* with regularly coiled trichomes.



Figs. 5, 6. Dense colony of *Anabaena discoidea* (Fig. 5.: top view; Fig. 6: side view).

Fig. 7. Regularly arrangement of filaments within a colony of *Anabaena discoidea*.

Asterisc: Heterocyst.

Fig. 8. Spirally arrangements of filaments within a colony of *Anabaena flos-aquae*.

Asterisc: Heterocyst.

Results

The lake water at the study site was colored yellow by the cyanobacterial bloom (Fig. 2). Cyanobacterial gyre or colony diameters ranged from 0.5-1mm. The biomass of dominant cyanobacteria species and other phytoplankton groups in sample 1 was as follows:

Anabaena flos-aquae 216.7 mg L⁻¹

Anabaena discoidea 52.4 mg L⁻¹

Microcystis aeruginosa 2.9 mg L⁻¹

Bacillariophyceae 4.8 mg L⁻¹

Chlorophyceae 2.7 mg L⁻¹

Dinophyceae 1.3 mg L⁻¹

Euglenophyceae 1.4 mg L⁻¹

Total phytoplankton biomass 282.2 mg L⁻¹

In total, 95.4 % of the biomass was due to *Anabaena* spp. The number of *Anabaena* cells in the sample was 800 million L⁻¹. In formaldehyde-fixed samples, the colonies of *Anabaena* disintegrated and lost their typical morphology. However, in Lugol's-fixed samples, the colonies appeared to remain stable. Two different morphotypes could be distinguished: colonies with irregularly spiralled trichomes which were determined as *A. flos-aquae* (Lyngbye) Brébisson ex Bornet et Flahault (Fig. 3), and regularly coiled trichomes (Fig. 4). The latter trichomes often accumulated to very dense coiled rope-like colonies and were determined as *A. discoidea* (Schmidle) Ostenfeld (Fig. 5, 6). The shape and size of the vegetative cells (spherical to slightly oval, 5 x 5-7 µm) and heterocysts (spherical, 6-8 µm in diameter) in both taxa were identical (Figs 7, 8). Akinetes were not observed.

Four structural variants of microcystin, were detected by HPLC-PDA and the masses confirmed by MALDI-TOF: microcystins -RR, -LR, -LA and -LF (Table 1). The concentrations of microcystins in both samples were similar. The total concentration of microcystins in the filtered material was 39.15 µg g⁻¹ dry weight in sample 1 and 41.4 µg g⁻¹ dry weight in sample 2. The material did not contain detectable dhb-microcystin-LR, microcystin-LW, microcystin-YR or anatoxin-a. In all samples tested the dissolved cyanobacterial toxins were below detection limit of 1 µg L⁻¹.

Table 1. Structural variants of microcystins in a plankton sample from Lake Victoria detected by HPLC-PDA and MALDI-TOF analysis.

Sample	Toxin	HPLC-PDA- Retention time [min]	Mass m/z	Concentration [$\mu\text{g g}^{-1}$ DW]
Sample No. 1 05.11.01	Microcystin-RR	9.16	1037	2.05
	Microcystin-LR	12.15	994	12.5
	Microcystin-LA	15.55	909	11.2
	Microcystin-LF	19.85	986	13.4
Sample No. 2 05.11.01	Microcystin-RR	9.17	1037	2.7
	Microcystin-LR	12.16	994	11.2
	Microcystin-LA	15.6	909	8.7
	Microcystin-LF	19.9	986	18.8

Discussion

According to more recent data, *Anabaena flos-aquae* is the most common species of this genus in Lake Victoria (Talling, 1987; Gophen et al., 1995). *Anabaena discoidea* is only mentioned in classical studies. Schmidle (1902) described this cyanophyte as *A. flos-aquae* f. *discoidea*. Ostenfeld (1908) raised this taxon to the level of a species. This was because of its apparently unique character of having dense coiled rope-like filaments with wide convolutions and close parallel arranged trichomes. Wołoszynska (1914) re-discovered *A. discoidea* and stated that this species could be endemic for the Great Lakes of Africa. Bachmann (1933) reported that the species did not exist in the samples collected by E.B. Worthington towards the end of the 1920s (Worthington, 1930). Interestingly, in modern phytoplankton lists of the African Great Lakes, *A. discoidea* is absent. Our findings confirm that this taxon is morphologically different from *A. flos-aquae* with regard to the arrangement of the trichomes within the colonies. Nevertheless, up to now no other diacritical characteristic has been found. For example, it would be useful to study akinete morphology in *A. discoidea* filaments, however, these ontogenic cells have never been found. Hence the issue of whether the two species should be considered as separate taxa still remains unclear.

The history of cyanobacterial blooms in Lake Victoria can be traced to the time of the classical studies of Ostenfeld (1908). Talling (1966) has reported on the remarkable seasonal

variation in the occurrence of cyanobacterial blooms at this lake. Maximum bloom development was observed during the dry season when the water column was thermally stratified. Between October and December 1960, more than 10 million cells of *A. flos-aquae* per liter were recorded at an offshore station ("open lake") on the Ugandan part of Lake Victoria (Talling, 1966). In our samples, the cell count of 800 million cells L⁻¹ for the *Anabaena* taxa was remarkable higher. However, this is attributable to our sampling close to the shoreline. Bootsma and Hecky (1993) have reported that dominance in the phytoplankton communities of Lake Victoria has switched to the cyanobacteria. Komárek and Kling (1991) have attributed the increase in filamentous cyanobacteria with heterocysts to eutrophication. Evidence of a progressive rise in eutrophication was based on a comparison of the Secchi disc measurements of 1920 (7-8 m: Worthington, 1930) with data for the beginning of 1990 (1.5-2.5 m: Hecky in Komárek and Kling, 1991). Ochumba and Kibaara (1989) and Lung'Ayia et al. (2000, 2001) have observed that blooms strongly dominated by cyanobacteria are a common phenomenon in Lake Victoria's Nyanza Gulf.

Cyanobacterial blooms often produce cyanotoxins which are health hazards (Carmichael, 1997; Codd et al., 1989, 1999; Chorus, 2001) and which can have serious ecological impacts on aquatic food webs (Christoffersen, 1996). Our findings of microcystins in the filtered Lake Victoria cell fraction are regarded as an underestimation of the total microcystin concentration present in the samples. Although no extracellular toxin was detectable this fraction is typically a small percentage of the total microcystin pool (e.g. < 10%) during the early stages of toxigenic population development, this can become the majority fraction in ageing and lysing cyanobacterial populations (Codd et al., 1989; Chorus, 2001). Whether the microcystins detected in the particulate material are attributable to all of the cyanobacterial species found, is not clear at present. The *A. flos-aquae* and *M. aeruginosa* members of the bloom are well established as microcystin-producers (Carmichael, 1997; Codd et al., 1989, 1999; Chorus, 2001), although not all strains of these species produce the toxins. *A. discoidea* has apparently not been hitherto recorded among toxigenic cyanobacteria (Skulberg et al., 1993). The assignment of the microcystins detected, to the individual species of the Lake Victoria cyanobacteria awaits further investigation after this report of cyanotoxins in one of the Great Lakes of Africa.

Zusammenfassung

Cyanotoxin-produzierende Wasserblüte von *Anabaena flos-aquae*, *Anabaena discoidea* und *Microcystis aeruginosa* (Cyanobakterien) im Nyanza Golf des Victoriasees, Kenia.

Eine Blaualgenblüte, die von *Anabaena flos-aquae*, *Anabaena discoidea* und *Microcystis aeruginosa* dominiert war, wurde im Nyanza Golf des Victoriasees bei Kisumu, Kenia untersucht. Nach klassischen Literaturangaben ist *A. discoidea* endemisch für die Großen Seen Afrikas. Die morphologische Charakteristik dieser Art wurde beschrieben und im Vergleich zu *A. flos-aquae* diskutiert. Die beiden untersuchten Wasserproben enthielten die Cyanotoxine Microcystin-LR, -RR, -LA und -LF.

Acknowledgements

The authors express their thanks to Government of Kenya, for providing research permission (No. MOEST 13/001/31 C 90). We are grateful to the German Federal Ministry of Education and Research for financial support in the frame of the BIOLOG programme (grant No. 01LC0001), and Stephanie Pütz and Stephen Mwangi Gichobi for excellent technical assistance.

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