III

Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya

Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya

Abstract

Cyanobacterial mats at hot springs on the shore of the alkaline Lake Bogoria, Kenya, were investigated regarding species community and cyanobacterial toxin content. The hepatotoxins microcystin -LR, -RR, -LF and -YR, and the neurotoxin anatoxin-a were present. The mats were dominated by *Phormidium terebriformis*, *Oscillatoria willei*, *Spirulina subsalsa* and *Synechococcus bigranulatus*. The concentration of microcystins in mat samples, ranged from 221 to 845 µg microcystin-LR equivalents g^{-1} DW of mat. Anatoxin-a concentrations ranged from 10 to 18 µg g^{-1} DW of mat. A contribution of the cyanobacterial toxins from the hot spring-mats to the mass mortalities of Lesser Flamingos is suggested by: *a*, the presence of hot spring cyanobacterial cells and cell fragments, and high concentrations of the cyanobacterial hepato- and neurotoxins in flamingo stomach contents and faecal pellets; *b*, observations of neurological signs of bird poisoning at the lake. Cyanobacterial toxins in stomach contents, intestine and fecal pellets were 0.196 µg g^{-1} FW for the microcystins and 4.34 µg g^{-1} FW for anatoxin-a. Intoxication with cyanobacterial toxins could occur by uptake of detached cyanobacterial cells from the mats, as the flamingos need to drink fresh- or brackish water, and to wash their feathers daily, which they do in the vicinity of the hot springs, where salinity is lower than in the main waterbody of the lake.

Keywords: Anatoxin, Cyanobacteria, Hot springs, Lake Bogoria, Lesser Flamingo, Microcystin

Introduction

The Rift Valley Lakes of Africa are among the natural wonders of the world and give an insight into the past history of Earth. Lake Bogoria, one of the harshest volcanic places in the region, is literally a hot-spot of cyanobacterial life. The alkaline water of the lake supports mass populations of *Arthrospira fusiformis* (Voronichin) Komárek (syn. *Spirulina fusiformis* Voronichin) [1]. Numerous thermal springs and geysers are active on the shore of L. Bogoria

[2]. These extreme environments are colonized by mats rich in thermophilic cyanobacteria [3]. Like other Rift Valley soda lakes, L. Bogoria is home to the *Arthrospira*-consuming Lesser Flamingo (*Phoeniconaias minor* Geoffrey). The daily feeding rate of an adult bird is about 72 g dry weight (DW) of food cyanobacteria, mostly *A. fusiformis* [4]. The population densities of this bird on the Rift Valley lakes fluctuate widely depending mainly on changes in food quantity and breeding activity [4]. According to Owino et al. [5], the number of Lesser Flamingos at Lake Bogoria between 1991 and 1999 varied from 175 000 to 1 074 000, with a mean of 521 925 individuals. These observations on L. Bogoria represented the highest population densities of flamingos at the Kenyan lakes. However, massive deaths in recent years, especially at Lakes Nakuru and Bogoria, threaten the flamingo populations in the Rift Valley [6, 7]. For example, Vick [8] reported about 30 000 dead flamingos at L. Bogoria in the second half of 1999. According to calculations of the World Wildlife Fund, Lesser Flamingo populations have been falling by 10% per decade [7]. Reasons for this dramatic decline are not fully understood. During the last decade, a multidisciplinary study has provided insights into this highly complex phenomenon. The main causes of the mass mortalities are thought to be infections by mycobacteria [9] and poisoning by heavy metals and pesticides [7,10,11]. The present study is the first attempt to evaluate the contribution of cyanobacterial poisoning to the flamingo deaths. Here, we report that the cyanobacterial mats in the hot springs at the shore of L. Bogoria contain microcystins and anatoxin-a, which may contribute to the mysterious deaths of the Lesser Flamingos.

Materials and methods

Study site

Lake Bogoria (formerly Lake Hannington) is situated within a depression of the Gregory Rift Valley. Its geographic position is 00°15'N and 36°05'E at an elevation above sea level of 963 m. A comparison of the main physico-chemical characteristics of the alkaline Rift Valley lakes inhabited by Lesser Flamingos was provided by Vareschi [4]. The water of L. Bogoria is very saline and alkaline. The hot springs at the shore discharge water with different physico-chemical conditions into the lake: from fresh to moderately saline [2]. During the present study, comparative data on physico-chemical properties of the lake and the hot spring water were obtained near the hot springs Visitors' Viewing Point on the western shore:

Lake: pH 10.0, conductivity 67 200 µS cm^{-1}, salinity 45.6‰, alkalinity 1 020 meq l^{-1}, total nitrogen 1.4 mg l^{-1}, total phosphorus 5.4 mg l^{-1}.

Hot springs: pH 9.0, conductivity 6 410 µS cm^{-1}, salinity 3.5‰, alkalinity 78 meq l^{-1}, total nitrogen < 0.5 mg l^{-1}, total phosphorus 0.019 mg l^{-1}. The temperature of the hot springs ranged from boiling water down to 35°C.

Samples 11HS and HS1 were collected at the hot springs Visitors' Viewing Point. Samples HK1 and HK2 were taken at the geyser and hot spring area (Fig. 1A) about two kilometers south of the Viewing Point.

Sampling design and microscopy

Cyanobacterial samples were taken in June and November 2001. Every sample contained about 15 ml of the fresh cyanobacterial mat scraped from the ground of the rivulets of the hot springs. The wet, paste-like samples were placed, using a spatula, onto glass fibre filters (Whatman GF/C; Whatman International Ltd, Maidstone, England). The filters with the cyanobacterial mass were air-dried and stored at room temperature for later cyanobacterial toxin analysis. From each sampling site, a formaldehyde-fixed mat sample was taken for microscopy. Dominant species of cyanobacteria were determined and photographically documented using an Eclipse E600 light microscope (Nikon Corporation, Tokyo, Japan). In addition, Lesser Flamingo faecal pellets, collected from lake shorelines, and stomach contents from dead birds were examined microscopically for cyanobacterial cells and remains. We sampled two dead flamingos, one on 13 June 2001 and another on 29 March 2002.

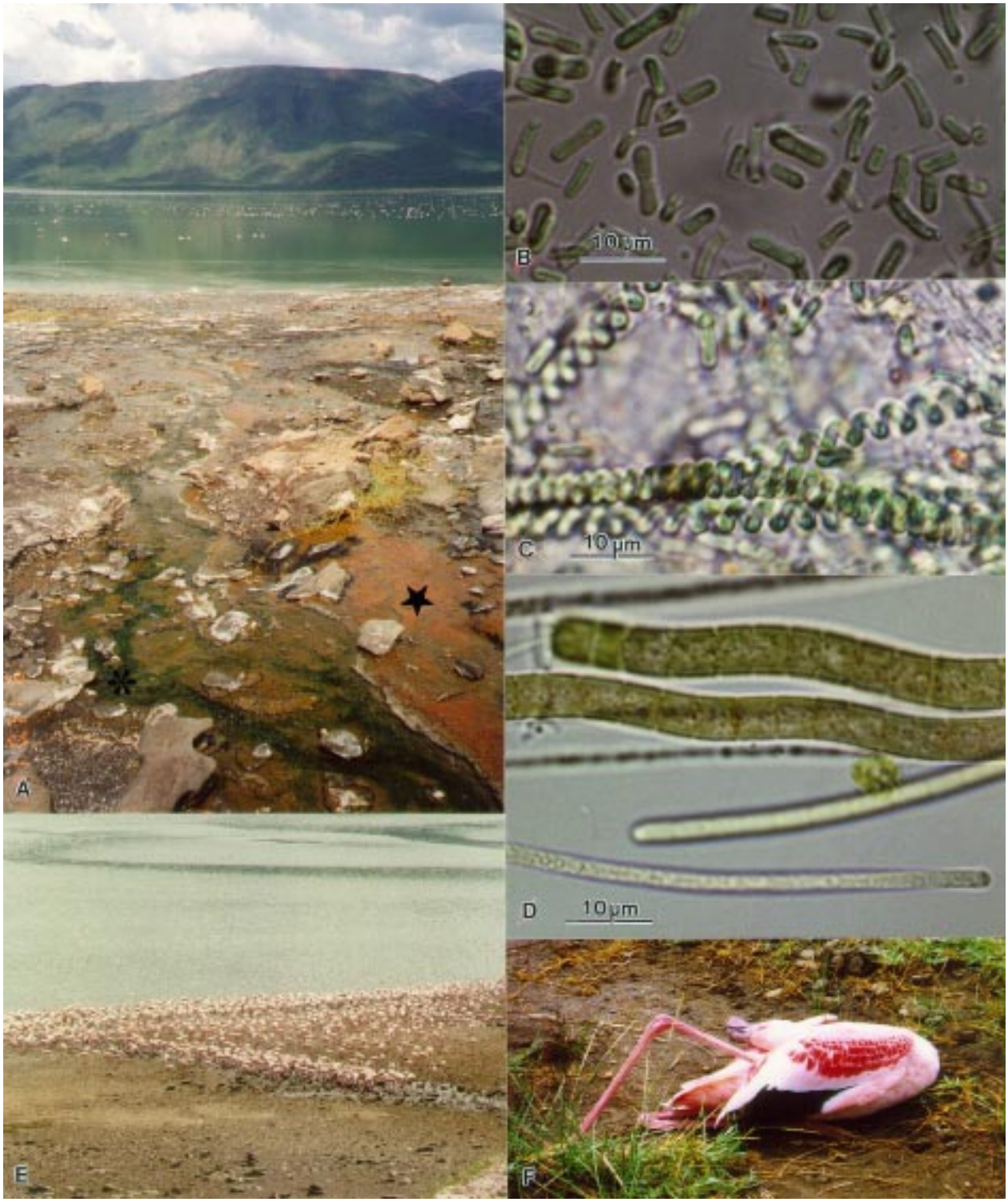

Fig. 1A: Sampling sites of hot spring cyanobacteria at Lake Bogoria. The star indicates the sampling site of sample HK1, dominated by *P. terebriformis* and *O. willei*; the asterisk indicates the sampling site of sample HK2, dominated by *S. subsalsa* and *S. bigranulatus*. B-D: Dominant cyanobacteria in hot springs at L. Bogoria. B: *S. bigranulatus*. C: *S. subsalsa* accompanied by *S. bigranulatus*.
D: *P. terebriformis* (above) and *O. willei* (below). E: Groups of Lesser Flamingo drinking water from the hot springs flowing into the lake. F: Dead Lesser Flamingo with convulsed position of extremities and neck (opisthotonus).

Determination of cyanobacterial toxin composition and concentration

Filtered cyanobacterial mat samples were extracted by adding 10 ml of 70% v/v aqueous methanol, followed by ultrasonication on ice for 15 minutes, and finally constantly shaken 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 nitrogen. The dried residues were redissolved in 1 ml 70% methanol [12]. Samples from flamingo carcasses (1.5 g stomach contents, and 0.5 g empty intestine and 2.0 g faecal pellets; fresh weights [FW]) were extracted with 10 ml of 70% methanol with the addition of 1% v/v trifluoroacetic acid, homogenized with an Ultra –Thorax for 5 min under constant cooling, and centrifuged for 5 min at 5000 rpm to remove particulate material. The supernatants were dried under nitrogen and redissolved in 500 µL 70% methanol for toxin analysis. 50 µl subsamples were used for analysis by high performance liquid chromatography with photodiode array detection (HPLC-PDA) and MALDI-TOF analysis [13]. Purified reference toxins used were: microcystin-LR (gravimetric standard) and dehydrobutyrine (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). Microcystins are heptapeptides, occuring in more than 60 structural variants, which mainly differ in two amino acids, indicated by the international abbreviations: L (leucine), R (arginine), Y (tyrosine), W (tryptophan), F (phenylalanine).

Results

Characterization of dominant cyanobacteria

The cyanobacterial communities in the hot springs at the shore of Lake Bogoria were dominated by four species (Table. 1) and were characterized as below:

Synechococcus bigranulatus Skuja (Figs. 1B, C)

Cells solitary, after cell division in pseudofilaments of two or four cells, rod-shaped straight or slightly curved, 3-11 µm long, 1.3-2.4 µm broad, with rounded poles, within the protoplast sometimes with granule-like incorporations near the pole, with no mucilaginous sheath; chromatoplasm pale to deep blue-green.

Spirulina subsalsa Oersted (Fig. 1C)
Filaments solitary, 1.2-1.4 µm in diameter, often more than 100 µm long, regularly spiraled, width of helices 3.5-4.5 µm, distance between helices 0.2-2.5 µm, with or without a mucilage envelope, cell contents homogeneous, no cross walls visible, bright blue-green.

Phormidium terebriformis (Agardh ex Gomont) Anagnostidis & Komárek (Fig. 1D)
Filaments solitary, often very closely attached to each other, 4.5-6 µm in diameter, often more than 100 µm long, slightly undulated, seldom covered by a sheath, cross walls within filaments very distinct, cells nearly cylindrical, as long as wide or slightly shorter than wide, terminal cells mostly with rounded ends, seldom long attenuated and pointed; cell contents clearly differentiated into chromato- and caryoplasm, with many inclusions. Filaments and mats of a characteristic brown-green colour.

Oscillatoria willei Gardner (Fig. 1D)
Filaments solitary, 2-2.5 µm in diameter, often more than 150 µm long, straight, with rounded ends, cross-walls visible, cylindrical cells 2.5-6 µm long, grey-green.

Species distribution at the different sampling sites

In samples 11HS, HS1 from the hot springs Visitors' Viewing Point, we observed a mixture of all four species. The colour of the mat samples was brownish-green. The other samples (HK1, HK2) from the geyser area could be differentiated visually by their colouration (Fig. 1A). Sample HK1 was brown and dominated by *Phormidium terebriformis* and *Oscillatoria willei*. Sample HK2 was strikingly blue-green and dominated by *Spirulina subsalsa* and *Synechococcus bigranulatus*. In all samples, about ten to twenty other coccoid and filamentous cyanobacterial taxa, described by Hindák [3] occurred as minor components of the assemblage.

Stomach contents and faecal pellets

Microscopic examination of the stomach contents and faecal pellets from the two carcasses of the Lesser Flamingos collected and examined revealed intact filaments and fragments of *Arthrospira fusiformis*, as well as members of the hot spring cyanobacterial mat community, especially *Oscillatoria willei*.

Cyanobacterial toxins

Cyanobacterial hepatotoxins (microcystins) and neurotoxin (anatoxin-a) were present in all cyanobacterial mat samples analysed (Fig. 2; Table 1). The number of microcystin structural variants detected per sample ranged from 2 to 4, and included microcystins -LR, -RR, -LF and -YR. Total microcystin concentrations ranged from 221 to 845 µg microcystin-LR equivalents g^{-1} DW of mat (Table 1). Anatoxin-a concentrations were considerably lower (10 to 18 µg g^{-1} mat DW).

Analysis of the stomach content, an emptied piece of the intestine from dead flamingos as well as faecal pellets showed the presence of different variances of microcystins as well as anatoxin-a (Table 2). In the stomach content, all four types of microcystin which also were detected in the cyanobacterial mats were present. The total microcystin content was 0.196 µg microcystin-LR equivalent g^{-1} FW. High concentration of 4.349 µg g^{-1} FW anatoxin-a was detected in the stomach content. In the intestine only microcystin-LR and anatoxin-a were present. In the faecal pellets all toxins were found with the exception of microcystin-LF.

Table 1: Cyanotoxins in cyanobacterial mats of hot springs at Lake Bogoria [a]

Sample code	Sampling date	Toxin type and concentration (µg g^{-1} DW)					Dominant cyanobacteria
		MC-LR	MC-RR	MC-LF	MC-YR	Anatoxin-a	
11 HS	13 June 2001	198	n.d.	n.d.	23	18	*Phormidium terebriformis, Oscillatoria willei Spirulina subsalsa, Synechococcus bigranulatus*
HS1	10 Nov. 2001	50	58	375	352	15	*Phormidium terebriformis, Oscillatoria willei Spirulina subsalsa, Synechococcus bigranulatus*
HK1	14 Nov. 2001	23	35	207	14	10	*Phormidium terebriformis, Oscillatoria willei*
HK2	14 Nov. 2001	n.d	n.d	177	150	10	*Spirulina subsalsa, Synechococcus bigranulatus*

MC = microcystin; n.d. = not detected.
[a] No sample contained detectable dhb-MC-LR, MC-LA or MC-LW.

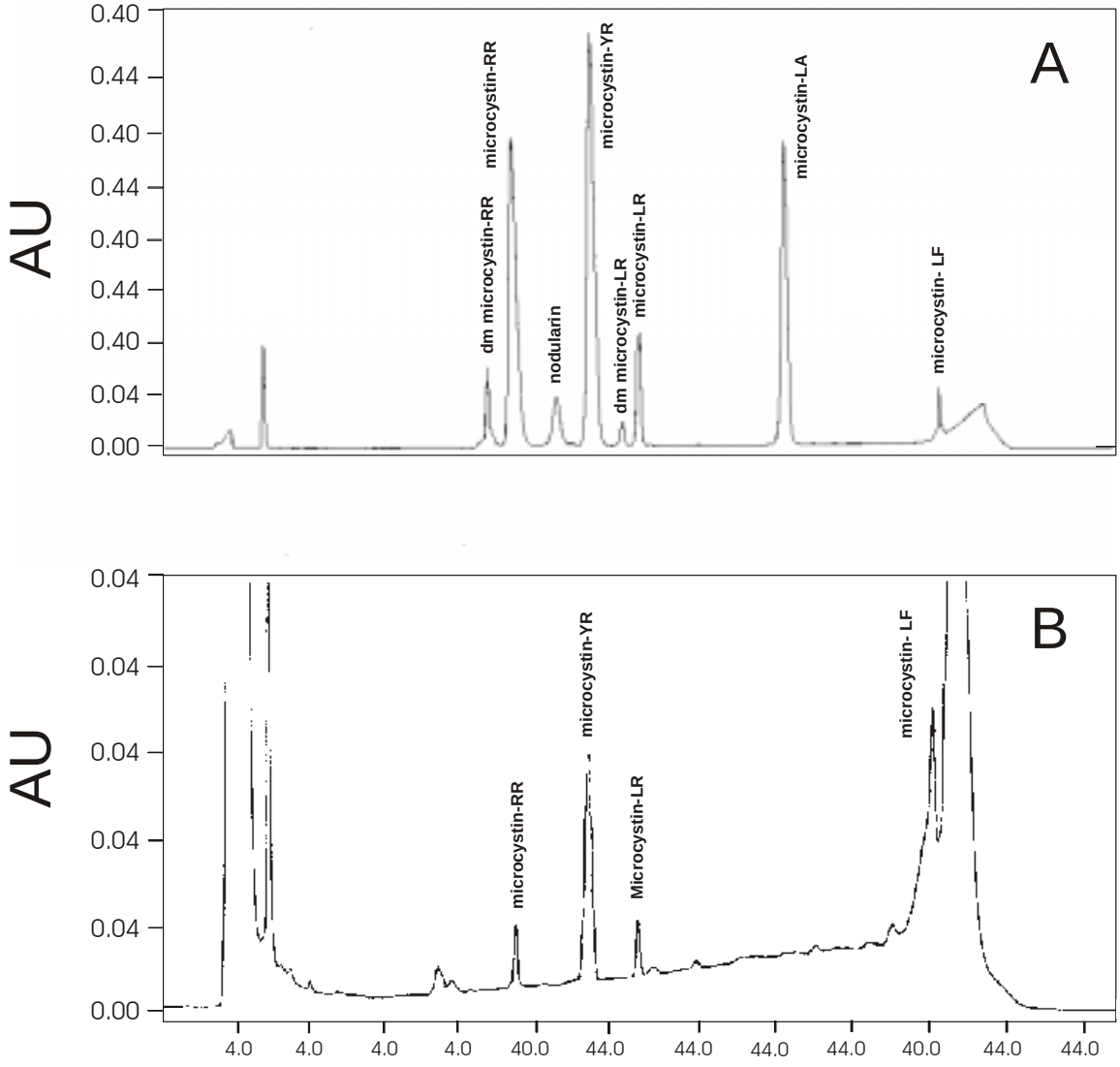

Fig. 2: Determination of cyanobacterial toxin content of sample HS 1 from a hot spring near the Visitors' Viewing Point at Lake Bogoria by HPLC-PDA. Comparison and detection of the toxins were done with reference to purified toxin retention times (A), PDA spectra and by MALDI-TOF analysis using the same extract (detected m/z in brackets after each respective toxin). Toxins detected in the sample (B) were microcystin-RR (m/z 1037), microcystin-YR (1044), microcystin-LR (994) and microcystin-LF (986). (Abbreviations: AU = absorption units, m/z = mass, PDA = photodiode array, MALDI-TOF = matrix assisted laser desorption/ionization time of flight mass spectrometry, dm = desmethyl-).

Table 2: Cyanotoxins in stomach content and faecal pellets of Lesser Flamingos carcasses from Lake Bogoria[a]

Sample	Toxin type and concentration (µg g^{-1} FW)				
	MC-LR	MC-RR	MC-LF	MC-YR	Anatoxin-a
Stomach content	0.016	0.021	0.112	0.047	4.349
Intestine	0.036	n.d.	n.d.	n.d.	0.762
Faecal pellet	0.013	0.008	n.d.	0.027	0.245

MC = microcystin; n.d. = not detected.
[a]No sample contained detectable dhb-MC-LR, MC-LA or MC-LW.

Discussion

The cyanobacterial communities in our samples closely resembled the findings of Hindák [3], who studied a sample collected by Dr. Liselotte Schulz in October 1981 at the hot springs at Lake Bogoria. The dominant species in our samples belong to the most common cyanobacteria in thermal habitats. *Synechococcus* spp. are found in hot springs worldwide [14, 15]. *Synechococcus bigranulatus* especially typically occurs at temperatures from 20 to 75 °C. Thermophilic model strains of *S. bigranulatus* are often mistakenly designated as *S. elongatus* [14]. Molecular methods have revealed high biodiversity in cyanobacterial mats. For example, numerous different 16S rRNA genotypes of *Synechococcus* have been recorded from the Yellowstone National Park hot springs [16]. A study on 16S rRNA sequences of 38 *Synechococcus* taxa from different continents, revealed the polyphyly of the genus [17].

Spirulina subsalsa, and the closely related taxon *Spirulina subtilissima* Kützing ex Gomont, which Hindák [3] reported to be the dominant species in L. Bogoria hot springs, have a wide ecological distribution. The two species occur in thermal springs and in mesophilic brackish and marine habitats [18]. Few cyanobacterial genera are so well adapted to a wide range of habitats as *Spirulina* [19]. It should be noted that the taxonomy of *Spirulina*/*Arthrospira* is under revision [20, 21]. Tomaselli *et al.* [22] strongly support the separation of these genera. *Arthrospira* (commercially available under the misleading designation "*Spirulina*"), with a

relatively high nutritional value, can clearly be differentiated from true *Spirulina* taxa with lower, or no nutritional value.

Phormidium terebriformis, which in ecological literature is mostly designated *Oscillatoria terebriformis* Agardh ex Gomont, has been classified as a cosmopolitan member of thermal and sulphur springs [18]. A "thermal-red" form of *O. terebriformis* is often found dominating the cyanobacterial mats in North American hot springs from 40 to 54°C [23]. *Oscillatoria willei* has previously been noted as a common taxon in the Bogoria hot springs [3]. This species also occurs in Lake Chad and is regarded as pantropical [24].

Our study shows that the cyanobacterial mat communities of the shoreline hot springs at L. Bogoria contain cyanobacterial toxins: at least 4 microcystins and anatoxin-a. This is apparently the first report of microcystin and anatoxin occurrence in hot springs, almost all knowledge of cyanobacterial toxin occurrence having been derived from samples and strains from mesophilic environments. Previous indicators that cyanobacteria from warm (and cold) environments can produce cyanobacterial toxins include [L-Har^2]nodularin in *Nodularia* PCC 7804, from a freshwater warm spring [25], and microcystin-LR and nodularin in benthic cyanobacterial mats in Antarctic ice-water melt ponds [26].

The exact origin of the anatoxin-a and microcystins among the cyanobacteria in the Bogoria hot spring mats remains unclear. Anatoxin-a, and the related neurotoxin, homoanatoxin-a, have been identified in benthic *Oscillatoria* and *Phormidium* spp. [27, 28]. Microcystins were also detected in cyanobacterial mat samples dominated by *Oscillatoria* and *Phormidium* spp. in Swiss alpine ponds which are sites associated with cattle poisoning [29]. If hot spring cyanobacterial toxins have deleterious effects on animal health, then some thermostability of the toxins may be expected to be necessary. This requirement is fulfilled: incubation of anatoxin-a-producing cultures of the mesophile *Anabaena flos–aquae* at 100°C for 60 min reduces acute neurotoxicity in bioassays by less than three-fold [30]. Microcystins withstand boiling for several hours [31] and are stable over weeks at 40°C [32]. The cyclic heptapeptide microcystins accumulate in the liver, leading to necrosis and intrahepatic bleeding [33, 34, 35]. Anatoxin-a is a bicyclic alkaloid, causing symptoms including muscle fasciculation, loss of coordination, gasping, convulsions and death by respiratory arrest [36].

Mass poisonings of wild and domestic birds, associated with cyanobacterial blooms and scums, have been recorded for several years in North America, Europe and Australia [37]. Matsunaga *et al.* [38] provide a recent example involving toxic *Microcystis aeruginosa* in the mass deaths of wild ducks in Japan. Takahashi & Kaya [39] report a LD_{50} of 256 µg microcystin-RR per kg quail obtained by intraperitoneal administration. Toxicity of anatoxin-a to birds has been studied using chicks, mallards, and ring-necked pheasants treated with lyophilized *Anabaena flos-aquae* NRC-44-1 suspensions. This resulted in intraperitoneal LD_{90} values from 50 – 120 mg kg^{-1}, and from 350 – 850 mg kg^{-1} by oral administration [40]. Based on the LD_{50} for microcystin-RR which is a less toxic microcystin [41], an estimate of the quantity of toxic cyanobacteria to be consumed by a 2 kg body weight Lesser Flamingo to have a lethal effect is about 22 g DW. This is 30% of the daily feeding rate of 72 g DW consumed by a flamingo [4]. For anatoxin-a, this computation leads to 0.6-1.6 g cyanobacterial DW (2% of the daily feeding rate), which would have to consist of anatoxin-a producing cyanobacteria. These estimates of the scale of necessary food consumption to cause toxic effects in the flamingos indicate that intoxication of the birds by feeding is feasible. However, these estimates are constrained by a lack of information about the specific susceptibility of flamingos to cyanobacterial toxins in laboratory trials and by the natural variabilities in susceptibility between species and different exposure routes.

Behavioral studies of Lesser Flamingos at high alkaline Rift Valley lakes have shown that each bird needs to drink some freshwater, or water of low salinity once per day and to wash its feathers [42]. At the shore of L. Bogoria, we observed large groups of flamingos drinking and washing near the inflow of the hot springs water into the lake (Fig. 1E). It is possible that during these activities, the flamingos feed on portions of cyanobacteria that have detached from the mats. In this way, the birds may consume sufficient quantities of the toxic cyanobacteria to result in chronic and acute toxic effects. The presence of hot spring cyanobacterial cells, fragments and cyanobacterial toxins in stomach contents and faecal pellets supports the possibility that these toxins contribute to the flamingo mass mortalities. The ophistotonus behaviour of flamingos, especially the convulsed position of extremities and neck in the dying phase (Fig. 1F) indicates neurotoxic effects. Similar symptoms were described after administering neurotoxic cyanobacterial extracts containing anatoxin-a, to mallards [36]. The high concentrations of microcystins and anatoxin-a in the stomach contents

and intestine of dead flamingos from L. Bogoria, and in the faecal pellets indicate that cyanobacterial hepatotoxins and neurotoxin(s) can contribute to the bird deaths. These findings may provide one additional explanation for the mass deaths of the Lesser Flamingos, in addition to the reported contributions of bacterial pathogens, metals and pesticides [7-11]. However, a close relationship has clearly evolved at ecosystem level between the flamingos and the hot spring cyanobacteria of L. Bogoria which has persisted over many years. Whether flamingo populations at these sites were exposed to cyanobacterial toxins before their habitates were subjected to the present high levels of human influence is unknown. However it is possible that the flamingos reaching L. Bogoria, especially from L. Nakuru have already been weakened by other anthropogenic toxicants and this increases their susceptibility to cyanotoxin poisoning. Currently, the causes of the flamingo deaths are the focus of multidisciplinary investigations in pathology, toxicology, ecology and ethology. These are further necessary to help to formulate steps to mitigate these problems. Because of the frequent movement of Lesser Flamingos between Rift Valley alkaline lakes, as dictated by their feeding and breeding preferences, the interpretation of the situation at L. Bogoria should cover all of the alkaline lakes of the Rift Valley. According to Vareschi [4], all Lesser Flamingos in this area could belong to the same single population. Hence, it is more than likely that the flamingos are subjected to multiple stresses at other Rift Valley lakes.

Acknowledgements

We thank the 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 (grant No. 01LC0001). Our sincere appreciation is also due to the County Councils of Koibatek and Baringo Districts for granting free access to L. Bogoria and for their collaboration and helpful assistance. In particular, we thank William Kimosop (Senior Warden, Lake Bogoria National Reserve), Oliver Nasirwa (WWF Bogoria), Daniel Koros (WWF Nakuru) and Ramesh Thampy (WWF Nairobi) for providing valuable information on the flamingos of the Rift Valley lakes. Logistical support was provided by Don Adams (State University of New York, Plattsburgh). Christine and Peter Casper (UBA/IGB Stechlin-Neuglobsow) assisted in sampling.

References

[1] Hindák, F. (1985) Morphology of trichomes in *Spirulina fusiformis* Voronichin from Lake Bogoria, Kenya. Arch. Hydrobiol., Suppl., Algological Studies 38/39, 201-218.

[2] Cioni, R., Fanelli, G., Guidi, M., Kinyariro, J.K. and Marini, L. (1992) Lake Bogoria hot springs (Kenya): geochemical features and geothermal implications. J. Volcanol. Geoth. Res. 50, 231-246.

[3] Hindák, F. (2001) Thermal microorganisms from a hot spring on the coast of Lake Bogoria, Kenya. Nova Hedwigia, Beih. 123 (Algae and Extreme Environments), 77-93.

[4] Vareshi, E. (1978) The ecology of Lake Nakuru (Kenya) I. Abundance and feeding of the Lesser Flamingo. Oecologia 32, 11-35.

[5] Owino, O.A., Oyugi, J.O., Nasirwa, O.O. and Bennun, L.A. (2001) Patterns of variation in waterbird numbers on four Rift Valley lakes in Kenya, 1991-1999. Hydrobiologia 458, 45-53.

[6] Bennun, L. and Nasirva, O. (2000) Trends in waterbird numbers in the southern Rift Valley of Kenya. Ostrich 71, 220-226.

[7] Wanjiru, J. (2001) Kenya's pink flamingos weighted down by heavy metals. Environment News Service, July 16, 2001. wysiwyg://4/http://www.ens-news.com/ens/jul2001/ 2001L-07-16-04.html.

[8] Vick, K. (2000) Kenya's pink death. Washington Post, March 7, 2000: A12. http://www.epa.gov/earlink1/earthlink/00janfeb.htm.

[9] Kock, N.D., Kock, R.A., Wambua, J., Kamau, G.J. and Mohan, K. (1999) *Mycobacterium avium*-related epizootic in free-ranging Lesser Flamingos in Kenya. J. Wildlife Dis. 35, 297-300.

[10] Kairu, J.K. (1996) Heavy metals residues in birds of Lake Nakuru, Kenya. Afr. J. Ecol. 34, 397-400.

[11] Nelson, Y.M., Thampy, R.J., Motelin, G.K., Raini, J.A. DiSante, C.J. and Lion, L.W. (1998) Model for trace metal exposure in filter-feeding flamingos at alkaline Rift Valley lakes, Kenya. Environm. Toxicol. Chemist. 17, 2302-2309

[12] Fastner, J., Flieger, I. and Neumann, U. (1998) Optimized extraction of microcystins from field samples – a comparison of different solvents and procedures. Water Research 32, 3177-3181.

[13] Pflugmacher, S., Wiegand, C., Beattie, K.A., Krause, E., Steinberg, C.E.W. and Codd, G.A. (2001) Uptake, effects and metabolism of cyanobacterial toxins in the emergent reed plant *Phragmites australis* (Cav.) Trin. ex. Steud. Environm. Toxicol. Chemist. 20, 846-852.

[14] Komárek, J. and Anagnostidis, K. (1999) Cyanoprokaryota 1. Teil: Chroococcales. In: Süßwasserflora von Mitteleuropa (Ettl, H., Gärtner, G., Heynig, H. and Mollenhauer, D., Eds.), pp. 548. Gustav Fischer, Jena, Stuttgart, Lübeck, Ulm.

[15] Copeland, J.J. (1936) Yellowstone thermal Myxophyceae. Ann. New York Acad. Sci. 36, 1-232.

[16] Ward, D.M., Ferris, M.J., Nold, S.C. and Bateson, M.M. (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiology and Molecular Biol. Rev. 62 1353-1370.

[17] Robertson, B.R., Tezuka, N. & Watanabe, M.M. (2001) Phylogenetic analyses of *Synechococcus* strains (cyanobacteria) using sequences of 16S rDNA and part of the

phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content. Int. J. Syst. Evol. Microbiol. 51, 861-871.

[18] Geitler, L. (1930-32) Cyanophyceae. In: Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz (R. Kolkwitz, Ed.), pp. 1196, Akademische Verlagsgesellschaft Leipzig.

[19] Anagnostidis, K. and Golubic, S. (1966) Über die Ökologie einiger *Spirulina*-Arten. Nova Hedwigia 11, 309-335.

[20] Castenholz, R.W. (1989) Subsection III. Order Oscillatoriales. In: Bergey's Manual of Systematic Bacteriology Vol. 3 (Staley, J.T., Bryant, M.P., Pfennig, N. and J.G. Holt, Eds.), pp. 1771-1780. Williams & Wilkins, Baltimore, Hong Kong, London, Sydney.

[21] Castenholz, R.W., Rippka, R., Herdman, M. and Wilmotte, A. (2001) Subsection III. (Formerly Oscillatoriales Elenkin 1934). In: Bergey's Manual of Systematic Bacteriology Vol. 1 (Boone, D.R., Castenholz, R.W. and Garrity, G.M., Eds.), pp. 539-562. Springer New York, Berlin, Heidelberg, Barcelona, Hong Kong, London, Milan, Paris, Singapore, Tokyo.

[22] Tomaselli, L., Palandri, M.R. and Tredici, M.R. (1996) On the correct use of the *Spirulina* designation. Algol. Stud. 83, 539-548.

[23] Castenholz, R.W. (1978) The biogeography of hot spring algae through enrichment cultures. Mitt. Intern. Verein. Limnol. 21, 296-315.

[24] Compère, P. (1974) Algues de la région du lac Tchad II. Cyanophycées. Cah. ORSTOM Hydrobiol. 8, 165-198.

[25] Beattie, K.A., Kaya, K. and Codd, G.A. (2000) The cyanobacterium *Nodularia* PCC 7804, of freshwater origin, produces [L-Har^2]nodularin. Phytochemistry 54, 57-61.

[26] Hitzfeld, B.C., Lampert, C.S, Spaeth, D., Mountfort, D., Kaspar, H. and Dietrich D.R. (2000) Toxin production in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Toxicon 38, 1731-1748.

[27] Edwards, C., Beattie, K.A., Scrimgeour, C.M. and Codd, G.A. (1992) Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. Toxicon 30, 1165-1175.

[28] Skulberg, O.M., Carmichael, W.W., Andersen, R.A., Matsunaga, S., Moore, R.E. and Skulberg, R. (1992) Investigation of a neurotoxic Oscillatorialean strain (Cyanophyceae) and its toxin: isolation and characterization of homoanatoxin-a. Environm. Toxicol. Chemist 11, 321-329.

[29] Mez, K., Beattie, K.A., Codd, G.A., Hanselmann, K., Hauser, B., Naegeli, H. and Preisig, H.R. (1997) Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. Eur. J. Phycol. 32, 111-117.

[30] Al-Layl, K.S.J. (1989) Studies on the toxicity of the cyanobacteria *Anabaena flos-aquae* and *Microcystis aeruginosa.* PhD Thesis. University of Dundee. Dundee, Scotland.

[31] Codd, G.A. and Bell S.G. (1996) The occurrence and fate of blue-green algal toxins in Freshwaters. R & D Report 29, National Rivers Authority. Her Majesty's Stationery Office, London, pp. 30.

[32] Harada, K.-I., Tsui K. and Watanabe, M.F. (1996) Stability of microcystins of cyanobacteria – III. Effect of pH and temperature. Phycologia 35, 83-88.

[33] Falconer, I., Beresford, A.M. & Runnegar, M.T.C. (1983) Evidence of liver damage by toxins from a bloom of the blue-green alga *Microcystis aeruginosa*. Med. J. Aust. 1, 511-514.

[34] Eriksson, J.E., Toivola, D., Meriluoto, J.A., Karaki, H., Han Y.G. and Hartshorne, D. (1990) Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases. Biochem. Biophys. Res. Comm. 173, 1347-1353.

[35] Råberg, C.M.I., Bylund, G. & Eriksson, J.E. (1991) Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa* on common carp (*Cyprinus carpio*). Aquat. Toxicol. 20, 131-146.

[36] Carmichael, W.W., Biggs, D.F. and Gorham, P.R. (1975) Toxicology and pharmacological action of *Anabaena flos-aquae* toxin. Science 187, 542-544.

[37] Yoo, R.S., Carmichael, W.W., Hoehn, R.C. and Hrudey, S.C. (1995) Cyanobacterial (blue-green algal) toxins: A Resource Guide. American Water Works Association Research Foundation, Denver, Colorado. pp. 229.

[38] Matsunaga, H., Harada, K.I., Senma, M., Ito Y., Yasuda, N., Ushida, S. and Kimura, Y. (1999) Possible cause of unnatural mass death of wild birds in a pond in Nishinomiya, Japan: Sudden appearencee of toxic cyanobacteria. Natural Toxins 7, 81-84.

[39] Takahashi, S. and Kaya, K. (1993) Quail spleen is enlarged by Microcystin-RR as a blue-green algal compound. Natural Toxins 1, 283-285.

[40] Carmichael, W.W. and Biggs, D.F., (1978) Muscle sensitivity differences in two avian species to anatoxin-a produced by the freshwater cyanophyte *Anabaena flos-aquae* NRC-44-1. Can. J. Zool. 56, 510-512.

[41] Harada, K.I. (1996) Chemistry and detection of microcystins. In: Toxic *Microcystis*. (Watanabe, M.F:, Harada, K., Carmichael, W.W., Fujiki, H. (Eds.), CRC Press, Boca Raton, pp. 103-148.

[42] Mari, C. and Collar, N. (2000) Pink Africa. The Harvill Press, London. 207 pp.