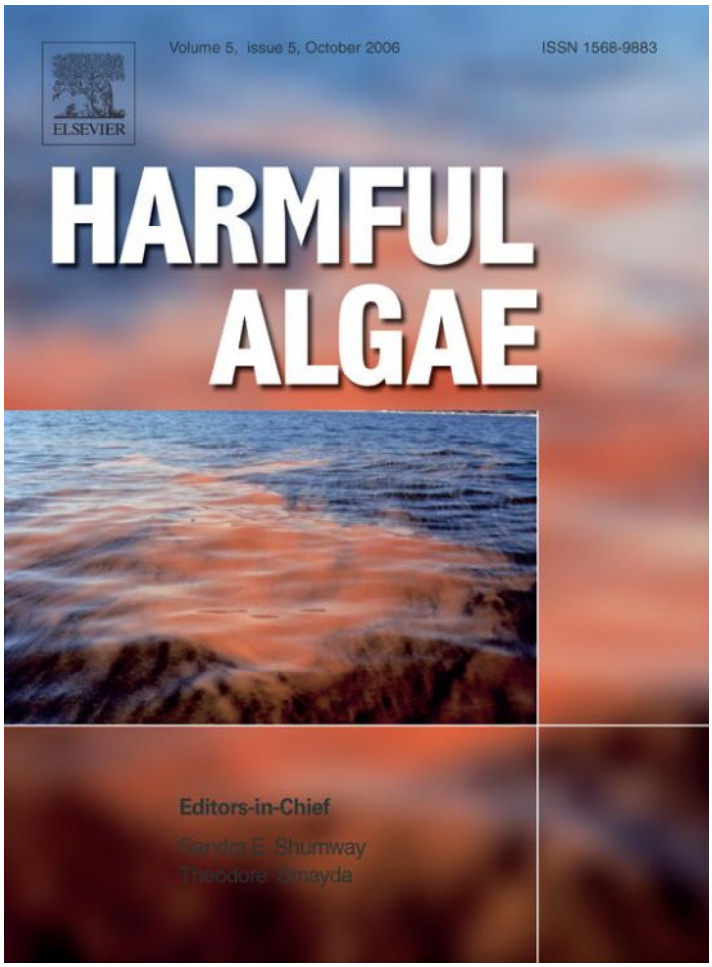
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Cyanobacteria blooms—A possible cause of mass mortality of Lesser Flamingos in Lake Manyara and Lake Big Momela, Tanzania

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Abstract

Limnological studies were conducted in three alkaline lakes (Lake Big Momela, Manyara and Embagai) with the aim of investigating the cause of mass mortality of the Lesser Flamingos in Lake Manyara and Lake Big Momela during July–August 2004. High concentrations, up to 150 million filaments per liter of the potentially toxic planktonic cyanobacterium *Arthrospira fusiformis* were found in surface scum of Lake Big Momela where Lesser Flamingos were dying at a rate of between 15 and 50 individuals per day during the study period. Gut content analyses indicated that *A. fusiformis* was the main food item in moribund flamingos. Mouse bioassay suggested that the crude microalgal extract dominated by *A. fusiformis* was toxic with all mice close to death becoming lethargic, with loss of balance, uncoordinated movements, intermittent tremors, dyspnoea with gasping followed by respiratory arrest. This observation gives circumstantial evidence that *A. fusiformis* at such high concentrations was toxic to the Lesser Flamingo in Lake Big Momela.
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Keywords: Lesser Flamingos; Cyanobacteria; Lake Big Momela; Lake Manyara; Tanzania

1. Introduction

In recent years, there have been frequent reports of mass mortality of the Lesser Flamingos in the Kenyan alkaline lakes (e.g. Kairu, 1996; Nelson et al., 1998; Kock et al., 1999; Wanjiru, 2001). In the year 2000, an estimated number of over 30,000 birds died in Lake Bogoria, Kenya (Vick, 2000). In Tanzania, flamingo deaths were uncommon until recently. During 2003, about 800 birds died in Lake Embagai, Tanzania (N. Magoma, personal communication). Between 3rd and 6th July 2004, about 700 flamingos were recorded at Lake Big Momela in Arusha National Park, thereafter, the arrival of more flamingos was observed. The first death of Lesser Flamingo at Lake Big Momela was recorded on the 29th July 2004 and since then the mortality rate gradually increased to about 50 per day (A. Mziray, personal communication) before going down to an average of 15 deaths per day by the time we visited the lake (9th–18th August 2004). Since Lake Manyara is nearest to Lake Big Momela, it was speculated that deaths occurring at Lake Big Momela were a result of contaminated flamingos from Lake Manyara. The death toll at Lake Manyara reached more than 15,000 individuals (E.M. Kihwele, personal

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communication) during the same period of July–August 2004.

Infection by mycobacteria, causing avian tuberculosis, and poisoning by heavy metals and pesticides have been thought to be major contributors to the observed deaths in Kenyan Lakes (Kairu, 1996; Nelson et al., 1998; Kock et al., 1999). More recently, however, poisoning from cyanotoxins was found to contribute to the observed deaths in the Kenyan volcanic lakes (Krienitz et al., 2003; Ballot et al., 2004). It was reported that hot spring cyanobacteria from Lake Bogoria, Kenya, comprising *Phormidium terebriformis*, *Oscillatoria willei*, *Spirulina subsalsa* and *Synechococcus bigranulatus* produced both the hepatotoxins microcystin-LR, -RR, -LF and -YR, and the neurotoxin anatoxin-a (Krienitz et al., 2003). The authors were able to detect these toxins in stomach contents, intestine and fecal pellets of the dead Lesser Flamingos in the lake. They concluded that intoxication with cyanobacterial toxins could occur by uptake of detached cyanobacterial cells from mats, as the birds need to drink fresh or brackish water and to wash their feathers, which they do in the vicinity of the hot springs. Moreover, Ballot et al. (2004) found microcystins-YR and anatoxin-a in isolated *Arthrospira fusiformis* from Lake Bogoria while the *A. fusiformis* from Lake Nakuru was found to produce anatoxin-a. The current study set out to investigate the microalgal composition and abundance in the water column and in sediment samples from the soda lakes of northern Tanzania following mass and prolonged deaths of the Lesser Flamingos in Lake Manyara and Lake Big Momela between July and August 2004.

2. Materials and methods

Three lakes were sampled during the course of this study. These included Lake Big Momela, Lake Manyara and Lake Embagai. The position at the sampling points on each lake is indicated in Table 1. Momela Lakes are a series of seven small lakes close to one another in Arusha National Park. Momela Lakes have no external inflow but depend on precipitation and underground water. During our visit to Momela Lakes, only Lake Big Momela was sampled which had Flamingos at the time. Lake Manyara has an estimated area of 520 km^2. The source of water in Lake Manyara is precipitation and floods from River Manyara. Since most of the lake was dry, samples were collected from the middle parts of the lake where there was still some water. Lake Embagai is a crater lake on mount Embagai. It has a maximum depth of about 85 m and depends on precipitation and underground water as its water source (Fig. 1).

A 20-μm mesh sized plankton net was used to concentrate phytoplankton samples for qualitative

Table 1
Sampling date (during August 2004), place, geographic position, elevation and physical–chemical parameters at the sampling point

Date	Place	Position	Altitude (m)	Salinity (‰)	Temperature (°C)	Nitrate μg-at NO_3/l	Phosphate μg-at PO_4/l
9th	Big Momela (S1)	03°13′02″ S 036°54′20″ E	1449	28.5	21	nd	nd
9th	Big Momela (S2)	03°13′11″ S 036°54′15″ E	1449	29	20.5	nd	nd
9th	Big Momela (S3)	03°13′08″ S 036°54′19″ E	1449	29	20	nd	nd
14th	Big Momela (S1)	03°13′02″ S 036°54′20″ E	1449	29	20	1.634	10.781
14th	Big Momela (SS)	03°13′02″ S 036°54′13″ E	1449	nd	nd	nd	nd
15th	Manyara pool	03°25′31″ S 035°51′15″ E	964	360	38	1.864	17.826
15th	Manyara water	03°25′19″ S 035°50′33″ E	963	2	21	2.209	4.911
16th	Embagai	02°54′37″ S 035°51′47″ E	2228	20	21	1.611	60.660
18th	Momela (S1)	03°13′02″ S 036°54′20″ E	1449	29	21	nd	nd

nd, not determined.

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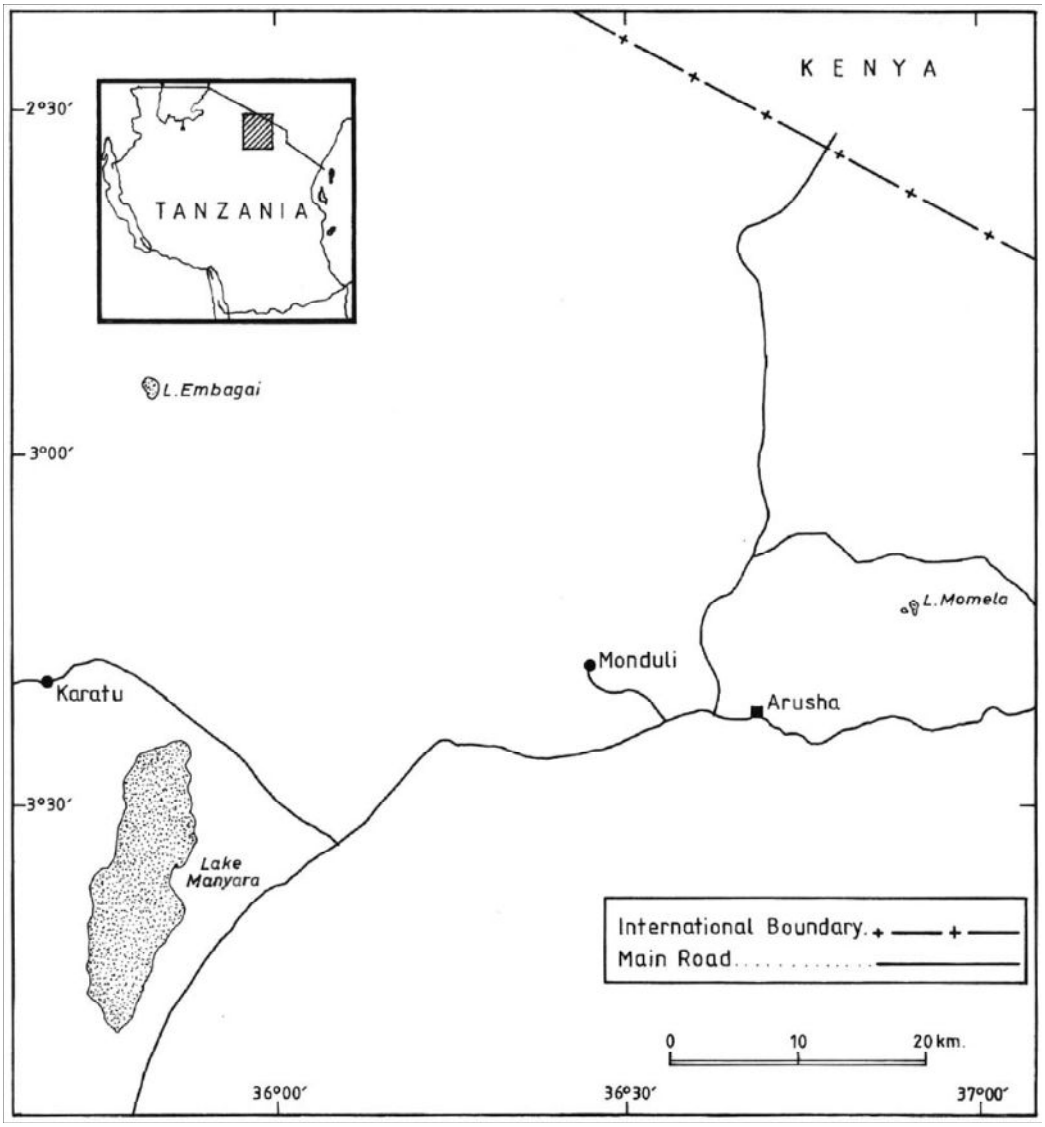


Fig. 1. Map showing the locations of Momela Lakes, Lake Manyara and Lake Embagai (*inset*: map of Tanzania with a shaded region to indicate the relative location of the study area).

analysis. The samples were kept in 50 ml dark glass bottles and fixed using formalin to a final concentration of 4%. One water sample for phytoplankton numerical abundance was taken at the surface using 50 ml dark glass bottles from each sampling point. Lake Big Momela was sampled on three different dates on three sub-stations (S1, S2, S3 and SS) while Lake Manyara and Lake Embagai were sampled only once (Table 1; Fig. 2). The samples were immediately fixed using 0.5 ml Lugol's solution and formalin added to a final concentration of 1%. All samples for phytoplankton analyses were stored at room temperature in a dark box. Visible biofilms/microbial mats were sampled by scraping the sediment surfaces. The samples were kept in 15 ml falcon tubes and fixed using formalin to a final concentration of 4%.

Microalgae identification was done using a Leitz microscope at 100 magnification following the morphological description given by Desikachary (1959); Prescot (1978); Anagnostidis and Komárek (1985,1988); Komárek and Anagnostidis (1986) and Hasle and Syverstsen (1997). Phytoplankton cells and *A. fusiformis* trichomes were counted using a Sedgewick Rafter cell as described by Woelkerling et al. (1976). *Arthrospira* trichomes were counted to a confidence limit of 90% (i.e. at least 400 cells and/or trichomes) according to Andersen and Throndsen (2003).

Water samples for inorganic nutrient analyses were taken by filtering the lake water through 0.22 μm pore sized Millex-GS Millipore filters (France). The filtered water samples were kept in 50 ml acid-cleaned plastic vials and immediately kept cool in an icebox for transport

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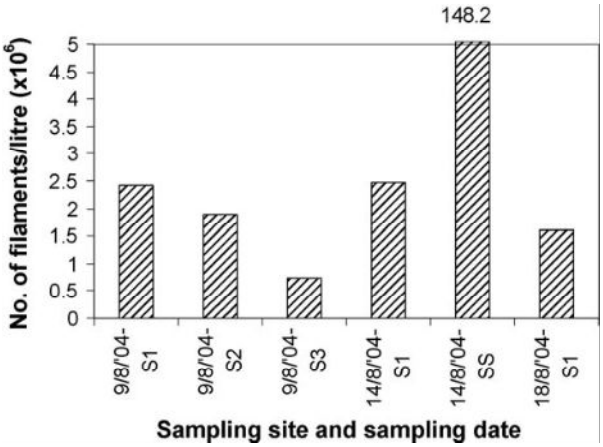


Fig. 2. Concentration of *A. fusiformis* in Lake Big Momela during three different sampling dates in August 2004 and at three sites points, site 1 (S1), site 2 (S2) and site 3 (S3). SS means a sample collected from a surface scum close to site S1.

to a deep-freezing facility. In the laboratory, analyses for NO_3 and PO_4 were done using a spectrophotometer as described by Parsons et al. (1984). Salinity and temperature were measured in situ using a hand refractometer and a thermometer, respectively.

Surface microalgae samples dominated by *A. fusiformis* for toxicity tests were collected from Big Momela Lake Station 1 (S1) and concentrated on membrane filters (0.45 μm pore size). The filters were stored in mini-grip labeled plastic bags and transported to the laboratory in an icebox. In the laboratory, the filters were kept frozen at −20 °C prior to analysis. The filters were thawed and cyanotoxin extraction was done in methanol/water (70:30, v/v). After replicate extractions, the organic solvent was removed in a rotary evaporator. The crude extract was diluted in sterile normal physiological saline (0.9%). Healthy laboratory mice (five for every treatment) of either sex (20–23 g) were administered intra-peritoneally with 1 mg/100 μl of the crude extract at concentrations of 0.1, 0.5, 1.0 and 10 mg using a microlance 21G needle. Control mice received saline alone. For each mouse, the time of injection, quantity injected, signs of intoxication and time of death were recorded. For the first 6 h, the mice were closely monitored followed by frequent observations for 24 h (Fernandez et al., 2003).

3. Results

3.1. Physical–chemical parameters

Environmental parameters recorded during the study period are shown in Table 1. The studied lakes are found in the highlands of northern Tanzania with an altitude ranging from 963 m above sea level at Lake Manyara to 2228 m above sea level at Lake Embagai. Salinity in the lakes ranged from 2‰ in Lake Manyara to 29‰ in Lake Big Momela. A high salinity (360‰) was recorded from an isolated drying pool in Lake Manyara. Water temperature ranged from 20 °C in Lake Big Momela to 38 °C in an isolated drying pool in Lake Manyara. Nitrate concentration was lowest in Lake Embagai (1.6 μg-at NO_3/l) and highest in Lake Manyara (2.2 μg-at NO_3/l) while phosphate concentration was lowest in Lake Manyara (4.9 μg-at PO_4/l) and highest in Lake Embagai (60.7 μg-at PO_4/l).

3.2. Microalgae composition

A. fusiformis was the dominant microalgae in Lake Momela and Lake Embagai during the study period. Other microalgae found in the water column in Lake Momela included a cyanobacterium of the genus *Pseudoanabaena* and some pennate diatoms like *Amphora*, *Navicula* and *Diatomella* spp., while at Lake Embagai apart from *Arthrospira* there were *Oscillatoria*, *Navicula* and *Hantzschia* species but these occurred at relatively low numbers. Pennate diatoms mostly of the genus *Navicula*, *Amphora* and *Hantzschia* dominated species composition in Lake Manyara. Cyanobacteria found in the water column of Lake Manyara were *Pseudoanabaena*, *Oscillatoria* and *Aphanocapsa* but these were observed quite infrequently.

A diverse species composition of microalgae was obtained on top of the sediments at all sampling sites (Table 2). In Lake Big Momela and Lake Manyara, *Oscillatoria jenensis* and *P. terebriformis* were the most common cyanophytes frequently forming biofilms and/or microbial mats at the surface. Other cyanophyte species that were seen on sediment were *S. subsalsa*, *S. labyrinthformis* and *Aphanocapsa* sp. In Lake Big Momela and Lake Embagai, *A. fusiformis* filaments were also found among microbial mats but these may have settled down from the plankton where they are dominant. *A. fusiformis* filaments were also seen on sediment from the dry parts of Lake Manyara. In Lake Embagai, the dominant benthic microalgae were the pennate diatoms of the genus *Navicula* and *Hantzschia* species. Other microalgal species were the cyanophytes *Aphanocapsa*, *Oscillatoria* and *Spirulina* species.

3.3. Microalgae abundance

The number of *A. fusiformis* in Lake Big Momela ranged from 736,000 on the 9th August to 2,475,000 filaments per liter on the 14th August 2004 (Fig. 2).

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Table 2
List of microalgae identified in the water column and on top of the sediments at the sampling sites

Microalgal taxa	Lake Momela	Lake Manyara	Lake Embagai	Water column	Sediment
Bacillariophytes					
Amphora sp.	x	x	x	–	x
Gyrosigma sp.				–	x
Diatomella sp.	x	–	–	x	–
Fragilaria sp.	–	x	–	–	x
Hantzschia sp.	x	x	x	x	x
Navicula sp	x	x	x	x	x
Nitzschia sp.	–	x	–	x	x
Surirella sp.	–	x	–	x	–
Chlorophytes					
Spirogyra sp.	–	x	–	–	x
Cyanophytes					
Arthrospira fusiformis	x	x	x	x	x
Arthrospira sp. 2	–	x	–	–	x
Aphanocapsa sp.	x	x	x	x	x
Chroococcus sp.	–	–	–	–	x
Komvophoron schmidlei	x	x	–	–	x
Oscillatoria jenensis	x	x	x	–	x
Oscillatoria sp.	x	x	x	x	x
Phormidium sp.	x	x	x	–	x
Pseudoanabaena sp.	x	x	–	–	x
Spirulina subsalsa	x	x	–	–	x
S. labyrinthformis	–	–	x	–	x

x, present/seen; –, not seen.

However, in accumulated surface scum found close to the shore on the 14th August 2004, the number of *Arthrospira* reached 148,200,000 filaments per liter (Fig. 2). During the study period, Lake Momela had the highest concentration of *A. fusiformis* followed by Lake Embagai that had 735,000 filaments per liter. There were no *A. fusiformis* in the water column at the sampling point in Lake Manyara (Fig. 3). The abundance of other cyanobacteria filaments (apart from *A. fusiformis*) ranged from 1000 filaments per liter in Lake Embagai to 3000 filaments per liter in Lake Big Momela (Fig. 4). Also, the abundance of diatoms ranged from 10,000 cells per liter in Lake Big Momela to 150,000 cells per liter in Lake Manyara (Fig. 4).

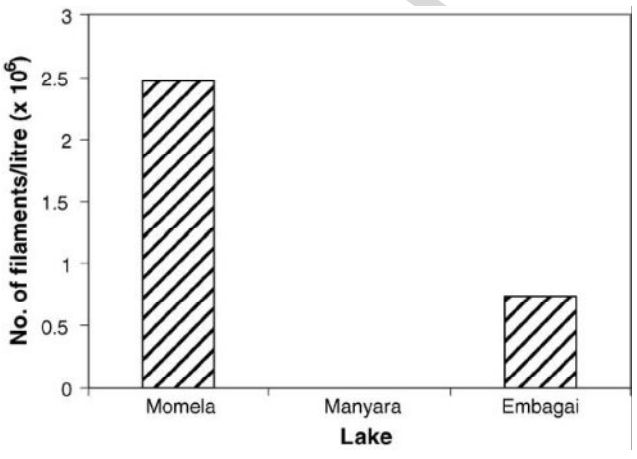


Fig. 3. Abundance of *A. fusiformis* in the water column of Lake Big Momela (S1, 14th August), Lake Manyara (15th August) and Lake Embagai (16th August).

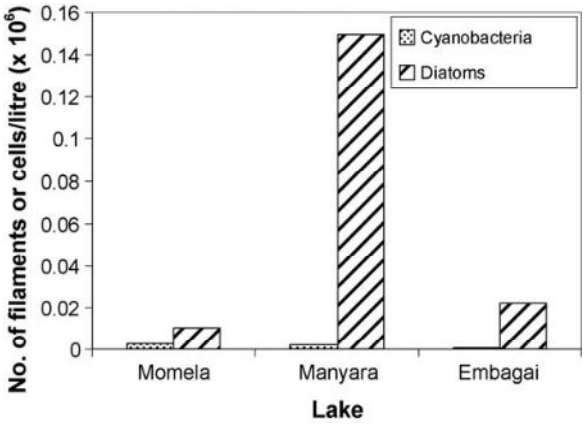


Fig. 4. Abundance of other cyanobacteria (apart from *A. fusiformis*; expressed as number of filaments per liter) and diatoms (number of cells per liter) in the water column of Lake Big Momela (S1, 14th August), Lake Manyara (15th August) and Lake Embagai (16th August).

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3.4. Gut content analysis

Three fresh carcasses of Flamingos were dissected for gut content analyses at Lake Big Momela. The only food item that could be microscopically identified in the guts (gizzards) of the carcasses was partially digested fragments of the cyanobacterium *A. fusiformis*. Feces of the bird also contained identifiable partially digested fragments of *A. fusiformis*. This suggests that the cyanobacterium was the main food item of the Lesser Flamingos during the study period in Lake Big Momela.

3.5. Mouse bioassay

All mice injected with crude extract showed signs of irritation, scratching all over the body and huddling behavior was evident. Mice injected with 10 mg of crude extract died within 90 min while those injected with 5 mg died within 4 h. The lethal dose (LCD_{50}) at which 50% of the mice survived during the 24 h test was 1 mg of crude extract as determined from the arithmetic plot. In the two groups of mice administered with 0.1 and 0.5 mg extract, the mortality was <10% after 24 h, but increased to 30% after 96 h. All mice close to death became lethargic, with loss of balance, uncoordinated movements, intermittent tremors, dyspnoea with gasping followed by respiratory arrest.

4. Discussion

Our results show very high concentrations (Fig. 2) of the *A. fusiformis* in Big Momela Lake where Lesser Flamingos were dying at a rate of between 15 and 50 individuals per day compared to the concentrations that were found at Lake Embagai (Fig. 3) where there were no deaths during the study period. Gut content analyses also indicated that *A. fusiformis* was the main food item of the dying flamingos that were dissected at Lake Big Momela. Mouse bioassay showed that at acute doses the crude microalgae extract dominated by *A. fusiformis* was toxic. This observation gives circumstantial evidence that *A. fusiformis* at such high concentration was toxic to the Lesser Flamingo in Lake Big Momela. Confirmation for this requires further investigation to ascertain that the Lesser Flamingos actually died from eating the cyanobacterium. Other cyanobacteria species were also found in the water column but these posed small risks due to their relative low concentration (Fig. 4).

A. fusiformis is the natural most important food item of the Lesser Flamingos in the soda lakes of eastern Africa (Vareschi, 1978) and for long time has been regarded as non-toxic species (Ciferri, 1983; Jassby, 1988). However, it was recently observed that *A. fusiformis* isolated from Lake Bogoria produced both microcystins-YR and anatoxin-a, and that from Lake Nakuru produced only anatoxin-a while the *A. fusiformis* isolate from Lake Elmenteita had no toxins (Ballot et al., 2004).

These findings raise several questions. The primary question is about the risk posed by this cyanobacterium to its main grazer, the Lesser Flamingos and other wild animals drinking in the lake waters, particularly when the cyanobacterium is occurring in very high concentrations in the water column. The second question is whether there are two strains of this cyanobacterium in the east African soda lakes, a toxic and non-toxic *A. fusiformis*. The third question is whether the species only produces toxins under certain endogenously or externally driven conditions, i.e. the species becomes toxic under certain circumstances. The fourth question is whether toxin production by toxic *A. fusiformis* takes place throughout the life span of an individual only to reach high concentrations capable of killing Lesser Flamingos at certain concentrations of the cyanobacterium in the water column.

Water sample from Lake Manyara, where there had been mass mortality of the Lesser Flamingos also, contained no *A. fusiformis*. However, *Arthrospira* was seen in the sediment samples from the dried part of the lake suggesting that the cyanobacterium existed when there was water on that part of the lake. The salinity in one pool in the drying part of the lake was very high (360‰) while in the middle parts of the lake where there was water the salinity was only 2‰. This suggests a very high salinity gradient between the upper parts of the lake and the lower parts of the lake or a high variation in salinity as the water dries up. Though *Arthrospira* is common in all soda lakes, it is known to occupy very selective habitats. The main ecological demands are stable alkaline, saline waters, warm growth temperatures, high irradiance and eutrophic conditions (Vonishak and Tomaselli, 2000). It is possible that a bloom of *A. fusiformis* had occurred before the lake dried up in the upper parts when there was an optimum condition for the cyanobacterium rapid growth. Another possibility is that *A. fusiformis* is limited to the peripheral fringes of Lake Manyara where salinities are high. Such a bloom could have resulted in mass mortality of the Lesser Flamingos just before the lake dried up.

It is also possible that the birds at Lake Manyara as well as in Lake Big Momela were intoxicated by toxic benthic cyanobacteria found within the lakes or from

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elsewhere such as in freshwater ponds, rivers or hot spring where these birds may visit to drink fresh water or take a swim as was recently reported by Krienitz et al. (2003). In this study, benthic aggregations comprising of several cyanobacterial genera such as *Phormidium*, *Oscillatoria* and *Spirulina* similar to those reported by Krienitz et al. (2003) in the Kenyan lakes were commonly encountered in both lakes. These were seen in the field to form visible biofilms/microbial mats and sometimes were observed to float on the water surfaces after they have detached off the sediment. Floating mats can be easily taken by the filter feeder Lesser Flamingos and intoxicate the bird if the cyanobacteria are toxic.

The occurrence of a bloom is a function of the environmental conditions and the resource requirements of the organism and many species of phytoplankton can form blooms under suitable conditions. The temperature values recorded during the study period are close to the optimal temperatures (30–38 °C) for laboratory cultivation of *Arthrospira* (Vonishak and Tomaselli, 2000) and within the optimal values of around 25–28 °C for bloom forming cyanobacteria in nature (Whitton and Potts, 2002). Nutrient levels were relatively low compared to the values recorded by Machiwa et al. (2004) from Magu Bay, Lake Victoria but are comparable to those reported by Ballot et al. (2004) in Lake Bagoria, Kenya and characterized as hypertrophic. This suggests that the study period was favorable for the growth of *A. fusiformis* in the lakes visited. Also, *A. fusiformis* as is the case with many other cyanobacteria, forms gas vacuoles composed of hollow proteinaceous vesicles filled with air. These facilitate buoyancy regulation enabling access to both near surface light energy and the more nutrient-rich deeper waters (Walsby, 1978, 1994). Gas vesicles may be the reason why cyanobacteria often form scum, which results from wind action on the shore regions of a lake. Theoretically, the number of cyanobacteria cells in the open water need not be high to form scum on the shore. As few as 10^3 cells ml^{-1} are enough to form a significant scum near the shore if moved horizontally and accumulated by wind over a larger distance from the open water to the shore (Walsby, 1994). Wind action may be the reason to the observed differences in concentration of *A. fusiformis* among different sampling points of Lake Big Momela on 9th August 2004 (Fig. 2). More studies, however, need to be conducted in order to explain the bloom dynamics of *A. fusiformis* and other microalgae in the Tanzanian soda lakes.

Although the current study points to cyanobacterial intoxication of the Lesser Flamingos in Lake Big Momela and Lake Manyara during the July and August 2004 mass mortality, other possible causes particularly those that have been associated with similar deaths of the bird in the Kenyan lakes (Kairu, 1996; Nelson et al., 1998; Kock et al., 1999) cannot be ruled out. In our opinion, heavy metal pollution in Lake Big Momela and Lake Embagai is very unlikely since these lakes have no influence from rivers and are located in remote areas away from major towns and in strictly managed National Parks. However, the possibility for heavy metal pollution exists in Lake Manyara, which is close to a small town of Mtowambu and Tanzanite mines at Merelani. Also Lake Manyara is influenced by river runoff from undetermined catchment area. Certainly an investigation on the possible anthropogenic pollutant contaminations to these lakes needs to be done. Infectious bacterial or viral diseases, as reported by Kock et al. (1999), are another possibility that may lead to such high death rates of the birds in the Tanzanian lakes. It is also possible that there are synergistic effects of different stress factors such as anthropogenic pollutants that have weakened the flamingos and are now unable to tolerate the toxins contained in their otherwise long time natural food.

5. Conclusion

The deaths of Lesser Flamingos in Lake Big Momela and Lake Manyara during July–August 2004 were most likely caused by cyanobacterial intoxication. The current study corroborates earlier reports that *A. fusiformis* may be toxic and cause flamingo deaths and suggests that the problem may be widespread in the east African lakes.

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Conservation in particular for providing laboratory space.[SS]

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