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SHORT RESEARCH NOTE

Does the potentially toxic cyanobacterium *Microcystis* exist in the soda lakes of East Africa?

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Abstract Presently, the food chains of the famous saline alkaline flamingo-lakes of East Africa are the focus of intense scientific discussion as the lakes host toxic cyanobacteria, which when consumed by Lesser Flamingos, weaken the birds and therefore make them susceptible to attacks by infective diseases. The distribution, genetic and toxicological aspects of *Microcystis* in Kenya has been studied extensively. Although there are reports on the occurrence of *Microcystis* in Kenya's hypersaline alkaline lakes, they have not been confirmed. Our investigations carried out over a 10-year period in about 50 inland waters showed that *Microcystis* occurs exclusively in freshwaters, but never in the hypersaline alkaline lakes. Microscopic examinations of the phytoplankton of these lakes revealed the presence of *Anabaenopsis abijatae* (Nostococales) whose lumpy structure makes it roughly similar to *Microcystis* when viewed under an inverted microscope. We conclude that the possible occurrence of *Microcystis* in hypersaline alkaline lakes is doubtful and, as such, confirmatory studies including microphotographic documentation of findings should be carried out.

Keywords *Anabaenopsis* · East Africa · Lesser Flamingo · *Microcystis* · Soda lakes · Toxic cyanobacteria

Members of the bloom- and scum-forming cyanobacterial genus *Microcystis* are worldwide found in inland waters where they are the main toxin-producers (Codd et al., 2005). The toxins produced by *Microcystis* are hepatotoxic microcystins. These are cyclic heptapeptides in more than 60 structural variants with the potential to cause considerable ecotoxicological effects (Wiegand & Pflugmacher, 2005). The species concept of *Microcystis* is still under discussion and to date, no correlation between morphology, phylogeny, and geography has been established (Otsuka et al., 1999; Wilson et al., 2005). *Microcystis aeruginosa* (Kützing) Kützing, an extremely polymorphic species (Komárek & Anagnostidis, 1998), is the most common and best studied taxon of the genus. In East Africa, *Microcystis* is widely distributed in fresh and subsaline inland waters (Wood & Talling, 1988; Kebede, 2002; Okello et al., 2010b). *M. aeruginosa* is genetically (Haande et al., 2007) and toxicologically well studied in the region (Okello et al., 2010a, b). Another common morphospecies of *Microcystis* in African lakes is

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M. botrys Teiling (Komárek & Anagnostidis, 1998). This species is often misidentified as *M. aeruginosa* (see Komárek & Komárková, 2002, and Hindák, 2006 for the delineation of the taxa). Cronberg & Van Baalen (2004) suspect that *M. botrys* could be identical to *M. toxica* Stephens, which was described from South Africa where its blooms caused cattle and sheep mortalities (Stephens, 1948).

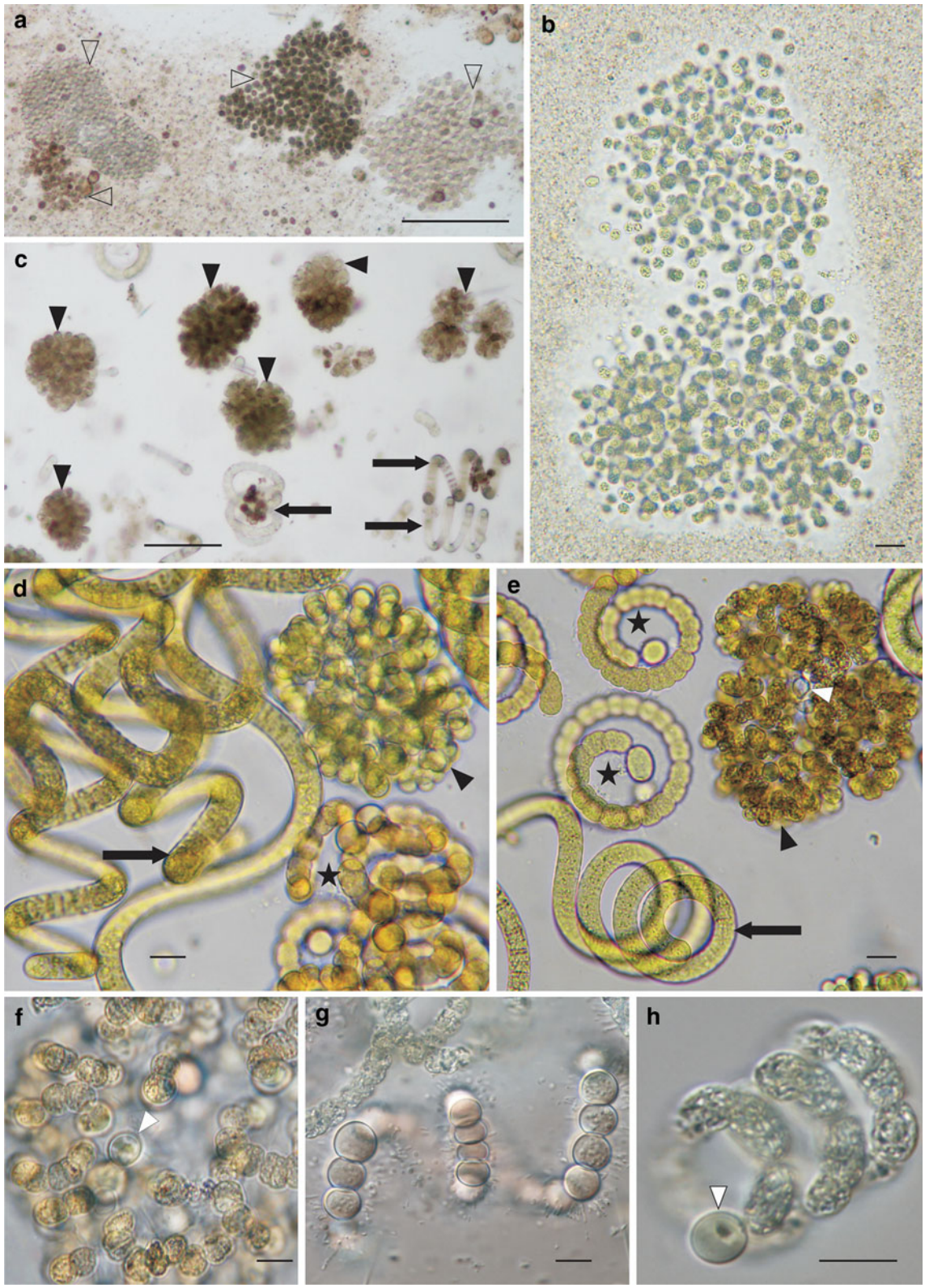
However, the question that appears to have received a less satisfactory answer is whether *Microcystis* also exists in hypersaline alkaline lakes of East Africa. Presently, the food chains of these lakes is the focus of intense scientific discussion as the lakes host toxic cyanobacteria, which when consumed by Lesser Flamingos, weakens the birds, and therefore makes them susceptible to attacks by infective diseases (Motelin et al., 2000; Codd et al., 2003; Krienitz et al., 2003; Ballot et al., 2004, 2005; Koenig, 2006; Kotut et al., 2006; Lugomela et al., 2006; Krienitz & Kotut, 2010). Recently, high densities of *Microcystis* in lakes Bogoria, Elmentaita, and Nakuru were reported and related to mass die offs of Lesser Flamingos (Githaiga, 2003; Ndetei & Muhandiki, 2005; Stewart et al., 2008). For the last 10 years, we have discussed with colleagues involved in planktological research and those in wildlife protection agencies across different countries of East Africa, the possible occurrence of *Microcystis* in alkaline saline lakes and came to the conclusion that there is a high level of uncertainty. Whereas a number of planktologists are of the opinion that *Microcystis* does not occur in African soda lakes, others have reported the occurrence of *Microcystis* in the saline alkaline lakes investigated. Unfortunately, these reports were not supported by microphotographic documentation. In our assessment, the presence of colonial cyanobacteria in alkaline saline lakes appears to be a source of confusion for the majority of researchers investigating these lakes. Because of the ecological significance of *Microcystis* blooms, it is essential to re-assess the situation regarding their existence in the famous flamingo-lakes of East Africa. In order to stimulate further discussion on the subject, this article presents our experience from 10 years phytoplankton research work in inland waters of Kenya.

The phytoplankton of about 50 inland waters of Kenya were studied, among them where:

Fig. 1 Cyanobacteria from inland waters of Kenya. **a** Fixed water sample from freshwater lake Baringo under the inverted microscope, containing different (dark and pale) colonies of *Microcystis* spp. (*empty arrowheads*). The *dark* colony in the centre is *M. botrys*. Scale bar 50 µm. **b** *Microcystis botrys* from freshwater lake Baringo under an upright microscope. The colony is surrounded by a hyaline, mucilaginous envelope which is made visible by silt particles and picoplanktonic cyanobacteria and green algae located outside the jelly cover. Scale bar 10 µm. **c** Fixed water sample from hypersaline alkaline lake Nakuru under the inverted microscope containing colonies of *Anabaenopsis abijatae* (*arrowheads*), and coiled filaments of *Arthrospira fusiformis* (*arrows*). Scale bar 50 µm. **d–h** Fresh cyanobacterial samples covered by a cover slip under an upright microscope. Scale bar 10 µm. **d–g** Samples from hypersaline alkaline lake Nakuru. **d** A sample containing a large drop of water and under low cover slip pressure. This reveals the three-dimensional nature of the cyanobacteria species present: *coiled* filaments of *Arthrospira fusiformis* (*arrow*), loose colonies of *Anabaenopsis arnoldii* (*star*), and compact colonies of *Anabaenopsis abijatae* (*arrowhead*) that are morphologically similar to *Microcystis* colonies. **e** The first indication of the filamentous nature of the colonies of *Anabaenopsis abijatae*. This follows a reduction in the amount of water in the mounted sample and increases the pressure of the cover slip. This loosens the compact colony of *A. abijatae*, hence revealing its filamentous nature. A heterocyte is visible in the middle of the colony (*white arrowhead*). **f** *Anabaenopsis abijatae* colony under the high pressure of a cover slip in a drying sample. A heterocyte (*white arrowhead*) is visible at the end of a filament. **g** *Anabaenopsis rippkae* with chains of akinetes (*arrow*) within the coiled filamentous colony. **h** *Anabaenopsis elenkinii* from the moderately saline alkaline Lake Oloidien. At the end of the coiled filamentous colony is a hyaline heterocyte (*white arrowhead*)

(i) freshwater lakes; Victoria, Baringo, Naivasha, and numerous small dams and ponds, such as Nakuru sewage ponds, and
(ii) alkaline saline lakes; Bogoria, Elmentaita, Magadi, Nakuru, and crater lakes Simbi and Sonachi.

These waters were sampled at irregular intervals over a 10-year period (2001–2010). On average, each water body was sampled at least twice a year during the decade long investigation that covered both wet and dry seasons. In the first and tenth year of this study period, we investigated the lakes once every 2 months. In each visit, samples were drawn with a scooper and used directly or concentrated with a plankton net (mesh size 25 µm). Initial identification of live samples was carried out in the field while samples for further laboratory investigation were fixed with Lugol's iodine solution or formaldehyde. Details of the phytoplankton and physico-chemical characteristics of these lakes are reported elsewhere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(Ballot et al., 2003, 2004, 2005, 2009; Kotut et al., 2006, 2010; Krienitz et al., 2002; Krienitz & Kotut, 2010). In this article, we focus on the occurrence of the chroococcalean *Microcystis* and morphological similar colonial nostocalean cyanobacteria in order to clarify some of the misconceptions held by researchers. Samples were studied in sedimentation chambers (Hydro-Bios Apparatebau GmbH, Kiel, Germany) under the inverted microscope Eclipse TS 100 or under an upright microscope Eclipse E 600 (Nikon Corporation, Tokyo, Japan). Microphotographs were taken with a Nikon Digital camera DS-Fi1, and Nikon software NIS-Elements D.

Under the inverted microscope, colonies of *Microcystis* in sedimentation chambers were dark or pale blue-green with an amorphous and mostly lumpy morphology (Fig. 1a). When viewed under an upright microscope, the coccoid and non-filamentous organisation of cells in *Microcystis* colonies became evident. The colonies formed lacked heterocytes (Fig. 1b). This is a typical feature of members of the Chroococcales. In our study, we found *Microcystis* exclusively in freshwaters such as lakes Victoria, Baringo and Naivasha, but never in the alkaline saline lakes. In Lake Victoria, *Microcystis* is a common member of phytoplankton (Ostenfeld, 1908; Ochumba & Kibaara, 1989). The first report on the occurrence of microcystins in Lake Victoria was made by Krienitz et al. (2002). Subsequently, several other reports on the presence of cyanotoxins in different parts of Lake Victoria have been made (Sekadende et al., 2005; Okello et al., 2010a; Semyalo et al., 2011). Okello et al. (2010b) reported the presence of *Microcystis* in 12 freshwater lakes in Uganda and based on molecular detection of the cyanotoxin genes, concluded that *Microcystis* is the major source of microcystins. In Ethiopia, *Microcystis* was found to be a rare to abundant taxon in five freshwater lakes (Kebede, 2002) and a common phytoplankton in the subsaline Lake Langano (salinity 2.4 g l^{-1}, alkalinity 12.5 meq l^{-1}). A study of chemical and algal relationship in a series of 28 lakes in Ethiopia revealed that *Microcystis* can exist up to a salinity of about 3 g l^{-1} as in the case of Lake Turkana (Wood & Talling, 1988). According to Hammer et al. (1983), *Microcystis aeruginosa* covers a broad range of salinities in Saskatchewan (Canada) lakes; up to a salinity of about 100 g salt l^{-1}. However, alkalinity does not play a major role in these lakes owing to the very low concentration of calcium (Ca^{+}) and bicarbonate (CO_3^{2-}) ions and the dominance of sodium (Na^{+}), sulfate (SO_4^{2-}), and potassium (K^{+}) ions (Bowman & Sachs, 2008). In comparison, the salinity and alkalinity ranges in the Kenyan soda lakes Nakuru and Bogoria were 17–55 g salt l^{-1} and 438–1760 meq l^{-1} respectively (Krienitz & Kotut, 2010). It can, therefore, be concluded that *Microcystis* can not exist in the soda lakes of East Africa because of the high alkalinity. Several detailed studies of the alkaline saline lakes of East Africa never found *Microcystis* in these lakes. These studies include a study of Lake Nakuru in the 1970s by Vareschi (1978), studies of Lake Bogoria in 1972–1978, and 2000–2003 by Harper et al. (2003) and the phytoplankton investigation of lakes Bogoria, Elmentaita and Nakuru by Oduor & Schagerl (2007) and Schagerl & Oduor (2008) over the period 2003–2005. The latter study was fairly detailed with a sampling frequency of once very 2 months. Molecular phylogenetic studies on environmental samples from Kenyan soda lakes recovered eight different phylotypes belonging to the genera *Anabaenopsis* (Nostocales), and *Umezakia* (Nostocales), *Arthrospira* (Oscillatoriales) and *Chroococcidiopsis* (Chroococcales). However, no phylotype belonging to the genus *Microcystis* was recovered (Dadheech et al., 2009). Occurrence of cyanotoxins that appeared to have adversely affected flamingo populations in three alkaline lakes of Tanzania was reported by Lugomela et al. (2006). However, the cyanotoxin effects were not induced by *Microcystis* but by *Arthrospira* blooms.

Although we did not find any *Microcystis* in the Kenyan soda lakes, we recorded the presence of colonial cyanobacteria, which were very roughly similar to *Microcystis* in morphology. A careful examination of these colonial cyanobacteria revealed that were indeed *Anabaenopsis abijatae* Kebede et Willén. When viewed with an inverted microscope under low power magnification, they were difficult to identify because of their lumpy structure (Fig. 1c). However, under a higher magnification using an upright microscope, the filamentous structure and the presence of heterocytes became clearly visible (Fig. 1d–f). *A. abijatae* was first described in the Ethiopian soda lakes by Kebede & Willén (1996) and their micrographs of the botryoid colonies (loc. cit. p. 4, Fig. 2) were comparable to our findings.

According to Kebede (2002), *Anabaenopsis abijatae* was abundant only in Lake Abijata, the locus classicus. Apart from *A. abijatae*, other *Anabaenopsis* species present in the soda lakes of Kenya were *A. arnoldii* Aptekarj (Fig. 1d, e), *Anabaenopsis* sp. (see Krienitz & Kotut, 2010) and *A. rippkae* (Florenzano, Sili, Pelosi et Vincenzini) Komárek (Fig. 1g). *A. elenkinii* (Fig. 1h) was found in the moderately saline alkaline waters of Lake Oloidien. These other cyanobacteria species had a clearly spiral filamentous structure as compared to *A. abijatea* and could not therefore be confused with *Microcystis*. Molecular studies of *Anabaenopsis abijatae* and *A. elenkinii* cultures isolated from samples collected in Kenyan soda lakes confirmed their nostocalean nature (Ballot et al., 2008).

Our findings contradict the observations made by Githaiga (2003) and Ndetei & Muhandiki (2005) who reported on the occurrence of *Microcystis* in lakes Bogoria, Elmentaita and Nakuru and attributed the flamingo mass die offs in 1993, 1997, and 2001 to *Microcystis* toxins. The two studies did not provide any photographic documentation of the *Microcystis* species observed to allow for independent confirmation. In our opinion, a better understanding of the dynamics of primary producers in soda lakes of East Africa and their interaction with Lesser Flamingo population requires that the taxa present in the lakes should be well documented for correct identification so as to prevent erroneous conclusions. One common cause of the erroneous identification is that most planktologists only study fixed material in sedimentation chambers under the inverted microscope. This method requires a lot of experience based on the observation of living field samples to bring out diacritical features of *A. abijatae* such as the filamentous structure and presence of heterocytes. When observations are made under an upright microscope, the cover slip presses the colonies flat and reveals the diacritical features of *Anabaenopsis*. It is important to note that the value of ecological data is to a large extent dependent on the correct identification of the organisms present, using the most appropriate methods. The value of incorrectly identified samples can not be improved by any statistical treatment or any other sophisticated method.

However, anything is possible in nature! We cannot state with certainty why there are no strains of the cosmopolitan *Microcystis* that are adapted to the unique conditions of the alkaline saline lakes. Although our decade long field investigations did not record *Microcystis* in the soda lakes, we may not at this stage completely rule out such a possibility. More effort should therefore be directed at closely monitoring the species changes in these lakes while avoiding drawing conclusions based on doubtful observations made in the past. It would be of great scientific interest if the occurrence of *Microcystis* in these lakes can be confirmed. Because of the interest that such a finding will generate, any colleague who finds real *Microcystis* in the soda lakes should carefully document and save such findings for future considerations.

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