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Toxic cyanobacteria at Nakuru sewage oxidation ponds – A potential threat to wildlife

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ABSTRACT

Phytoplankton composition and biomass, and microcystin content were determined on diverse dates between November 2001 and June 2006 in the final oxidation pond of the Nakuru town sewage treatment plant. The oxidation ponds as well as the rivulet that drains the same are important water sources for some wildlife in the park. The phytoplankton composition of the pond studied mostly comprised coccoid green alga species. However, occasionally cyanobacteria or euglenoids were dominant. Among the cyanobacteria, *Microcystis* sp. made periodic appearance in the phytoplankton, and was the dominant species on some occasions. Total phytoplankton biomass varied widely from 48 to 135 mg L^{-1} (wet weight) while cyanobacteria biomass ranged from undetectable levels to 130 mg L^{-1}. Most phytoplankton biomass was due to one or a few species. Detectable cyanotoxin concentrations (sum of microcystins) of up to 551.08 μg mg^{-1} dry weight (DW) of cyanobacteria biomass or 0.28 μg mg^{-1} DW of total phytoplankton biomass were recorded in samples collected on different dates. Microcystin content did not appear to correspond to the biomass of cyanobacteria suggesting that toxin production is possibly triggered by environmental changes or changes in the proportion of toxic strains. An occasional presence of microcystins in the pond water suggests that the wildlife species, which regularly use the ponds as drinking water sources are potentially exposed to intoxication. A close monitoring of pond water phytoplankton composition is necessary to accurately quantify the potential impact of cyanotoxins on these wildlife species.

Introduction

Lake Nakuru National Park is world famous for its massive flock of flamingos that fringe the shores of the soda lake. The park also abounds with game: there are huge herds of waterbuck, zebra, buffalo, the endangered Rothschild Giraffe and more. Additionally, it is an important sanctuary for the rhino.

Threats facing the lake ecosystem include a decline in river inflows and an increase in pollutant discharge mainly from the town settlements and wastewater outlets. Nakuru town sewage treatment plant is located within Lake Nakuru National Park. The partially enclosed sewage plant discharges its wastes into Lake Nakuru via a rivulet that runs through the park. Owing to water scarcity within the park, especially during the dry season, the wastewater ponds as well as the rivulet are important sources of water for some wildlife species. The sewage ponds also harbor a diverse avifauna and act as a temporary refuge for some flamingos when the conditions in the lake are not favorable. Hence the quality of water in the ponds is important to the wildlife within the park. Elsewhere in the country, although regulations restricting use of wastewater from sewage plants exist, a general scarcity of water makes such water important sources of water for illegal small-scale irrigation of food crops.

Warm temperatures and stable and high nutrient levels, suitable for algal blooms, usually characterize sewage maturation ponds in tropical areas. Hence algal blooms are a common feature of these ponds (Vasconcelos and Pereira 2001). However, the dominant bloom forming species varies with the actual load of nutrients and organic matter, influence of wind induced stirring, and temperature changes among other environmental factors. A common bloom forming group is the cyanobacteria, which comprises opportunistic, nuisance and frequently toxic micro-organisms that adversely affect human health and the quality of water resources. Worldwide, over 40 species of cyanobacteria, mostly found in freshwaters, have been implicated in the generation of toxic blooms (Carmichael 1997) whose cyanotoxins have been linked to the mass poisonings of wild and domestic animals for several years in North America, Europe and Australia (Yoo et al. 1995; Codd and Beattie 1991; Lawton and Codd 1991). Blooms of toxic cyanobacteria in water reservoirs have also been

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linked to adverse human health effects in these countries (Haider et al. 2003). Globally, incidents associated with toxic cyanobacteria appear to be on the rise (Luckas 2000), hence cyanotoxins are now recognized by international and national health and environment agencies as significant health hazards (Metcalf et al. 2006).

In Africa, most of the available reports on cyanobacteria and their toxins have in the past been limited to cases reported from South Africa, where records of animal poisoning have been in existence for more than 50 years (Harding 1997; Harding et al. 1995; Scott 1991; Van Ginkel 2003; Van Halderen et al. 1995; Wicks and Thiel 1990). Animal poisoning has mostly occurred in highly eutrophic man-made reservoirs with cyanobacteria blooms (Wray and Strauss 2007). Elsewhere in the continent, the last 10 years have witnessed an increased interest in the presence of cyanotoxins in inland water bodies. Occurrence of cyanotoxins mostly linked to the cyanobacterium *Microcystis aeruginosa* have been reported for various drinking water reservoirs in North African countries such as Algeria (Nasri et al. 2004), Morocco (Oudra et al. 2002) and Ghana (Addico et al. 2006).

In eastern Africa, studies carried out on the alkaline saline lakes of the Rift Valley following episodes of Lesser Flamingo die-offs in the lakes have confirmed the presence of cyanotoxins in the lakes, and hence their possible contribution to the flamingo die-offs (Ballot et al. 2004, 2005; Krienitz et al. 2003, 2005; Kotut et al. 2006; Lugomela et al. 2006). In east Africa's freshwaters, cyanotoxin occurrence has recently been reported in a number of important water sources such as Lakes Victoria (Krienitz et al. 2002) and Baringo (Ballot et al. 2003) and several reservoirs (Mwaura et al. 2004).

Most sewage treatment plants in Kenya employ an aerobic pond system as the secondary treatment system for sewage effluents. In these ponds, the natural process of algal photosynthesis and bacterial oxidation of organic matter exist in a mutually dependent relationship. Sewage plant management programs usually focus on assessing nutrient and microbial quality of the water. Investigation on the occurrence of toxic algae and toxin measurements are rarely carried out. In water scarce countries where wastewater is immediately put into use, the presence of cyanotoxins needs to be monitored. This paper focuses on the phytoplankton composition and toxin levels in the final aerobic pond prior to wastewater release in the Nakuru town sewage treatment plant.

Materials and methods

Physico-chemical conditions

Water temperature, conductivity, pH and salinity were measured directly in the field using a WTW Multiline P4 meter (Weilheim, Germany). Nutrient analyses (total nitrogen and total phosphorus) were carried out using a field nanocolor test kit and a field photometer (Nanocolor 300 D, Macherey, Germany). Total alkalinity was determined titrimetrically using mixed bromocresol green – methyl red indicator and standard hydrochloric acid (APHA 1998).

Phytoplankton sample collection

Samples for the determination of phytoplankton composition, biomass and toxin concentration were collected on diverse dates between November 2001 and June 2006 from the final pond of the maturation pond system. The pond studied is roughly 10 m wide and 70 m long with a depth of 1 m. Water residence time in the pond when the system is operating at its optimum capacity is 3 days. However, the flow rate is on most occasions lower, hence a greater residence time. Surface water samples were collected from at least 3 m away from the shore, near the centre of the pond, and about 5 cm below the water surface using a water scooper with a 3 m extendible pole. Samples for phytoplankton enumeration were fixed with Lugol's iodine solution and stored in the dark. Determination of phytoplankton species composition was based on net samples obtained by concentrating approximately 2 l of pond water using plankton net with a mesh size of 25 μm and fixed with formaldehyde (final concentration 1%).

Phytoplankton identification and biomass

Phytoplankton species identification was carried out using an Eclipse E600 light microscope (Nikon Corporation, Tokyo, Japan). Determination of phytoplankton abundance and biomass was performed using the Utermöhl technique (Utermöhl 1958). Enumeration of phytoplankton taxa was carried out 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 the computerized counting program Opticount (Hepperle 2000). The program provides an estimate of wet weight from biovolume assuming a specific density of 1 g cm^{-3}.

Cyanotoxin analysis

At each sampling site, a 1 l pond water sample was filtered using a 1.2 μm pore size Whatman Glass Fiber (GF/C) filters in a shaded area in the field. The seston retained by the filters was then air-dried and stored in the dark on transport to Germany for toxin analyses. Toxins in the filter materials were extracted with 70% methanol. Toxin concentrations were determined by the high-performance liquid chromatography with photodiode detection array (HPLC-PDA) and confirmed with the matrix laser desorption/ionization time flight mass spectroscopy (MALDI-TOF) (Pflugmacher et al. 2001). Intracellular toxin concentration was computed per unit volume of water filtered as well as per unit dry weight (DW) of phytoplankton and per unit DW of cyanobacterial biomass. Phytoplankton DW was estimated as 10% of its wet weight (Ruttner 1938). The following reference toxins were used to determine sample toxin concentration: microcystin-LR (gravimetric standard) and dhb-microcystin-LR provided by G.A. Codd (University Dundee, UK); microcystin-LA 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).

Results

Physico-chemical properties

Pond water quality varied widely during the study period. Daytime water temperatures ranged from 20.5 to 28.5 °C. Where dissolved oxygen content was mostly supersaturated with concentrations of up to 14.6 mg L^{-1}, nearly anoxic values were recorded on a number of occasions. Electrical conductivity and total alkalinity varied slightly with a range from 623 to 1440 μS cm^{-1} and 244 to 500 mg L^{-1} $CaCO_3$, respectively. Water pH was constantly high with values ranging from 8.8 to 10.4. High nutrient levels characterized the pond water with TP and TN ranging from 1.4 to 5.2 mg L^{-1} and 4.0 to 8.0 mg L^{-1}, respectively (Table 1). (Figs. 1 and 2)

Phytoplankton composition and biomass changes

The phytoplankton composition in the two ponds mostly comprised of coccoid green alga species. However, there were times when the cyanobacteria or the euglenoids were present in large numbers, with each of the two occasionally becoming the dominant group. The most common green alga species were *Actinastrum* spp., a number of species of *Desmodesmus*, *Micractinium* spp. and other coccoid green algae species (Table 2, Fig. 3). Other genera like *Pediastrum*, *Dictyosphaerium* were always present in low densities. Among the cyanobacteria, *Arthrospira fusiformis* was the dominant species occurring almost as a monoculture once in March 2004 (Fig. 4). However, cyanobacterium *Microcystis* sp. made periodic appearance in the phytoplankton, at times registering the highest density (Fig. 5). Common euglenoids included the genera of *Lepocinclis, Euglena* and *Phacus*. In general, the pond phytoplankton was characterized by a few very dense species whose composition varied with time (Figs. 3–5).

Most phytoplankton biomass in the pond was contributed by one or a few species, which were mostly different during each sampling period. With the exception of *Microcystis* spp., *Arthrospira fusiformis* and *Euglena* spp., which accounted for the highest biomass on some occasions, all the other species that assumed dominance at different times belonged to the Chlorophyta division. These included species such as *Desmodesmus* spp., *Micractinium* spp., and other coccoid green algae species such as *Oocystis* spp. The highest biomass was recorded when the euglenoids were dominant.

Common phytoplankton divisions in the pond included the Bacillariophyta, Chlorophyta, cyanobacteria, Euglenophyta and Xanthophyta. However, most biomass was due to the cyanobacteria, Chlorophyta and Euglenophyta (Fig. 1). The Chlorophyta, which were represented by different species, frequently contributed the highest biomass. The cyanobacteria and Euglenophyta, each of which was represented by a few species, at times had the highest biomass (Fig. 1).

Toxin concentration

Toxin analyses recorded the presence of low concentrations of various variants of microcystins (MCs) on a number of occasions. To evaluate the potential threat posed by microcystins to different animals that utilize the pond water, toxin values are provided in μg L^{-1} (to highlight the threat posed to pond water drinkers or those living in the water). Additionally toxin content was calculated as μg mg^{-1} DW of the total phytoplankton biomass (to illustrate the potential assimilation by filter feeders) and in μg mg^{-1} DW of the total cyanobacteria biomass (Table 3). Overall, the presence of microcystins in the pond water was found to be a highly irregular event with total intracellular microcystin concentration (sum of microcystins) ranging from below the limit of detection to 1.66 μg L^{-1} of pond water (Fig. 2). Expressed in terms of phytoplankton biomass, the highest microcystin content was 0.28 μg mg^{-1} phytoplankton DW. Based on the total cyanobacteria biomass, the maximum toxin content was 551.08 μg mg^{-1} cyanobacteria DW (Table 3). The dominant MC

Table 1
Levels of selected physico-chemical properties at the final maturation pond at Nakuru town sewage treatment plant.

Date	10 June 2001	18 November 2001	02 February 2002	29 May 2002	06 February 2006	26 August 2006
Water temperature (°C)	25.0	21.3	20.5	28.5	27.3	21.9
Dissolved oxygen (mg L^{-1})	14.6	2.0	9.1	13.8	NM	NM
pH	9.7	9.1	9.9	9.0	10.4	8.8
Conductivity (μS cm^{-1})	723	623	656	682	1440	882
Secchi depth (m)	NM	0.41	0.28	0.44	0.30	0.42
Ammonia nitrogen (mg L^{-1})	0.04	2.20	0.07	4.84	NM	NM
Total nitrogen (mg L^{-1})	4.0	8.0	4.1	7.5	NM	NM
Total phosphorus (mg L^{-1})	1.4	5.2	2.8	4.8	NM	NM
Total alkalinity (mg L^{-1} $CaCO_3$)	500	305	290	244	NM	NM

NM – not measured.

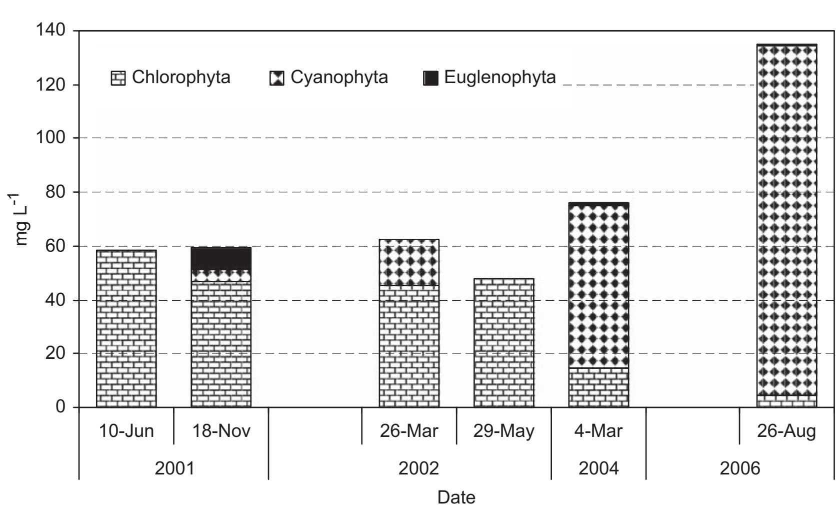


Fig. 1. Biomass of the major phytoplankton divisions at the final oxidation pond of the Nakuru town sewage reatment plant at diverse dates during the period 2001–2006.

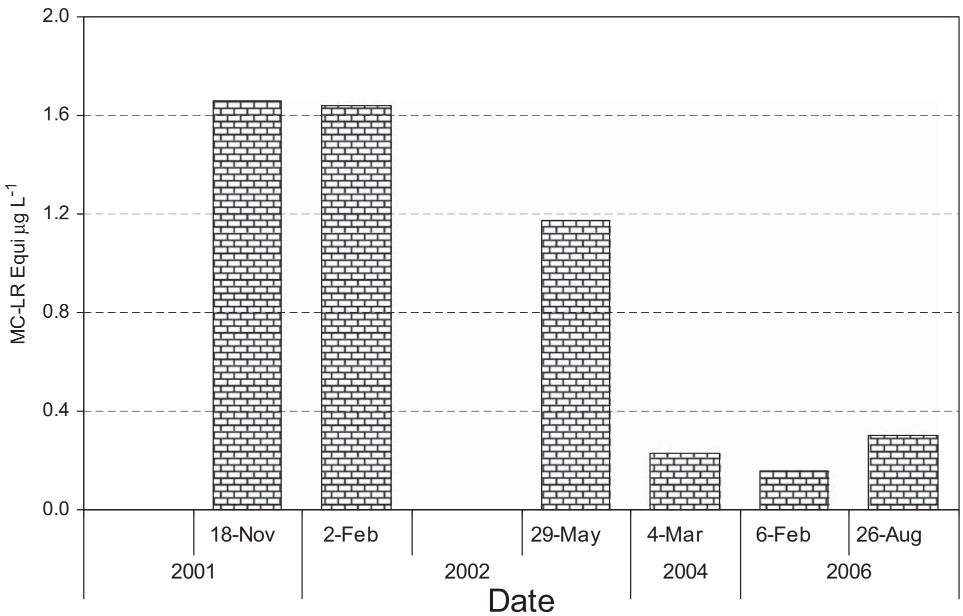


Fig. 2. Intracellular microcystin concentration (sum of MC per liter of pond water) in the final oxidation pond of the Nakuru town sewage treatment plant at diverse dates during the period 2001–2006.

variants varied between MC–LR and MC–RR. Where MC–LR was present in all samples, MC–RR presence was occasional. Microcystin occurrence did not correspond to cyanobacteria biomass. Periods with high toxin concentrations were not necessarily periods with a high biomass of cyanobacteria. For example, the highest pond intracellular microcystin concentration (sum of microcystins of $1.66\,\mu g\,L^{-1}$) was recorded on 18 November 2001 when the phytoplankton was dominated by coccoid green algae (Table 1). On the other hand, a *Microcystis* dominated water sample collected on the 26 August 2006 had a comparatively lower microcystin concentration of $0.3\,\mu g\,L^{-1}$ (equivalent to $0.02\,\mu g\,mg^{-1}$ phytoplankton DW or $0.02\,\mu g\,mg^{-1}$ cyanobacteria DW) (Table 3).

Discussion

A wide variation in dissolved oxygen is a characteristic feature of sewage oxidation ponds. Rapid photosynthesis during the day can result in supersaturation in dissolved oxygen while respiration and decomposition at night can result in anoxic conditions. Total alkalinity and electrical conductivity variation results from variation in the volume and concentration of wastewater introduced. During the wet season, some run off water finds its way into the sewer lines while biting water shortage is occasionally experienced in town owing to breakdown of water supply lines. Consistently high pH values are indicative of high algal activity in the ponds, which is supported by the high algal biomass.

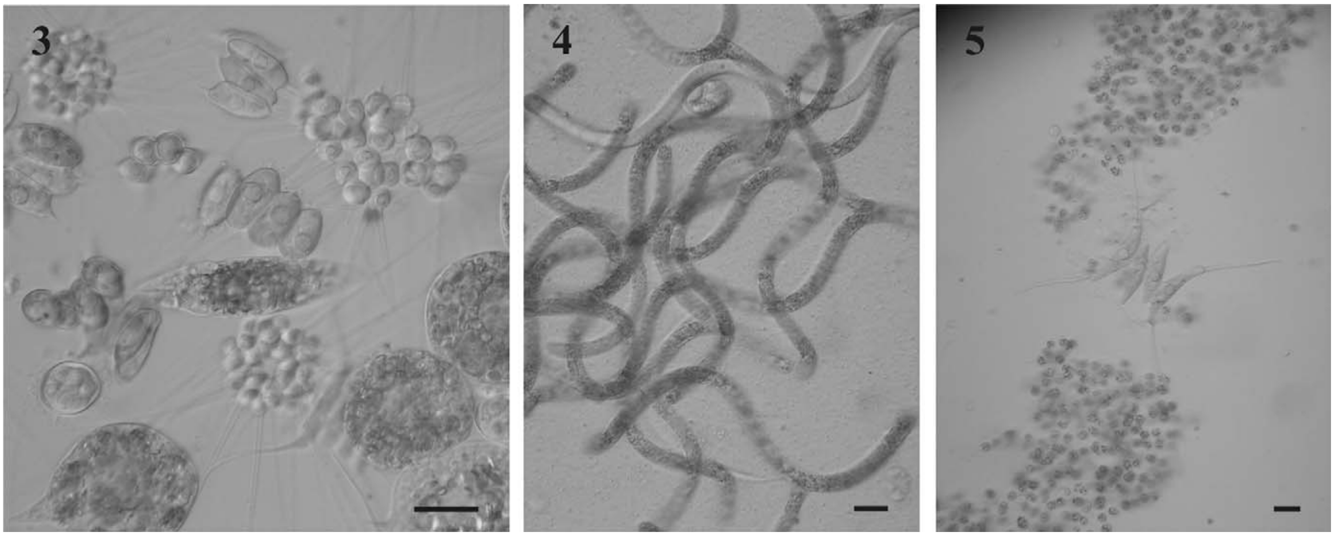
Variation in phytoplankton composition is closely linked to changes in the physico-chemical properties of the pond water. On occasions dominated by green algae, the pond water exhibited a dark green appearance, which is indicative of healthy algal population (Mara and Pearson 1986). However, a surface scum characterized the pond water when the cyanobacteria were dominant. Periodic biomass dominance by euglenoids possibly resulted from an increase in the organic matter load of the pond. In nature, this division has been shown to increase in standing waters enriched with organic compounds (Hoek et al. 1995).

Table 2
Phytoplankon species composition and relative abundance at the final oxidation pond of the Nakuru sewage treatment ponds.

Phytoplankton division	Relative abundance
Cyanobacteria	
Microcystis aeruginosa (Kützing) Kützing	3
Arthrospira fusiformis (Voronichin) Komárek et Lund	3
Euglenophyta	
Euglena div. spec.	3
Lepocinclis spec.	1
Phacus div. spec.	2
Strombomonas spec.	1
Trachelomonas hispida (Perty) Stein	1
Trachelomonas volvocina Ehrenberg	1
Chlorophyta	
Actinastrum aciculare Playfair f. *africanum* Compére	1
Actinastrum hantzschii Lagerheim	2
Coelastrum microporum Nägeli	1
Desmodesmus armatus (R. Chodat) Hegewald	1
Desmodesmus communis (Hegewald) Hegewald	1
Desmodesmus multiformis (Hegewald et Hindák) Hegewald	1
Desmodesmus opoliensis (P. Richter) Hegewald	2
Desmodesmus pannonicus (Hortobágyi) Hegewald	2
Desmodesmus protuberans (Fritsch et Rich) Hegewald	2
Dictyosphaerium ehrenbergianum Nägeli	1
Dictyosphaerium tetrachotomum Printz	1
Eudorina elegans Ehrenberg	1
Micractinium pusillum Fresenius	3
Oocystis div. spec.	1
Pandorina morum (O.F.M.) Bory	1
Pediastrum boryanum (Turpin) Meneghini	1
Scenedesmus obtusus Meyen	1
Scenedesmus ovalternus R. Chodat	1

Abundances: 1 = present; 2 = common; 3 = dominant.

Biomass dominance by one or a few species is a characteristic feature of extreme environments, which in this case resulted from a high nutrient load and presence of organic matter. Occasional dominance by cyanobacteria in the sewage ponds relates to variation in the controlling environmental conditions. Cyanobacteria are common in eutrophic natural waters where they are favoured by warm, stable and nutrient-enriched waters and may



Figs. 3–5. Light microscope micrographs showing the dominance shift in the phytoplankton composition of the final sewage maturation pond on some selected occasions: (3) sample collected on 18 November 2001 codominated by green algae (*Micractinium pusillum*, *Desmodesmus pannonicus*) and *Euglena* spp., (4) sample collected on 4 March 2004 dominated by *Arthrospira fusiformis*, (5) sample collected on 26 August 2006 dominated by *Microcystis* spp. Scale bar = 10 μm.

Table 3
Phytoplankton biomass (PB) and cyanotoxin concentration at the final oxidation pond of the Nakuru town sewage treatment plant.

	10 June 2001	18 November 2001	02 February 2002	26 March 2002	29 May 2002	04 March 2004	06 February 2006	26 August 2006
Sum of microcystins (μg L^{-1})	ND	1.66	1.64	NM	1.17	0.23	0.16	0.30
Phytoplankton biomass (mg L^{-1} DW)	5.82	5.98	NM	6.25	4.80	7.60	NM	13.50
Phytoplankton toxin content (μg mg^{-1} DW)	0	0.28	NM	NM	0.24	0.03	NM	0.02
Cyanobacteria biomass (mg L^{-1} DW)	0	0.42	NM	NM	0.002	6.07	NM	13.00
Cyanobacteria toxin content (μg mg^{-1} DW)	0	3.94	NM	NM	551.08	0.04	NM	0.02

NM – not measured, ND – not detected.

constitute an important part of the phytoplankton community in wastewater treatment plants (Vasconcelos and Pereira 2001). The periodic appearance of the known toxin-producing *Microcystis* sp. may also be linked to its meroplanktonic growth habit, which is characterized by temperature dependent pelagic reinvasion from the sediments (Ihle et al. 2005). A near monoculture dominance of the cyanobacteria (about 80% of the total biomass) in March 2004 resulted from the invasion of the sewage pond by *Arthrospira fusiformis*, which was possibly introduced into the sewage ponds by Lesser Flamingos that visit the sewage ponds for freshwater. Some toxin-producing strains of *Arthrospira* have been isolated from some rift valley lakes including Lake Nakuru (Ballot et al. 2004, 2005).

The occasional presence of toxins recorded in the sewage ponds relates to the periodic appearance of cyanobacteria as was the case for the known toxin-producing *Microcystis aeruginosa.* An isolate of *M. aeruginosa* from the final oxidation pond of the Nakuru town sewage treatment plant recently investigated by Haande et al. (2007) was found not to produce microcystins. However, previous studies have shown that a single water body can have 2–6 genotypes and that genetic variation is common within and across habitats. According to Kurmayer et al. (2002), the total quantity of MCs produced by a cyanobacterial bloom varies in response to the proportion of MC producing genotypes within a specific population. It has also been suggested that the wax and wane of MC producing versus non-MC-producing strains is the most important factor regulating MC production in water (Sivonen and Jones 1999).

A lack of correspondence between biomass of cyanobacteria and toxin levels, even when the dominant cyanobacteria are known toxin-producing species suggests that toxin production may also be influenced by the prevailing environmental conditions. According to Jähnichen et al. (2007), microcystin production in *M. aeruginosa* is controlled by internal cell processes related to growth and/or photosynthesis and is therefore controlled by several environmental factors. An increase in microcystin concentration has been linked to temperature (between 24 and 28.5 °C), high concentration of dissolved nutrients and a high light intensity (PAR) (Albay et al. 2005). It has also been reported that microcystin production is largely controlled by pH (Jähnichen et al. 2001). A consistently high pH ranging from 9.1 to 10.4 was recorded at the final oxidation pond of the Nakuru town sewage treatment plant. Dominance changes between toxic and non toxic strains of cyanobacteria could have also contributed to the lack of correspondence between total cyanobacteria biomass and toxin concentration. Nonetheless, a number of field studies have reported significant linear relationships between MC net production and *Microcystis* biovolume (Naselli-Flores et al. 2007; Kurmayer et al. 2003).

A similar study carried out in cyanobacteria dominated facultative and maturation ponds in Esmoriz (North Portugal) reported a microcystin concentration (as MCYST-LR equivalents) range of between 2.3 and 56.0 μg L^{-1} based on the ELISA assay (Vasconcelos and Pereira 2001). Toxin presence albeit in low levels in the Nakuru town wastewater treatment plant indicates that animals using the pond water can be exposed to toxin intoxication.

Worldwide, the cyanobacteria are well known for their ability to produce potent toxins, which have been responsible for numerous animal deaths (Schwimmer and Schwimmer 1968; Carmichael et al. 1985; Falconer 1988; Beasley et al. 1989; Kuiper-Goodman et al. 1999). More recently, algal blooms dominated by *Microcystis* contributed to the death of at least 52 wild animals in the Krüger National Park (South Africa) (Wray and Strauss, 2007). In Ghana, preliminary toxicological analysis of intracellular toxin of samples from the raw water intakes of the two reservoirs indicated the presence of microcystin, with the highest concentration (3.21 μg L^{-1}) found in the Weija Reservoir (Addico et al., 2006). In Morocco, occurrence of toxic cyanobacteria blooms has been confirmed in some water bodies used for recreational and/or as drinking water reservoirs (Oudra et al., 2002). In Lake Nakuru National Park, limited availability of freshwater especially during the dry season makes the sewage ponds popular and convenient freshwater sources for wildlife. The ponds also support a rich avian population that includes the 'Near Threatened' Lesser Flamingos (IUCN 2006). Discharge of treated wastewater through a rivulet into Lake Nakuru and other smaller wetlands near the lake indicates that the Nakuru town sewage treatment plant may be a source of contamination of these water bodies with cyanobacterial toxins.

Conclusions and recommendations

The Nakuru town sewage oxidation ponds exhibit a wide variation in pond water physico-chemical properties caused by the variation in inflow volume. The phytoplankton species composition and biomass levels in the pond water were highly irregular with the Chlorophyta being the dominant division on most occasions with the cyanobacteria and Euglenophyta dominating periodically. Occasionally, microcystins are present in low concentration in the pond water. Hence the wildlife species, which regularly use the ponds as drinking water sources, are sometimes exposed to microcystins. Besides this, Lake Nakuru and the small wetlands around the lake feed by the sewage ponds may also be exposed to contamination. A close monitoring of pond water phytoplankton composition is therefore necessary to accurately quantify the potential impact of microcystins on these wildlife species. It may also be necessary to prevent wildlife from drinking the sewage ponds and its rivulet.

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