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The rise of potentially toxin producing cyanobacteria in Lake Naivasha, Great African Rift Valley, Kenya

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ABSTRACT

Lake Naivasha, an important inland water ecosystem and a crucial freshwater resource in the Great African Rift Valley, has displayed clear signals of degradation in recent decades. We studied the phytoplankton composition and biomass levels in the period 2001–2013 and noted a progressive increase in the occurrence of potentially toxic cyanobacteria. Analyses for the presence of cyanotoxins such as microcystins (MC), cylindrospermopsin (CYN) and anatoxin-a (ATX-a) were carried out on samples collected in 2008–2013. Among the cyanotoxins tested, low concentrations of MC were detected in the lake. This is the first record of the occurrence of MC in Lake Naivasha. For the first time, molecular phylogenetic investigations of field clones of cyanobacteria from Lake Naivasha were carried out to establish the taxa of the dominant species. Amplification of the aminotrasferase (AMT) domain responsible for cyanotoxin production confirmed the presence of the *mcyE* gene belonging to the microcystin synthesis gene cluster in field samples containing *Microcystis* and *Planktothrix* species. These findings suggest that toxin producing cyanobacteria could become a threat to users of this over-exploited tropical lake in the near future.

1. Introduction

Lake Naivasha is – beside Lake Victoria – the second largest freshwater body in Kenya. It is the coolest and the freshest of the smaller lakes in the Gregory Rift Valley (Worthington and Worthington, 1933; Harper and Mavuti, 2004). Some four decades ago, Lake Naivasha was praised as a crystal clear gem in the floor of the Great African Rift Valley. Enthusiastic naturalists described the lake as a "bird-watcher's and fisherman's paradise near Nairobi" (Brown, 1971, loc. cit. p. 82; Willcock, 1974). However, in the last 70 years, the lake's water quality has deteriorated significantly. At the end of the 1930s, a higher sediment accumulation rate induced by increased human activities in the catchment of the lake was recorded (Stoof-Leichsenring et al., 2011). In this phase, a shift in the diatom assemblage from littoral and periphyton to planktonic taxa has taken place, indicating a changing light regime characterized by loss of water transparency (Stoof-Leichsenring et al., 2012). Furthermore, the lake ecosystem of Naivasha was considerably degraded by the introduction of alien species, and a multitude of impacts leading to eutrophication. All in all, during the last century, about 23 exotic species, fishes, invertebrates and macrophytes entered the lake (Gherardi et al., 2011). The invading species established a complicated network of interactions, which led to considerable fluctuations in the population density of the primary producers. Notable among the invasive species is the water hyacinth, which has the ability to outcompete other macrophytes, and dominant phytoplankton.

The presence of microphytes, such as colonial and filamentous cyanobacteria, in Lake Naivasha were recorded by early surveys of the Cambridge Expedition to East African lakes in 1930 (Rich, 1933). However, first mass developments of cyanobacteria were witnessed in 1980 (Kalff and Watson, 1986) and subsequently in 2005 and 2006 (Harper, 2006). Nowadays, mass developments of cyanobacteria are common components of the phytoplankton communities in Lake Naivasha and hence influence the lake's water quality.

Lake Naivasha is located in a tropical semi-arid zone and subjected to dramatic fluctuations in lake level. Water level changes covering or exposing several metres of shoreline within a period of a few months occur in response to drought or flood events

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(Becht et al., 2006). These fluctuations have been exacerbated by excessive abstraction of lake water to support the geothermal power industry, the horticulture industry and water supply to human settlements in the catchment area (Harper et al., 2011). Consequently the lake looses much more water than it receives from rainfall and other inflows. During periods of low water level, the swamp vegetation found along the shoreline is exposed and this results in a dramatic decline of macrophyte community dominated by papyrus *Cyperus papyrus* L. Presently, only 10% of the area previously inhabited by papyrus remains available as the natural filter of sediments and eroded materials from the catchment (Morrison and Harper, 2009). The inflowing rivers, especially the Malewa, transport large quantities of silt and nutrients from the deforested agricultural land into the unprotected lake. Surface runoff from urban settlements, untreated wastewater from horticultural farms, wildlife and domestic animal droppings also contribute to nutrient loading of the lake.

One major consequence of the sustained degradation of the lake's environment is the progressive eutrophication, which makes the lake more vulnerable to cyanobacterial blooms (Kitaka et al., 2002; Harper et al., 2011). The occurrence of dense blooms of colonial coccoid cyanobacteria is indicative of the potential production of cyanotoxins in this lake. Cyanotoxins create health hazards both for humans (through the consumption of drinking water and fish from the lake), to livestock and wild animals watering at the lake shore.

In this paper, we present data on; (i) abundance of cyanobacteria in comparison to the entire phytoplankton community in Lake Naivasha between 2001 and 2013, (ii) characterization of uncultured field clones of the dominant cyanobacteria, (iii) detection of toxin genes in field samples, and (iv) toxin content in field samples.

2. Materials and methods

2.1. Lake Naivasha

Lake Naivasha is a eutrophic freshwater lake located 1890 m above sea level in the Gregory Rift Valley approximately 80 km North of Nairobi, the capital of Kenya. The lake has a surface area of 100–150 km^2 and its main basin is ±6 m deep (Harper et al., 2011). Detailed characteristics of the lake as well as a comprehensive picture of its present ecological challenges are provided in the proceedings of an international conference on Lake Naivasha held in 1999 (Harper et al., 2002) and a review by Harper et al. (2011).

2.2. Sampling

The sampling point was at distance of 20 m from the shore near the jetty of the Elsamere Field Study Centre in the south-western bay of the lake (00°45′24″ S, 36°17′07″ E). Phytoplankton samples for this study were taken on 21 different dates at irregular intervals spread over the period 2001–2013. The samples were collected directly with a sample bottle from a depth of about 10 cm below the water surface. An aliquot was fixed with Lugol's solution for cell counting. Samples for morphological analyses were concentrated with a plankton net having a mesh aperture of 25 μm and fixed with formaldehyde. For molecular analyses, 1 L of the fresh sample was filtered through membrane filters with a pore-size of 0.6 μm (Schleicher & Schuell GmbH, Dassel, Germany). To determine the toxin content of the water, 125 mL was filtered through pre-weighed glass fibre filters (Whatman GF/C, Whatman International Ltd, Maidstone, England). After drying the filter with sample, it was weighed again, and the dry weight of the phytoplankton on the filter calculated. A polycarbonate filter holder SM 16510 (Sartorius AG, Göttingen, Germany) and a manual vacuum pump VacuMan (Bürkle GmbH, Bad Bellingen, Germany) were used to carry out filtration in the field.

2.3. Microscopy

Phytoplankton was counted according to Utermöhl (1958) in sedimentation chambers (Hydro-Bios Apparatebau GmbH, Kiel, Germany) under an inverted microscope Eclipse TS 100 (Nikon Corporation, Tokyo, Japan). The phytoplankton biomass was calculated by geometric approximations using the computerized counting program OPTICOUNT (Opticount, 2008). The specific density of phytoplankton cells was taken as 1 g cm^{-3}. The phytoplankton were photographically documented under a Nikon Eclipse E 600 light microscope using a Nikon digital camera DS-Fi1, and Nikon software NIS-Elements D (Nikon Corporation, Tokyo, Japan).

2.4. Molecular analyses

Two samples containing the dominant populations of potentially toxic cyanobacteria were collected from the pelagic habitat of Lake Naivasha. Under the light microscope, the sample collected on 14 November 2010 was confirmed to be dominated by *Microcystis* while the sample of 19 November 2011 was mainly composed of *Planktothrix*. The genomic DNA was extracted from field samples using Dynabead DNA Direct System I (Invitrogen/Dynal Biotech, Oslo, Norway) following the steps outlined in the manufacturer's manual. The polymerase chain reactions (PCR) were performed in a Peltier Thermal Cycler PTC 200 (MJ Research Inc., San Francisco, USA). The volume and concentrations of PCR cocktail used were as described by Dadheech et al. (2012). Primers CYA361f and CYA785r (Mühling et al., 2008) were employed for amplification of V3-V4 regions of 16S rRNA gene. Amplification of 16S rRNA gene fragment was carried out as follows: initial 3 min at 94 °C; 30 cycles of 30 s at 94 °C; 30 s at 55 °C; 45 s at 70 °C: and a final elongation step at 72 °C for 3 min. The primers PCβf and PCαr (Neilan et al., 1995) were used for amplification of beta and alpha subunits including intergenic spacer (*cpc*BA-IGS) of the phycocyanin operon using the PCR protocol described by Ballot et al. (2008). The amplification of the aminotrasferase (AMT) domain responsible for cyanotoxin production was done using HEPF and HEPR primers (Jungblut and Neilan, 2006) and a PCR protocol described earlier (Dadheech et al., 2009). The amplified products were cleaned using QIAquick PCR purification column according to manufacturer's protocol and examined on a 1% agarose gel. Cleaned PCR products were cloned using the Zero Blunt® Topo® PCR cloning kit (Invitrogen, Germany) according to manufacturer's instructions. The positive clones selected were PCR amplified and then cycle sequenced to retrieve the sequence of 16S rRNA gene fragment, *cpc*BA-IGS and AMT domain. The uncultured *Microcystis* sp. clones sequenced were designated as nav_16S_ micro (16S rRNA gene), nav_cpc_micro and nav_ mcyE _micro. Similarly, uncultured *Planktothrix* sp. clones sequenced were designated as nav_16S_plank, nav_cpc_plank and nav_mcyE_plank. Both strands were sequenced on ABI 3100 Avant Genetic Analyzer using BigDye Terminator v3.1 using Cycle Sequencing Kit (Applied Biosystems, Applera, Deutschland, GmbH, Darmstadt, Germany) as described in the manufacturer's manual.

The sequences of 16S rRNA gene, *cpc*BA-IGS and AMT domain belonging to *Microcystis* and *Planktothrix* taxa were retrieved from nucleotide NCBI database and aligned with sequences obtained in the present study using MUSCLE software (Edgar, 2004). Alignment was checked visually using the Manual Sequence Alignment Editor, Align v05/2008 (Hepperle, 2008). The phylogenetic trees were constructed by the maximum likelihood (ML) method using the program MEGA v5.0 (Tamura et al., 2011) with default settings,