1 Introduction

Cyanobacteria are worldwide one of the most studied organism groups. They are photosynthetic prokaryotes with the ability to synthesize chlorophyll a. Like eukaryotic algae and plants they use H_2O as electron donor leading to the production of oxygen. The oldest fossil findings of cyanobacteria can be dated back to around 3500 million years ago (Schopf 2000). Due to these findings it can be suggested that in earth history cyanobacteria possibly played a major role in the change of the oxygenless atmosphere to an oxigenic one (Schopf 1994, Schopf 2000). Their long evolutionary history is considered as a reason for the success of cyanobacteria in many habitats and their wide ecological tolerance (Whitton and Potts 2000). Especially under eutrophic and hypertrophic conditions they are known for mass developments and blooms worldwide (Paerl 1996). The bloom causing phytoplanktic cyanobacteria are genera possessing gas vesicles. They vary in form and size from small filaments to large globular colonies. During a cyanobacterial bloom, occuring mainly under conditions of high water temperatures and reduced turbulence, they show a buoyant migration to the water surface (Hutchinson 1967, Reynolds and Walsby 1975, Robarts and Zohary 1987, Paerl 1996). Bloom forming cyanobacteria mainly belong to the genera *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Arthrospira*, *Cylindrospermopsis*, *Oscillatoria*, *Nodularia*, and *Microcystis* (Reynolds and Walsby 1975, Oliver and Ganf 2000). In temperate regions water blooms develop frequently during the warmer summer and autumn. With decreasing latitude towards the subtropics and tropics the changes in the daily solar input and temperature become less variable. The waterbodies become increasingly dominated by seasonal rainfall and runoff and vertical mixing due to the effects of changing wind regimes (Melack 1979, Beadle 1981). In the tropics cyanobacterial blooms can therefore occur at almost any time of the year, due to the relatively constant annual air temperature and solar radiation (Oliver and Ganf 2000). The limnology of small shallow waterbodies in tropical latitudes is often influenced only by the changes in the diel climate.

1.1 Cyanobacteria in East African waterbodies

1.1.1 Freshwater lakes

The freshwater lakes of East Africa have been objects of scientific research since the end of the 19th century. The investigations until 1955 are summarized in a bibliography by Brook et al. (1957). Later investigations have been focused on Lake Victoria and the greater lakes in the Western Rift Valley (Talling and Talling 1965, Talling 1966, Hecky and Kling 1981, Hecky and Kling 1987, Talling 1987, Ochumba and Kibaara 1989, Komárek and Kling 1991, Cocquyt et al. 1993, Evans 1997, Lung´ayia et al. 2000). The phytoplankton communities of most of the large and deep African lakes follow a common periodicity like freshwater lakes of temperate regions (Talling 1987). In the large and deep African lakes during mixing periods diatoms are the mostly dominating organisms and cyanobacteria only dominate during periods of restratification. This pattern could also be observed in Lake Victoria until the 1960s. Since then the abundance and diversity of cyanobacteria have increased considerably and cyanobacterial blooms have become common in the open water and near the shore areas of the lake (Talling 1987, Komárek and Kling 1991, Lung´ayia et al. 2000). The main cyanobacterial genera in observed blooms in L. Victoria are *Microcystis*, *Anabaena*, *Anabaenopsis*, *Lyngbya*, and *Merismopedia* (Talling 1966, Ochumba and Kibaara 1989, Lung´aiya et al. 2000, Kling et al. 2001).

The small and shallow Lake Baringo is also known for the dominance of cyanobacteria e.g. *Microcystis aeruginosa* (Kützing) Kützing since early investigations by Jenkin (1929). Later investigations by Beadle (1932), Patterson and Wilson (1995) and Oduor (2000) have confirmed the dominance of *M. aeruginosa*.

1.1.2 Alkaline lakes

The tropical East African alkaline-saline lakes like other soda lakes in the tropics and subtropics are known for a unique cyanobacterial community characterised by massive growth and blooms of filamentous cyanobacteria. Early investigations and general descriptions have mentioned persistent almost unicyanobacterial blooms of *Arthrospira fusiformis* (Vorochinin) Komárek (syn. *Spirulina fusiformis* Voronichin) as the typical characteristic feature of the East African and other alkaline soda lakes in the tropics and subtropics (Jenkin 1929, Rich 1931, Jenkin 1936, Beadle 1932, Iltis 1968, Iltis 1969,

Compère 1974, Beadle 1981, Melack 1996). Though most of the *Arthrospira* species have been described from alkaline-saline waterbodies, some findings also have been reported from freshwater habitats (Vonshak and Tomaselli 2000).

Beside *Arthrospira fusiformis* other filamentous cyanobacteria e.g. *Anabaenopsis arnoldii* Aptekarj and *A. elenkinii* V. Miller have been reported as abundant in alkaline lakes (Iltis 1969, Vareschi 1982, Melack 1988, Kebede and Willén 1998). From the Ethiopian Lake Abijata a new species of the genus *Anabaenopsis*, *Anabaenopsis abijatae* Kebede et Willén, has been described which occurs dominant or codominant beside *A. fusiformis* (Kebede and Willén 1996, 1998).

Arthrospira is the main food source for the characteristic bird of the alkaline-saline lakes, the Lesser Flamingo (*Phoeniconaias minor* Geoffroy) (Jenkin 1929, Ridley et al. 1955). It feeds by filtering the cyanobacteria from the water with its bill. The estimated daily feeding rate on cyanobacteria for an adult bird is 72 g DW (dry weight) (Vareschi 1978). Variations in the cyanobacterial community can have an impact on the food chain, especially on the Lesser Flamingo. The population density of the Lesser Flamingo in the Kenyan Rift Valley varies considerably due to changes in the food quantity and quality and breeding activities. The estimated number of flamingos for the whole Rift Valley varies from around one million to 3-5 million (Brown 1959, Bartholomew and Pennycuick 1973). Yearly countings of flamingos in the Rift Valley from 1991 to 1999 ranged from 337,000 to 1,470,000 individuals. The main flock could be found at L. Bogoria with numbers between 175,000 and 1,074,000 (Owino et al. 2001). However, massive deaths of flamingos could be observed in recent years at the Rift Valley lakes, mainly at L. Nakuru and L. Bogoria. 30,000 dead flamingos were reported from L. Bogoria in the second half of 1999 (Vick 2000). A multidisciplinary study has provided insight into this complex problem. The main causes are thought to be infections by mycobacteria, poisoning by heavy metals or pesticides or an interaction of these factors (Sileo et al. 1979, Oyugi 1994, Kairu 1996, Nelson et al. 1998, Kock et al. 1999, Wanjiru 2001).

1.1.3 Hot springs

Many lakes in the Kenyan Rift Valley (e.g. L. Baringo, L. Bogoria, L. Elmenteita, L. Magadi) are influenced by thermal springs due to recent volcanic activity (Beadle 1981, Schlüter 1997). The hot springs are situated in the vicinity of the lakes or in the lakes themselves.

These extreme environments are colonised by mats of thermophilic cyanobacteria. Cyanobacterial genera, described from hot spring environments worldwide are *Calothrix*, *Oscillatoria*, *Phormidium*, *Pseudanabaena*, *Synechococcus*, *Synechocystis*, and *Spirulina* (Castenholz et al. 1991, Garcia-Pichel et al. 1994, Komárek and Anagnostidis 1999, Ward and Castenholz 2000, Hindák 2001). Some of these genera also occur in the numerous hot springs and geysers active on the shore of L. Bogoria (Hindák 2001). Differences in species composition of hot spring cyanobacterial communities are likely due to differences in chemical composition, temperature and exposure to solar irradiance (Ward and Castenholz 2000).

1.2 Cyanobacterial toxins

Surveys on water blooms and mass developments of planktic and benthic cyanobacteria in different countries have shown that a high percentage (25 to 90%) of the blooms is toxic (Carmichael 1988, Baker and Humpage 1994, Codd 1995, Sivonen 1996, Codd 2000).

Mass developments of toxic cyanobacteria can have a severe impact on the food web of lakes (Christoffersen 1996). Laboratory experiments have shown that the growth of members of different phytoplankton groups (diatoms, cyanobacteria, Chlorophyceae and Cryptophyceae) and macrophytes was inhibited or reduced by toxic cyanobacteria (Kirpenko 1986, Bagchi et al. 1990, Christoffersen 1996). Behavioural changes and a higher mortality caused by cyanobacterial toxins are found in zooplankton organisms like copepods and cladocerans (Lampert 1981, Nizan et al. 1986, DeMott et al. 1991, DeMott and Moxter 1991). Acute effects of microcystins are also observed on fish, including liver damage, disturbed ionic regulation, behavioural changes, and mortality (Tencalla et al. 1994, Bury et al. 1995, Chorus 2001).

Cyanobacterial toxins can also cause serious health problems for wild and domestic animals and are an increasing hazard to human health (Carmichael 1992a, Carmichael and Falconer 1993, Carmichael 1997, Codd et al. 1999, Kuiper-Goodman et al. 1999, Chorus 2001). In North America, Australia and Europe mass mortalities of wild and domestic birds could be related to toxic cyanobacterial blooms and scums (Yoo et al. 1995, Matsunaga et al. 1999). In Danish lakes, bird kills occurred during blooms of *Anabaena lemmermannii* P. Richt. in which the neurotoxin anatoxin-a(s) was found (Henriksen et al. 1997, Onodera et al. 1997).

Mass deaths of flamingos and other water birds in the Spanish National Park Cota Donana due to the intake of toxic *Microcystis aeruginosa* and *Anabaena flos-aquae* (Lyngb.) Bréb. were observed (Alonso-Andicoberry et al. 2002). At SeaWorld Orlando, Florida, USA the death of ten captive Chilean flamingos (*Phoenicopterus chilensis*) was attributed to microcystins produced in a bloom of *Microcystis* sp. (Chittick et al. 2002). Mass mortalities of flamingos are also known for the Kenyan alkaline Rift Valley Lakes Bogoria and Nakuru in recent years. A possible influence of cyanobacterial toxins on the mass deaths of Kenyan flamingos is not investigated so far.

Cyanotoxins are bioactive secondary metabolites produced by cyanobacteria. They can be distinguished in the acute lethal poisonous hepatotoxins and neurotoxins and the less lethal cytotoxins (Dow and Swoboda 2000).

1.2.1 Hepatotoxins

The most common hepatotoxins worldwide are microcystins which are cyclic heptapeptides. They have been isolated from planktic and benthic cyanobacterial genera e.g. *Microcystis*, *Anabaena*, *Oscillatoria*, *Nostoc*, *Anabaenopsis*, *Phormidium*, and from the terrestrial cyanobacterium *Hapalosiphon* (Prinsep et al. 1992, Sivonen 1996, Mez et al. 1997, Codd et al. 1999, Chorus 2001). Fig. 1 shows the general structure of microcystin.

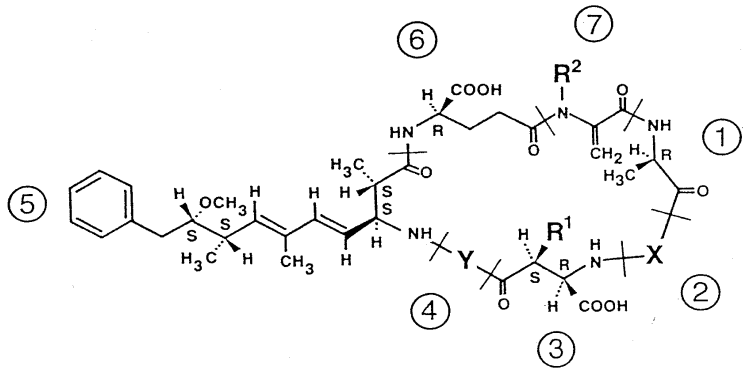

Fig.1: General structure of microcystin, cyclo(D-Ala[1]—X[2]—D-MeAsp[3]—Y[4]—Adda[5]—D-Glu[6]—Mdha[7]). X and Y represent variable L-aminoacids at positions 2 and 4. MeAsp is D-erythro-ß methylaspartic acid and Mdha is N-methyldehydroalanine. Adda is (2S,3S,8S,9S)-3-amino-9-methox-2,6,8-triethyl-10-phenyl-4,6-decadienoic acid.

Variation in the chemical structure is very common. Variations in the X and Y position account for 25% of microcystin variants, but substitutions have been reported for each amino acid (Sivonen 1996, Codd et al. 1999). At present more than 65 microcystin variants are known worldwide (Codd 2000). The hepatotoxicity results from the ability of microcystins to enter into hepatocytes via the bile acid transport system causing lethal intrahepatic haemorrhage, liver necrosis and destruction of parenchymal cells of the liver (Carmichael 1992a, 1994). Microcystins are inhibitors of eukaryotic protein phosphatases. These enzymes are vital to cell growth and tumour suppression. Therefore microcystins are possible potent cancer promoters (Luukainen et al. 1993, Carmichael 1994, Carmichael 1997).

Other cyanobacterial hepatotoxins are the nodularins which are cyclic pentapeptides and the cyclic guanidine alcaloid cylindrospermopsin (Sivonen 1996, Codd et al. 1999).

1.2.2 Neurotoxins

One of the most frequently found neurotoxins is anatoxin-a. It is a bicyclic secondary amine, 2-acetyl-9-azabicyclo-[4.2.1]non-2-ene (Fig. 2) (Huber 1972, Devlin et al. 1977, Carmichael 1988, Dow and Swoboda 2000). Anatoxin-a is a structural analogue to the neurotransmitter acetylcholine and binds to the nicotinic acetylcholine receptor with a higher affinity than acetylcholine. It acts as a postsynaptic depolarizing neuromuscular blocking agent and cannot be degraded by acetylcholinesterase thus causing muscle overstimulation (Carmichael et al. 1979). Signs of poisoning are staggering, muscle fasciculations leading to fatigue and paralysis, gasping, and opisthotonus in birds (Dow and Swoboda 2000). Anatoxin-a is mainly reported from blooms of *Anabaena*, *Oscillatoria*, *Phormidium* and on rarer associations with *Cylindrospermum*, and *Aphanizomenon* (Sivonen, 1996, Codd et al. 1999, Sivonen and Jones 1999, Chorus 2001). Fig. 2 shows the structure of anatoxin-a.

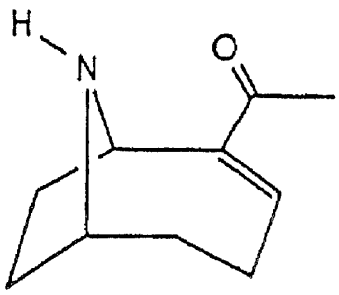

Fig. 2: The general structure of the neurotoxin anatoxin-a.

Investigations on cyanobacterial blooms, scums and toxins have been carried out in Australia (e.g. Baker and Humpage 1994, Steffensen et al. 1999, Falconer 2001), in Europe (e.g. Sivonen et al. 1995, Mez et al. 1997, Willén and Mattson 1997, Chorus 2001), in North America (e.g. Carmichael 1992b, Kotak et al. 1995, McDermott et al. 1995, Johnston and Jacoby 2003), in South America (e.g. Pouria et al. 1998, Jochimsen et al. 1998, Campos et al. 1999, Carmichael et al. 2001) and Asia (e.g. Park et al. 1993, 1998, Matsunaga et al. 1999, Oh et al. 2001). In Africa most studies on cyanobacteria were done in the frame of phytoplankton studies. In East Africa these phytoplankton investigations were mainly conducted in the 1970s and 1980s. Investigations on cyanobacterial toxins on the African continent have been done only in Morocco, Egypt, and South Africa (Wicks and Thiel 1990, Scott 1991, Mohamed and Carmichael 2000, Oudra et al. 2002). In East Africa and especially Kenya no toxicity studies on cyanobacteria existed prior to this study.

1.3 Molecular Phylogeny

The species and genus concept of cyanobacteria is changing from the traditional morphological classification to a concept using different phenotypic features e.g. biochemical and ultra structural characteristics and genotypic features (Anagnostidis and Komárek 1985, Komárek and Anagnostidis 1989, Castenholz et al. 2001, Wilmotte and Herdman 2001). For phylogenetic analyses and identification of bacteria and cyanobacteria 40 to 100 genes in the genome are suitable to fulfil the basic requirements of useful phylogenetic markers (Ludwig and Klenk 2001). One the most informative genes is the 16S rRNA gene, which was demonstrated by Woese and coworkers (Woese et al. 1976, Woese 1987). The 16S rRNA gene is universally present in bacteria and cyanobacteria and has a conserved function. The phylogenetic investigations using 16S sequences have shown that many unicellular and filamentous non heterocysteous cyanobacterial genera are probably polyphyletic and cannot be grouped as natural taxa, whereas heterocysteous strains form a monophyletic group (Giovannoni et al. 1988, Wilmotte 1994, Castenholz et al. 2001, Rippka et al. 2001, Wilmotte and Herdman 2001).

The genus *Arthrospira* is non heterocysteous and belongs to the Subsection III (formerly Oscillatoriales) according to Bergey´s Manual of Systematic Bacteriology (Castenholz et al. 2001). Due to the incorrect unification of the genus *Arthrospira* and the genus *Spirulina* by

Geitler (1932) the term "*Spirulina*" often is used instead of "*Arthrospira*". The divison in two separate genera has been suggested by several authors (Desikachary 1959, Rippka et al. 1981, Anagnostidis and Komárek 1988, Tomaselli 1997). Many morphological studies have shown differences between both genera e.g. in helicity and trichome size, cell wall structure, pore pattern and gas vesicles (Desikachary 1959, Hindák 1985, Guglielmi and Cohen-Bazire 1982, Guglielmi et al. 1993). Investigations of the 16S rRNA genes of two *Arthrospira* strains (PCC7345 and PCC8005) in comparison to one *Spirulina* strain (PCC6313) have shown that the genus *Arthrospira* is not closely related to the genus *Spirulina* (Nelissen et al. 1994). The separation into two genera has been accepted by Bergey´s Manual of Systematic Bacteriology (Castenholz 1989, Castenholz et al. 2001).

Investigations of the 16S rRNA gene of an *Arthrospira fusiformis* and *A. maxima* strain have shown that they are 100% identical (Li et al. 2001). Nelissen et al. (1994) found a 16S similarity of two *Arthrospira* strains (PCC7345 and PCC8005) of 99.7%. According to Castenholz et al. (2001) such a high similarity makes it likely that all *Arthrospira* strains are representatives of a single nomen species. A 16S rRNA gene sequence similarity value of more than 97.5 % is regarded as the level at which two strains can be regarded as congeneric or belonging to the same species (Devereux et al. 1990, Stackebrandt and Goebel 1994). Fox et al. (1992) mention that high 16S rRNA sequence identities are not necessarily a sufficient criterion to guarantee species identity, because very recently diverged species may not be recognizable with this tool. The 16S rDNA is a relatively conserved region and it has only an insufficient number of variable positions which can be used to study the relationship on the species level or below it. For this purpose other more variable non-coding sequences like the internal transcribed spacer region of the 16S-23S region or the intergenic spacer of the phycocyanin operon have been used (Neilan et al. 1995, Boyer et al. 2001, Baurain et al. 2002, Manen and Falquet 2002).