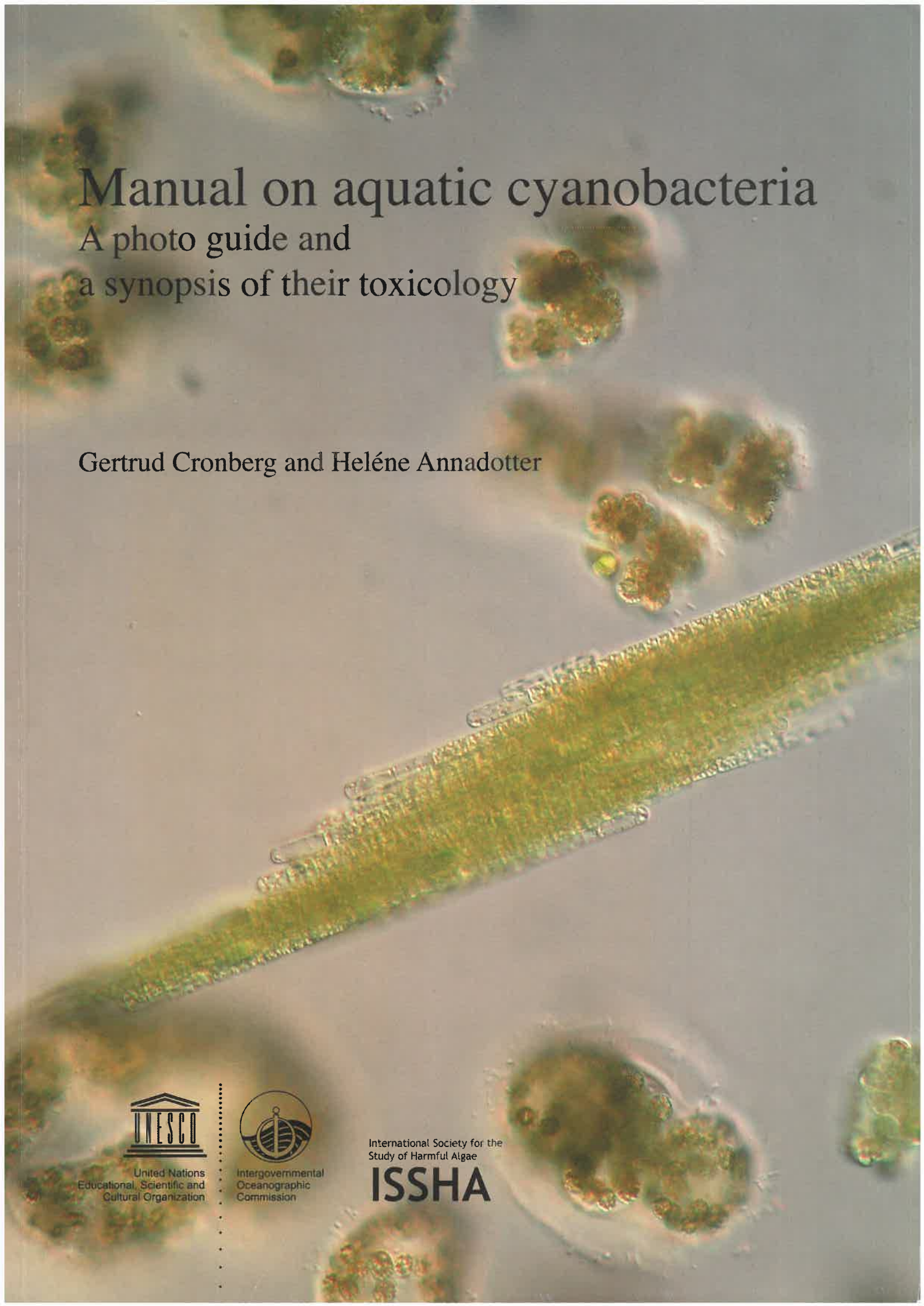

Manual on aquatic cyanobacteria

A photo guide and a synopsis of their toxicology

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United Nations Educational, Scientific and Cultural Organization

Intergovernmental Oceanographic Commission

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ISSHA

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Intergovernmental Oceanographic Commission of UNESCO
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Front cover image: The cyanobacteria *Microcystis viridis* and *M. wesenbergii* (at top and bottom); *Aphanizomenon flos-aquae* var. *klebahnii* (in the middle). (Photo: *Cronberg*).

Back cover images: A mallard swimming in dense cyanobacterial bloom in a park pond, Malmö, Sweden (Photo: *Cronberg*); Crocodile partly hidden in a waterbloom, South Africa (Photo: *Forssblad*); "The peoples fish", Lake Kariba, Zimbabwe; In the background, the cyanobacterium *Anabaena lemmermannii* (Photos: *Cronberg*).

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Preface

The Intergovernmental Oceanographic Commission (IOC) of UNESCO has since the early 1990s offered training opportunities and developed manuals and guides in the field of research and management of harmful microalgae. With this manual we wish to focus in greater detail on one of the groups of harmful algae, the cyanobacteria or blue-green algae, where the species are among the most difficult to identify and where the societal impacts are widespread and complex. Not only are large human populations subject to acute intoxication but often also subject to long-term exposure to low concentrations of toxins. Furthermore, there are aspects of the toxicology and potential impacts of cyanobacteria that are still not widely acknowledged, in particular in relation to safe drinking water resources. Furthermore, the International Hydrological Programme of UNESCO has in 2005 established a global network for cyanobacterial bloom and toxin risk management, CYANONET. This manual therefore responds to an identified need in both the marine, freshwater and drinking water scientific and managerial communities.

Potentially harmful cyanobacteria occur widespread in the aquatic environment and this manual treats their taxonomy, identification, and toxicology across freshwater, brackish and marine environments.

The material that has provided the basis for this manual has been compiled by the authors over many years as well as developed for the IOC training courses on identification of harmful microalgae held at the IOC Science and Communication Centre on Harmful Algae at University of Copenhagen since 1993. This manual is therefore in addition to serving as a handbook for researchers and managers, also intended as a source book for training and educational purposes.

In recent years the International Society for the Study of Harmful Algae (ISSHA) has become an important coherent factor for the scientific and managerial community working with harmful algae. With this joint IOC-ISSHA publication the publishers hope to facilitate the distribution and use of the manual.

The IOC and ISSHA are highly appreciative of the efforts of the two authors, Dr. Gertrud Cronberg and Heléne Annadotter at Lund University, Sweden, who have dedicated many hours of work to the preparation of the manuscript and a unique photo material.

The scientific opinions expressed in this work are those of the authors and are not necessarily those of UNESCO and the IOC nor ISSHA. Equipment and materials have been cited as examples of those most currently used by the authors. Their inclusion does not imply that they should be considered as preferable to other available at that time or developed since.

The production of this manual has been made possible through support from Lund University, Sweden, as well as from the Danish Environmental Research Institute and the Royal Danish Ministry of Foreign Affairs (DANIDA), though the IOC Science and Communication Centre on Harmful Algae at the University of Copenhagen.

Patricio Bernal
Executive Secretary IOC
Assistant Director-General UNESCO

Patricia Tester
President ISSHA

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Most of the micrographs in Chapter 2 have been taken by Gertrud Cronberg. However, Fig. 6 has kindly been put at our disposal by Antony Joosten, The Netherlands. Ian Games, Zimbabwe took the aerial photograph, Fig. 2 in Chapter 3 and Nguyen Thi Thu Lien, Vietnam took the microphotograph, Fig. 2 in the Epilogue. Glen MacGregor, Australia provided us with samples of *Lyngbya majuscula* for photography. Johan Forssblad, as indicated, has kindly permitted us to use his photographs, which we gratefully acknowledge.

Jiri Komárek and Jarka Komárková have given their time to review Chapter 2 and suggested improvements. In addition Øyvind Moestrup Denmark, Linda Lawton, UK and Olav Skulberg, Norway have reviewed the whole manual and we are thankful for their comments. Translator Karin Ryde has kindly corrected the English language.

Gertrud Cronberg
Heléne Annadotter

1. Introduction

History and paleobiology

Cyanobacteria are photosynthetic, prokaryotic autotrophs with the ability to synthesize chlorophyll *a* and the blue-green phycobilin pigment, phycocyanin.

Geologists and geochemists agree that cyanobacteria have a long evolutionary history that extends at least 3500 million years back in time. The Proterozoic Era, 2500-570 million years ago, has been called. "the Age of Cyanobacteria" since it is the period when most abundant cyanobacterial fossils have been recorded. (Schopf and Walter, 1982).

In organic-rich, Precambrian (2500 million years old) sediments, derivatives of 2-methylbacteriohopanepolyols, which occur in many modern cyanobacteria, have been found (Summons *et al.*, 1999).

A range of morphological features are similar in the fossil and modern species of cyanobacteria. These include the cell shape and the form and arrangement of originally mucilaginous cell-encompassing envelopes. They also exhibit comparable frequency distributions of dividing cells and comparable patterns of cellular development. Furthermore, they occur preserved in fossil material with species composition and biological diversity being equal to recent cyanobacterial communites (Golubic & Hofmann, 1976).

Distribution and habitats

Cyanobacteria are present in aquatic environments as well as in many terrestrial surroundings, and are especially prevalent in soils in arid regions (Forest and Weston, 1966). A high pH has been suggested as the most important environmental factor that favours cyanobacteria in soils (King and Ward, 1977) even though the reason for this still remains unexplained. (Komárek, 2003).

In inland and brackish waters, several cyanobacterial genera such as e.g. *Anabaena* and

Fig. 1. Stromatolites created by cyanobacteria in the intertidal of Shark Bay, Western Australia. (Photo *Cronberg*).

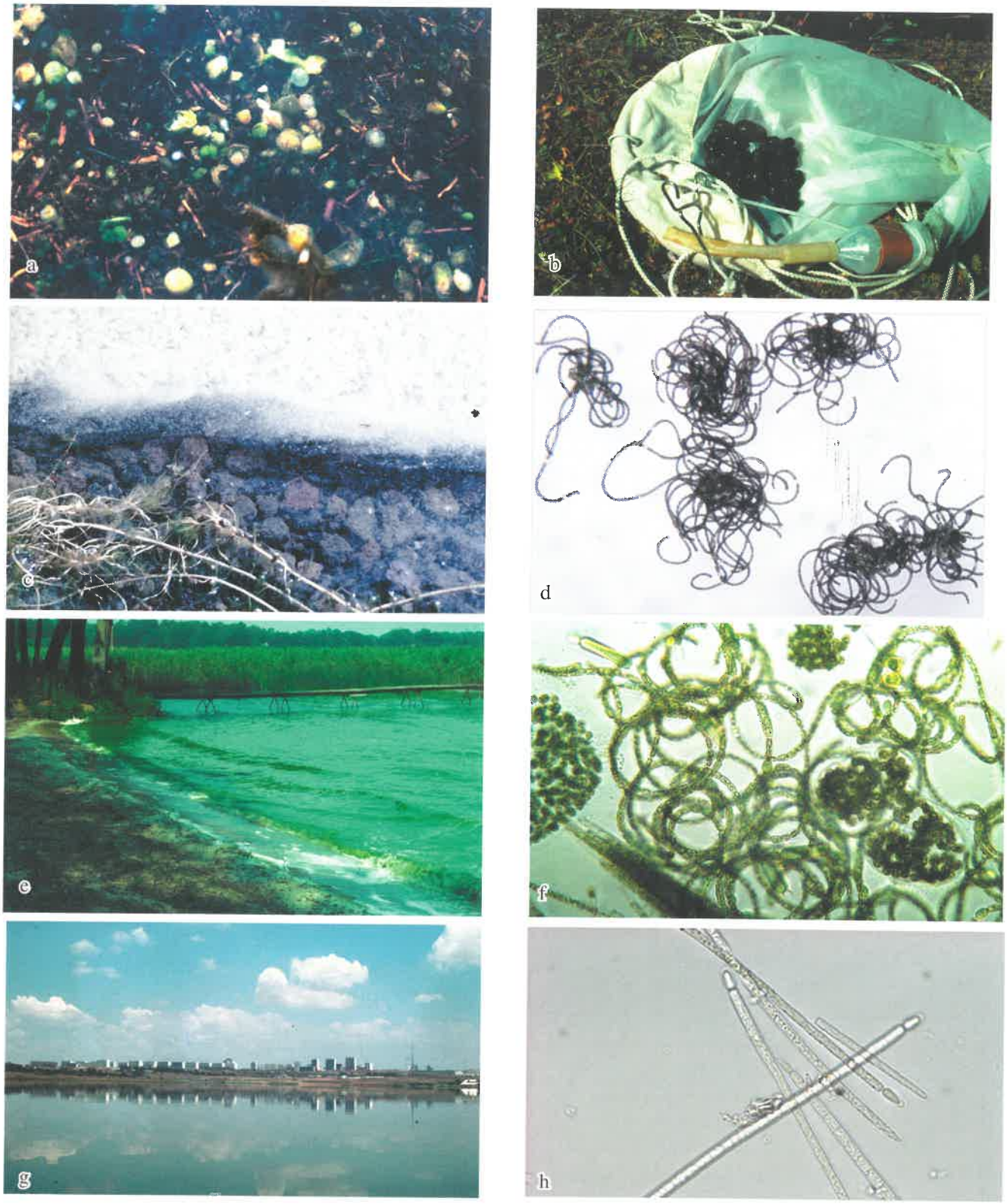

Plate 1. Developments of cyanobacteria at different latitudes. (**a,c,e,g**.) with microphotos (**b, d, f, h**). **a)** *Nostoc* sp. at the shore of a pond in Greenland. **b)** Balls of *Nostoc* sp. spread on a plankton net. **c)** A bloom of *Anabaena lemmermannii* var. *laxa* in the Icelandic, eutrophic Lake Myvatn (66°N) . **d)** *Anabaena lemmermannii* var. *laxa*. **e)** Lake East Ringsjön, Southern Sweden (56°N) with mass development of *Microcystis, Anabaena* and *Aphanizomenon*. **f)** Various species of *Microcystis, Anabaena* and *Aphanizomenon*. **g)** A monoculture of *Cylindrospermopsis raciborskii* proliferating in the tropical Lake Paranoa, Brasilia, Brazil (15°S). **h)** *Cylindrospermopsis raciborskii* (Photos *Cronberg*).

Nodularia are cosmopolitan while other species (e.g. *Aphanizomenon* and *Cylindrospermopsis*) have species with a limited geographical distribution (Komárek, 2003). Various species may have very different environmental requirements. Some cyanobacterial species occur in saline habitats, while others proliferate in freshwater of low salinity. In coastal pelagic waters and in the open oceans coccoid cyanobacteria of the genera *Synechococcus, Synechocystis* and *Prochlorococcus* have woldwide distribution. In segments of the warm oceans where nutrients are sparse, the filamentous *Trichodesmium* - a diazotrophic blue-green alga - may achieve vigorous growth in upper layers of the tropical and subtropical oceanic ecosystems.

Cyanobacteria are widely occuring in the flora of wetlands. In rice fields, species capable of fixing

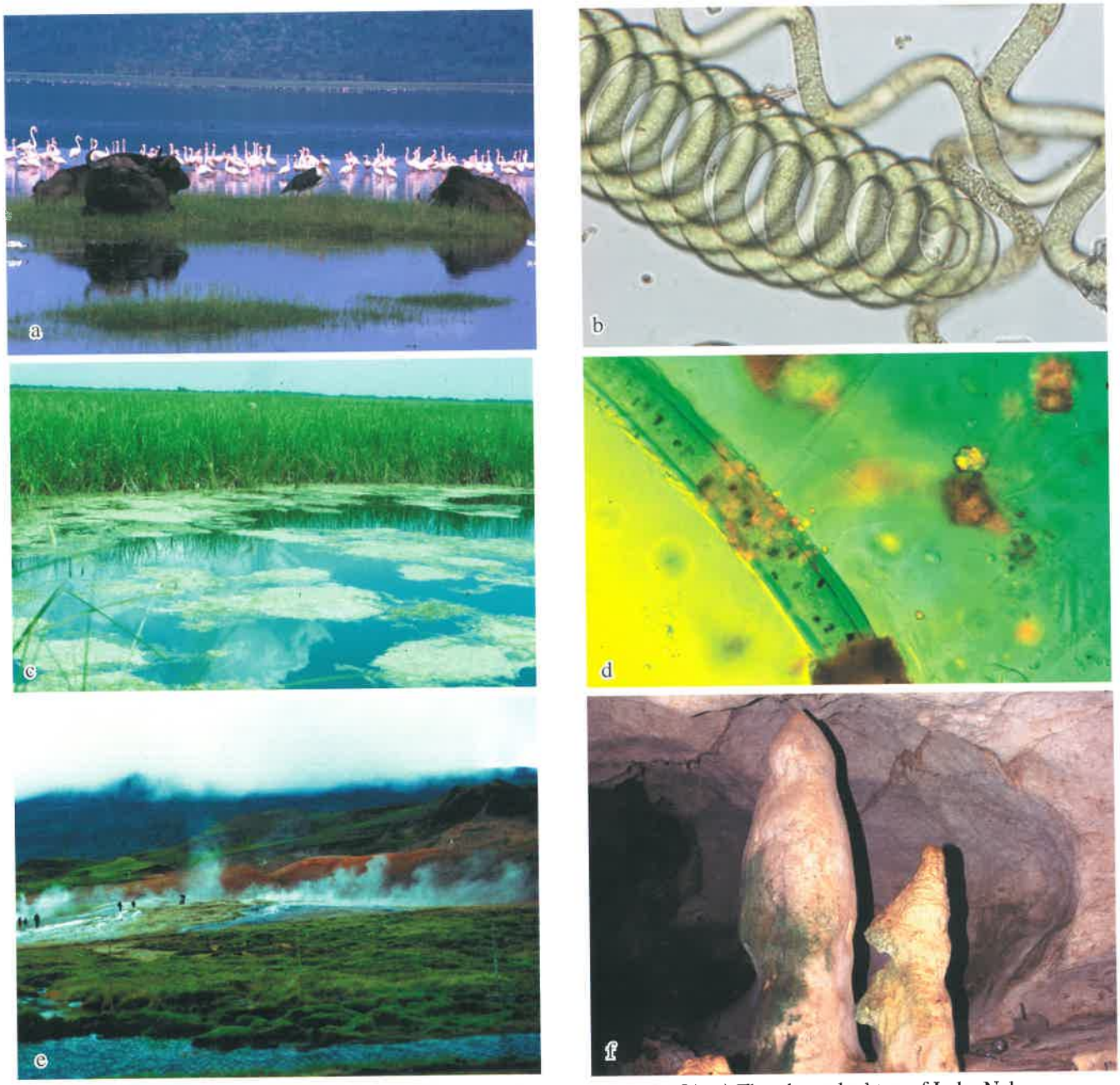

Plate 2. Cyanobacteria in extreme habitats (**a, c, e, f**) and microphotos (**b, d**). **a)** The phytoplankton of Lake Nakuru, a natron lake in the Rift Valley, Kenya, is dominated by the halophile and alkalophile cyanobacterium *Arthrospira* sp. **b)** *Arthrospira fusiformis*. **c)** Mass development of *Oscillatoria* sp. in a pool in the Black River morass, Jamaica. **d)** Lime-encrusted filaments of *Oscillatoria* sp. **e)** Hot sulphur springs, Iceland, are characteristic localities for acidotolerant and thermophilic cyanobacteria. **f)** Epilithic cyanobacteria growing on stalagmites in a cave in northern Jamaica. (Photos **a)** *Annadotter*; **b), c), d), e)** *Cronberg*; **f)** *Forssblad*)

nitrogen are especially prevalent. In calcareous swamps and bogs, cyanobacteria may become encrusted with calcium carbonate (Browder *et al.*, 1994) (Plate 2c and d). The most common cyanobacteria in wetlands include species of *Aphanothece, Chroococcus, Cyanothece, Eucapsis, Leptolyngbya, Lyngbya, Merismopedia, Phormidium, Rhabdoderma* and *Scytonema*.

In extreme environments, such as soda lakes and hot springs, cyanobacteria are often the dominating autotrophic organisms. Cyanobacteria may endure a temperature of 73°C (Ward and Castenholz, 2000). The most important high-temperature cyanobacterial genera include *Calothrix, Cyanothece, Leptolyngbya, Mastigocladus, Oscillatoria, Phormidium, Spirulina* and *Synechococcus*,

Cyanobacteria are conspicuous in many environments that are exposed to high solar irradiance. Several cyanobacterial strains have a high tolerance to ultraviolet-B and ultraviolet-C radiation, an ability probably evolved in the

Fig. 2. Mass development of *Microcystis* in the eutrophic Lake Eastern Ringsjön, Southern Sweden. (Photo *Cronberg*).

early Precambrian when the levels of ultraviolet radiation were high (Kasting, 1992). Different strategies evolved to cope with high ultraviolet radiation include the development of sunscreen pigments, generating systems to repair damaged DNA, downward migrations during periods of high solar irradiance and restitution of the photosynthetic machinery (Castenholz and Garcia-Pichel, 2000).

Bloom-forming Cyanobacteria

Surface blooms of cyanobacteria are often associated with calm conditions and reduced turbulence (Fig. 2, 3, 4a and b). Blooms usually consist of only one or a few species and are identified by the dominant phytoplankton type, e.g. *Microcystis* bloom, *Aphanizomenon* bloom, cyanobacterial bloom etc. A bloom is often defined in terms of the cell concentrations which represent a major inconvenience to humans, and

Fig. 3. A nuisance bloom of cyanobacteria in the eutrophic and polluted Lake Norra Bergundasjön, Southern Sweden. (Photo *Cronberg*).

a lower limit may be ca. 20.000 cells ml $^{-1}$ (Oliver and Ganf, 2000).

Blooms can appear suddenly, often within hours, and to the casual observer without prior warning of the presence of the organisms. Their sudden appearance, in fact, results from the upward migration from the sediment or deep water of an existing, dispersed population (Reynolds, 1971) and is not due to rapid cell growth.

The most frequent bloom-forming cyanobacteria are aerotope-bearing species. Aerotopes are also termed gas vesicles (or incorrectly gas vacuoles). These cyanobacteria are distributed within a number of genera and vary in form and size from small filaments to large globular colonies (Oliver and Ganf, 2000).

The extensive vertical migrations that the

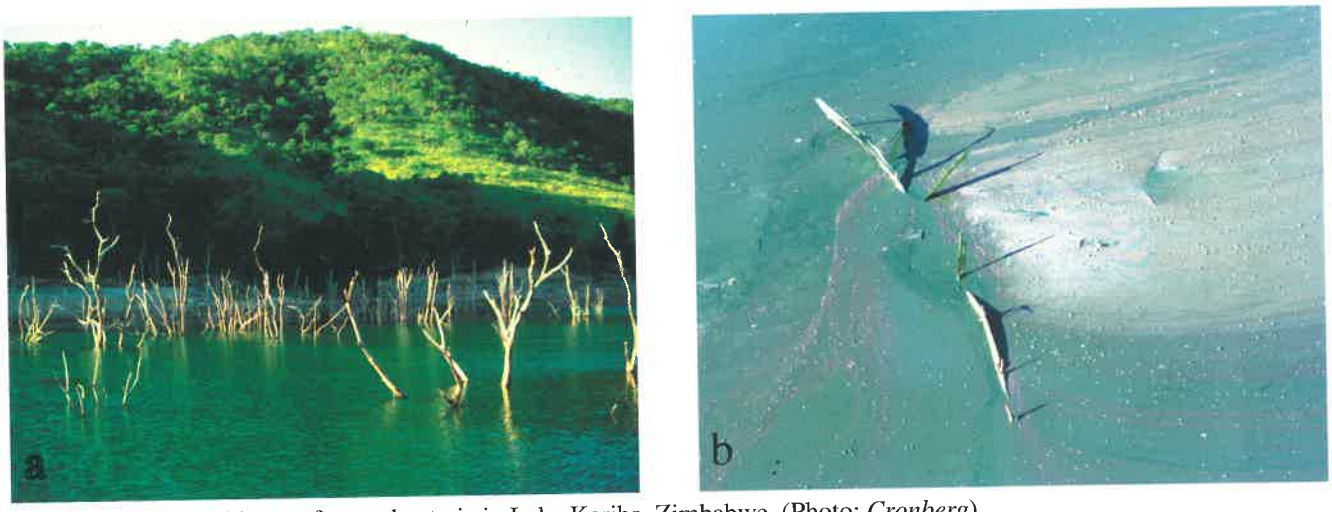

Fig. 4a and b. Waterbloom of cyanobacteria in Lake Kariba, Zimbabwe. (Photo: *Cronberg*).

bloom-forming species can perform (Kromkamp and Walsby, 1990) are made possible by the regulation of gas pressure in the aerotopes (Walsby, 1994), and density changes caused by intracellular carbohydrate dynamics (Gibson 1978a and b). During photosynthesis in the photic zone, the heavy carbohydrates counteract the floating forces of the gas vesicles, with the result that the colony sinks. The aggregates therefore sink from the photic into the dark, deeper zone. The carbohydrates are consumed during respiration, the cells become lighter and can float to the surface again.

Another crucial requirement for such long vertical transport is the size of the organisms (Mur *et al.*, 1999). All species of mass-developing cyanobacteria, such as *Anabaena, Anabaenopsis, Aphanizomenon, Microcystis, Gloeotrichia, Woronichinia, Planktothrix* and *Cylindrospermopsis* form either filaments or colonies. According to Stoke's law, the rate of sinking depends on the density difference between the cells and the water, as well as on the square of the size of the aggregates (d^2). This has the result that large colonies have the ability to perform vertical transfer faster than small colonies, and single cells can hardly make any vertical migration at all. Colonies of the cyanobacterium *Microcystis aeruginosa*, with a diameter <20 μm, scarcely move, whereas colonies up to 1 600 μm in diameter can accomplish transport of 10 m three times a day.

In tropical regions, mass developments of cyanobacteria can occur at almost any time of the year since the air temperature and the annual insolation are fairly constant. However, despite this relative constancy, the phytoplankton community in tropical lakes can undergo alterations due to periodic meterological and hydrographic changes. Similarities exist between deep tropical lakes such as Lakes Victoria, Lanao, Valencia, Tanganyika, Malawi, Kariba and Titicaca (Cronberg, 1997; Lewis, 1978; Talling, 1987; Talling and Lemoalle, 1998). Meterological events such as stormy weather may break the thermocline, which in turn triggers a development of diatoms. In calmer conditions, a thermocline is formed. As long as the nutrient-stress is not severe, chlorophytes dominate and when the nutrients are almost depleted, the cyanobacteria start to dominate.

Surface blooms of cyanobacteria (e.g. species of *Trichodesmium*) are encounterd in the open oceans of southern latitudes. Also in the pelagic salt waters the buoyancy regulation has an important role for the organisms when remaining in the radiant energy-rich euphotic zone. But the buoyancy needs to be finely managed to acquire a desireble vertical postion. It should not be too close to the surface of the water (the intensity of solar radiation could be detrimental), and not too far below the surface (where the availble illumination is too weak to support photosynthetic growth.

Different theories on the dominance of cyanobacteria

Many studies have addressed the question of why cyanobacteria are so successful in such a wide

range of environmental conditions. At present, the massive literature on this subject includes at least eight different, single-factor theories that postulate differences between cyanobacteria and eukaryotic phytoplankton which may explain cyanobacterial success:

The **TN/TP hypothesis** explains cyanobacterial dominance by a low ratio of total nitrogen to total phosphorus in the water (Schindler, 1977; Smith, 1983, 1986).

The **low light hypothesis** suggests that cyanobacteria are favoured by low light intensities (Mur *et al.*, 1978; Zevenboom and Mur 1980).

The **buoyancy hypothesis** explains cyanobacterial success as a result of their ability to regulate their buoyancy and thus their position in the water column (reviewed by Reynolds *et al.*, 1987).

According to the **elevated water temperature hypothesis**, cyanobacterial blooms are promoted by high water temperature (reviewed by Robarts and Zohary, 1987).

The **zooplankton grazing hypothesis** suggests that resistance against zooplankton grazing is an explanation for cyanobacterial dominance (reviewed by Haney, 1987).

The **trace element hypothesis** postulates that cyanobacteria require more trace elements compared to eukaryotic microalgae (reviewed by Reuter & Petersen, 1987).

The **storage strategy hypothesis** postulates that vertically migrating cyanobacteria gain a competitive advantage by bringing an internal store of phosphorus from the sediment (Pettersson *et al.*, 1993).

The **inorganic nitrogen hypothesis** suggests that non-nitrogen-fixing cyanobacteria are favoured by ammonium-nitrogen, whereas nitrate-nitrogen favours the development of eukaryotic microalgae. Nitrogen-fixing species are favoured by nitrogen scarcity (Blomqvist *et al.*, 1994).

A comprehensive review of factors determining cyanobacterial success is given by Hyenstrand *et al.* (1998).

However, no single hypothesis would be correct by its own.

Nitrogen metabolism

Nitrogen is of particular importance to the aerotope-bearing cyanobacteria, since it is an essential component in the synthesis of the aerotopes. Consequently, a deficit of nitrogen may not only affect cell metabolism negatively,

Fig. 5. The nitrogen-fixing cyanobacteria *Cylindrospermopsis raciborskii* and *C. curvispora* with heterocytes (indicated). (Photo: *Cronberg*).

but also the buoyancy of the organism (Oliver and Ganf, 2000)

Cyanobacteria can make use of nitrogen as nitrate, nitrite or ammonium. Several species are also able to perform fixation of atmospheric nitrogen (N_2). The order of preference is ammonium > nitrate > N_2 (Tandeau de Marsac and Houmard, 1993). When ammonium is available, cyanobacteria do not use other nitrogen sources (Turpin, 1991, Ochoa de Alda *et al.*, 1996)

The nitrogen-fixation occurs inside a special transformed, vegetative cell, the heterocyte (Fig. 5) (Wolk *et al.*, 1994) which is thick-walled, often with a nodule of cyanophycin, a polymer of aspartate and arginine in the pore channel at one or both ends of the cell. The nitrogen-fixing enzyme complex, nitrogenase, is functioning inside the heterocyte. Nitrogenase is inactivated by oxygen, and the heterocytes provide protection by enhanced respiration, and by the barrier of the heterocyte envelope (Wolk *et al.*, 1994).

During periods when environmental sources of combined inorganic nitrogen have been depleted, the nitrogen-fixing cyanobacteria become most competitive. The common distributed

freshwater genera that can fix nitrogen are the heterocyte-bearing, filamentous members of the Nostocales, including *Anabaena, Anabaenopsis, Aphanizomenon, Cylindrospermopsis* and *Gloeotrichia. Nodularia spumigena* is another nitrogen-fixing species that thrives in brackish water. *Trichodesmium* and *Richelia* (endo-symbiont of the diatoms *Rhizosolenia* and *Hemiaulus*) are nitrogen-fixing marine genera that proliferate as surface blooms in estuarine, coastal and oceanic waters (Paerl, 2000).

After having reviewed the literature on laboratory and field experiments, Horne & Commins (1987) concluded that the concentrations of total inorganic nitrogen must be lower than 50-100 $\mu g\ l^{-1}$ to induce nitrogenase activity.

So far, no eukaryotic alga is known to fix molecular nitrogen, so that the diazotrophic cyanobacterial group has a major advantage during periods of shortage of combined inorganic nitrogen in the water.

The cyanobacteria possess another important advantage. Unlike eukaryotic algae, they have the capacity to store large amounts of nitrogen in the cytoplasmic inclusions phycocyanin and cyanophycin functioning as reserve products (Kromkamp, 1987).

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2. 1. Methods and Taxonomy

Methods

Sampling

Cyanobacteria are omnipresent, living in fresh, saline and brackish waters. They develop in soil, on cliffs, on and even in stones, benthic or planktic in oceans, lakes, ponds, reservoirs and wetlands in all climate zones. The methods used to collect cyanobacteria must be adjusted to their habitats. In this book we mainly have focused on the free floating, (planktic) cyanobacteria. The sampling of these organisms is done preferably with plankton nets of various mesh sizes (5-45 μm) depending on their dimensions. The small, pico-planktic cyanobacteria (0.5-3 μm in diameter) must be collected unconcentrated (quantitatively), as even the finest plankton net (5 μm mesh) is too coarse. The best sampling method is to fill a bottle directly from the locality to be studied. The quantitative sample can then be concentrated by sedimentation or centrifugation.

The benthic or periphytic cyanobacteria can be sampled with their substratum. Benthic algae growing in large mats can be sucked up with a pipette or gathered with forceps and directly put into glass tubes.

It is important to note when and where the samples are collected, and to describe the sampling location (biotope) as exactly and carefully as possible (coordinates taken by GPS). Photographic documentation is valuable.

The biological material should preferably be studied alive and before preservation. The natural colouration of the cyanobacteria as well as the mobility of the cells, trichomes or hormogonia are observed in live samples. However, the preservation of samples is necessary for later studies.

Fig. 1. Sampling phytoplankton in a small pool in Vietnam. (Photo: *Forssblad*).

Preservation

The mode of preservation depends on the use intended for the collected material. Net samples should be preserved with formalin to a maximum concentration of 2%, as conservation with too much formalin often destroys the colouration of the cells, breaks colonies and can cause artefacts of the mucilage.

The quantitative samples for biomass estimation must be preserved with Lugol´s solution (Guillard & Sieracki, 2005). This preservative makes the cells heavier and they thus settle at the bottom of plankton chambers, used for counting of organisms. For long-term preservation glass bottles are recommended, and samples preserved in Lugol should always be collected in clear glass bottles and stored in the dark. The Lugol solution is quickly decomposed in plastic bottles and exposure to light.

Plankton material aimed for electron microscopical (EM) studies should be preserved with glutaraldehyde, 2% final solution and kept refrigerated till studied. Note that Lugol and glutaraldehyde preservation can only be used for short-term storage. For longer storage (several years) preservation should be in formalin.

Identification

For identification of the cyanobacteria first and foremost light microscopy (10-1000 times magnification) is used. The object should first be studied with bright field illumination to note its colour (blue, blue-green, green yellow, violet etc). The next step should be to use phase- and/ or interference contrast to observe mucilage and finer details. Sometimes a closer examination with electron microscopy (EM) is necessary, but this will not be discussed here.

Estimation of cyanobacterial biomass

For the estimation of algal biomass an inverted microscope with phase-contrast is necessary. The quantitative sample has to be preserved with Lugol´s solution. The quantitative collected sample is allowed to settle in sedimentation chambers of different sizes/depths. The sedimentation time depends on the size of the chamber and the nature of the organism in the sample. Longer time is needed for tall chambers and small-celled cyanobacteria. The recommended settling time is 4-12 hours.

An exception must be made for picocyanobacteria, which are too light and never settle completely in sedimentation chambers. Instead picocyanobacteria can be filtered onto Nuclepore filters and counted with epifluorescence microscopy (Stockner *et al.*, 2000).

Colonial cyanobacteria are difficult to count as the thickness of the colonies makes it impossible to estimate the number of cells. Therefore samples rich in cyanobacteria can be sonicated for 15-60 seconds with a sonication bar to split the bundles of *Aphanizomenon* into filaments, *Anabaena* into small chains and *Microcystis* into cells. The exact time needed for sonication must be determined in each case, since too long a treatment may destroy the cells. Newly developed colonies often need longer sonication than older ones, due to the fact that young colonies have more resistant mucilage. Sonication also disrupts aerotopes, which facilitates the sedimentation (Cronberg, 1982).

The micrographs used in the guide are reproduced in varying magnifications. This was decided due to expedience. No scale bars have been added to the pictures. But the dimensions of the objects are stated in the accompanying figure text describing relevant organism.

Size measurements

In Table 1-4 the diacritical features and dimensions of some common cyanobacterial genera and species are presented. The width, length, diameter are given for different cell types. The dimensions are written in different ways and consequently require an explanation. When for instance, dimensions of a cell width is written (2.5) 4-7(10), then the figures within brackets are extremely low (2.5 μm) or extremely high values (10 μm). The normal size range is 4-7.

The cell length and diameter of for example filamentous algae is somtimes written in another way. If the cell length of an algal species is 5-8-11 μm. Then the length can vary between 5-11, but

the figure in the middle (8) is the mean cell length value.

How to use the guide

In this guide 65 cyanobacterial species are photographed and described. They are organized into the different orders and are then as nearly as possible arranged alphabetically. However, similar species within a genus have been placed side by side to facilitate comparisons. No keys are given, as the guide primarily comprises common, and potentially toxigenic species, and not all species. However, for every genus presented, the total number of species described within the genus is given.

We have tried to picture common, mostly planktic cyanobacteria, which can produce cyanotoxins. In addition, other species with similar morphology have been included to point out distinguishing characters. Tables 1-3 contain schematic presentations of species belonging to the bloom-forming genera *Microcystis*, *Anabaena* and *Aphanizomenon*. A compilation is given of cell and colony dimensions, habitats and ecology, as well as cited literature for further study.

Mostly freshwater species are included in the guide, but some brackish and marine species are also mentioned. The material for the photographic documentation has been collected mainly from temperate regions, but additional material from the tropics has been included.

The genus *Microcystis* contains several potentially toxigenic species, which may sometimes be difficult to distinguish from each other. This genus is cosmopolitan, and forms water blooms during the summer in temperate regions, but can be in continual bloom in the tropics. As it may consist of both toxic and non-toxic organisms it is important to make correct identifications (Table 1 and Chapter 3), when assessing health risks.

The genera *Anabaena* and *Aphanizomenon* include many species. The descriptions of these are scattered in the literature and are often difficult to obtain. Thus Tables 2-3 may be helpful tools for the identification.

Sometimes, when special cells, heterocytes and akinetes, are missing on the trichomes even identification to genus level may present difficulties. This is the case with, e.g., *Aphanizomenon issatschenkoi, A. tropicale, Cylindrospermopsis raciborskii* and *Raphidiopsis mediterranea*. These species are common in warm water localities, and if heterocytes and/or akinetes are missing, there will be great difficulties in distinguishing them from each other. In Table 4 the distinguishing features for the species are summarized.

Taxonomy

The systematics and nomenclature of cyanobacteria are in a state of dilemma as two possible methodologies of biological classification are appropriate (respectively the International Code of Botanical Nomenclature and the International Code of Nomenclature of Bacteria). There has been a marked change in outlook on taxonomy of microorganisms over the past decades. The development and application of nucleic acid based techniques have to a high degree changed current methods of the characterization, identification and classification of the organisms. In modern systematics of the cyanobacteria significant genotypic, phenotypic and ecological traits are incorporated. A polyphasic, synthetic approach is now entering its scientific consensus. However, for monitoring and every day practical purposes a phenotypic based identification and classification of cyanobacteria is both appropiate and relevant (Compère, 2005; Oren & Tindall, 2005; Castenholz & Norris, 2005; Johansen & Casamatta, 2005; Hoffmann *et al.*, 2005).

The taxonomic system followed in the manual is founded on Anagnostidis & Komárek (1985,1988, 1990) and Komárek & Anagnostidis (1986, 1989, 1998, 2005).

The cyanobacteria are here divided into two groups, non-filamentous and filamentous organisms, which further are split into 4 orders.

NON-FILAMENTOUS GENERA

Order Chroococcales

Unicellular or colony-forming prokaryotes, which multiply mainly by binary fission in two, three or more planes. They do not form true filamentous, but sometimes pseudofilametous structures.

Species belonging to the following genera are pictured in the guide. Here follow brief descriptions of these genera, which are extracted from Komárek & Anagnostidis (1998, 2005).

Aphanocapsa

Colonies are microscopic, more or less spherical or flat, with cells sparsely to densely distributed in the colourless mucilage.The cells are spherical without aerotopes.

Aphanothece

Planktic multicellular colonies, micro- to macroscopic with cells irregular distributed in mostly colourless mucilage. The cells are elongated, rod-like mostly without aerotopes.

Chroococcus

Microscopic, more or less spherical, gelantinous colonies with cells often in packet-like, 2-8 celled groups. Mucilaginous envelopes mostly colourless or yellow often with well-marked, sometimes laminated margin. The cells are spherical to semispherical without aerotopes.

Merismopedia

Colonies are planktic or benthic with cells regularly arranged in one plane in the flat, square or quadrangular colonies. Cell division takes place in two planes. The cells are spherical or widely elliptical, arranged more or less densely in perpendicular rows. The colour of the cells is pale to bright blue-green, green or reddish. In a few species the cells might contain refractive bodies and a few aerotopes.

Radiocystis

Planktic colonies, more or less spherical with cells radiating in uniseriate rows from the centre of the colonies. The cells are embedded in colourless mucilage. Cells often in pairs with pale, blue-green colour and sometimes aerotopes.

Microcystis

This genus contains planktic, multicellular, micro- to macroscopic, mucilaginous colonies. The colonies are irregular, cloud-like with hollow spaces and sometimes with a well developed outer margin. The cells are spherical with many aerotopes. Cell division takes place in three planes. Very common, often forming water blooms.

Snowella

Colonies are planktic, spherical to irregular oval with homogenous mucilaginous envelopes. Within the colonies there is a system of mucilaginous thin, thread-like, branched stalks radiating from the centre. The cells are spherical to oval and attached to the outer ends of the stalks. They are grey to pale blue-green in colour and some species have a few distinct aerotopes.

Woronichinia

Colonies are planktic, spherical to oval in shape, often composed of subcolonies, with mucilaginous envelopes. In the colonies, there is a well developed stalk system radiating from the centre. The cells are attached to the unbranched stalks. The cells are usually elongate, oval or obovoid, in older colonies densely packed at the periphery, The cells are blue-green, green or purple in colour with many aerotopes.

For detailed information about the genera belonging to Chroococcales, see Komárek and Anagnostidis (1998).

FILAMENTOUS GENERA

Order Oscillatoriales

With unbranched filaments. Cells in the trichomes multiplying by binary fission in one plane only. Vegetative cells are not differentiated into heterocytes (nitrogen-fixing cells) and akinetes (resting spores).

Romeria

Planktic organisms with solitary, or few trichomes in mucilaginous clusters, short, fine, irregular with

1-8 (-18-32) cells. The trichomes are straight or slightly curved, The cells are elogated, cylindrical, always longer than wide, light green to blue-green without aerotopes.

Limnothrix

Planktic organisms with solitary, or in small clusters, isopolar, straight or slightly bent with many elongated, cylindrical cells of the same morpholgy, not constricted or slightly constricted at cross walls, 1-6 μm wide. The cells contain apical and/or central, large aerotopes, which are sometimes missing. The colour is pale blue-green, blue-grey, yellowish, red or pink,

Planktolyngbya

Planktic organisms with trichomes, solitary, straight, curved or spirally coiled with firm, thin, colourless sheaths. The cells are isopolar, uniseriate, immotile, not constricted or slightly constricted at crosswalls. Cells cylindrical, 1-3(5) μm wide, usually longer than wide, rarely isodiametric, mostly lacking aerotopes.

Planktothrix

Mostly planktic organisms with trichomes straight or slightly waved, not constricted at crosswalls, mucilaginous envelopes or sheath mostly missing, The trichomes might have attenuted ends sometimes with calyptra. Trichome width (2)3-12(15) μm. Cell length little shorter than wide or isodiametric.

Planktothricoides

Planktic organisms with trichomes solitary, straight, attenuated towards their ends, sometimes slightly bent near the apex, isopolar, ± constricted at crosswalls. The trichomes are (3.5) 6-11 μm wide, sometimes a colourless fine sheath is present. The cell width is larger than the cell length. It contains several small aerotopes scattered near the cell the periphery. Multiplication by hormogonia.

Lyngbya

Filaments are solitary, straight or slightly curved, forming mats on substrate, and mainly wider than 6 μm. The cylindrical, motile trichome is enclosed in varying thick, colourless sheath, or sometimes yellow-brown or reddish. The cells are discoid, short, always shorter than wide, rarely isodiametric, mostly without aerotopes. Apical ends are usually with thickened outer cell wall sometimes with calyptra.

Oscillatoria

Planktic, benthic, epiphytic or subaerophytic organisms. Trichomes cylindrical, isopolar, straight or slightly waved, motile with gliding oscillations. Cells wider than 6.8 μm (up to 70 μm), not constricted or slightly constricted at crosswalls. The cells are short, discoid, usually 3-11 times shorter than wide. Cytoplasma homogenous or sometimes containing prominent granules. Filaments with or without sheath, with mostly 1-2 (rarely more) trichomes. If sheath is present, it is wide gelatinous or firm, cylindrical, sometimes lamellated, and rarely coloured. False branching may occur in sheathed filaments. Cell division transversal. Reproduction by disintegration into hormogonia (necridia formation)

Tychonema

Benthic, tychoplanktic or planktic organisms solitary or in delicat mats. Trichomes cylindrical, pale greyish, purplish, reddish or dirty olive green, up to 5 mm long, 2-16 μm wide, mostly without sheath. Cells uniform, ± isodiametric, without aerotopes. Cell content pale with keritomized chromatoplasma. Apical cells are rounded with thickened cell wall or calyptra.

Pseudanabaena

Organisms planktic, solitary, or in slim mats. Trichomes straight, arcuated, rarely waved, cylindrical, narrow, mostly with few cells and constrictions at crosswalls. Trichomes are without sheath, but often in wide mucilaginous envelopes. Cells mostly cylindrical with rounded or barrel-shaped ends, longer than wide, rarely isodiametric, with or without polar aerotopes.

Trichodesmium

Planktic organisms mostly forming assemblages

with parallell or radially arranged fascicles joined by mucilage. Trichomes are without sheath, more or less straight or curved, 6-22 μm wide with cylindrical or tapering ends. Cells isodiametric with fine homogenous content and aerotopes, blue-green or reddish in colour. Apical cells straight, rounded or slightly capitate.

Arthrospira

Planktic, benthic or periphytic organisms. Trichomes solitary, regularly or rarely irregularly spiralled, 2.5-16 μm wide, usually relatively large diameter of spirals and more or less attenuated at the ends, mostly not constricted at crosswalls. Cells isodiametric or shorter than wide, with aerotopes, sheath absent or rarely present, blue-green, olive-green or reddish brown in colour.

Arthrospira species (*A. maxima* and *A. fusiformis*) often forms waterblooms, and are commonly used in mass cultures for producing biotechnological products, e.g. food complements, *Spirulina* tablets. These products are incorrectly named *Spirulina* (*S. platensis*). However, the genus *Spirulina* is different from the genus *Arthrospira*. The genus *Spirulina* is characterized by having more narrow trichomes (diameter 0.3-7.5 μm), more regularly coiled trichomes and greater mobility than *Arthrospira*. In addition aerotopes are missing in *Spirulina* (Komárek & Lund, 1990).

Order Nostocales:

Cyanobacteria with unbranched filaments (trichomes), cells multiplying by binary fission in one plane only. Vegetative cells can differentiate into heterocytes and akinetes. No true branching exists.

Raphidiopsis

Planktic organisms. Trichomes solitary, straight or slightly curved and without sheath. Cells are cylindrical, unconstricted or slightly constricted at cross walls, with aerotopes, intercalary, cylindrical akinetes produced but no heterocytes. Apical cells are pointed or slightly rounded.

Anabaena

Planktic, periphytic or benthic organisms. Trichomes are straight, curved or coiled, in some species with mucilaginous colourless envelopes, mat forming. Trichomes are mostly constricted at cross walls, uniseriate and isopolar. Cells are spherical, oval, cylindrical or barrel-shaped, with intercalary heterocytes and akinetes. The position of heterocytes and akinetes on the trichomes is an important taxonomical criteria.

Anabaenopsis

Planktic organisms. Trichomes bent, spirally coiled, rarely straight, no sheath, but can have mucilaginous envelopes. Heterocytes present in pairs. The trichomes break between heterocytes, thus apical heterocytes appear. Cells are mostly spherical, barrel-shaped or cylindrical, blue-green to brownish in colour, with aerotopes. Akinetes are spherical or ellipsoidal, mostly distant from heterocytes.

Aphanizomenon

Planktic organisms. Trichomes solitary or gathered in small or large fascicles with the trichomes arranged in parallel layers and can from macroscopic flakes in the water. Trichomes are isopolar, subsymmetric, straight with or without constrictions at the cross walls. Cells are cylindrical of varying length, pale blue-green to green with aerotopes. The end cells are often longer than the central cells. Heterocytes spherical, oval, cylindrical with two pores, always intercalary positioned and solitary. Akinetes cylindrical, adjacent or distant from the heterocyte.

Cylindrospermopsis

Planktic organisms. Trichomes are straight, bent or spirally coiled, isopolar or secondary heteropolar, subsymmetrical with or without constrictions at cross walls. Cells are cylindrical or barrel-shaped pale blue-green or yellowish, with aerotopes. End cells are often conical or bluntly to sharply pointed. The heterocytes are terminal and develop after unequal division of the end cells. Akinetes are ellipsoidal or cylindrical, formed intercalary, distant or near the end cells.

Gloeotrichia

Planktic or periphytic organisms, developing mucilaginous, spherical to hemisperical, microscopic or macroscopic colonies with many trichomes. Trichomes are heteropolar with basal hererocytes and apical hairs, radially arranged in the colony and inside the mucilaginous envelopes. Trichomes are more or less constricted at cross walls, tapering towards the apical end. Heterocytes are spherical to hemispherical or ellipsoid, positioned basaly or intercalarly. Akinetes are cylindrical, elongated with rounded ends, adjacent to the heterocytes and inside the sheath.

Nodularia

Planktic or benthic organisms. Trichomes are solitary or in groups. They are isopolar, unbranched, straight, curved or spirally coiled with fine two-layered sheath open at both ends. Trichomes are uniseriate, cylindrical with slightly attenuated ends, constricted at the cross walls, with metameric heterocytes. Cells are short barrel-shaped, with length never exceeding the width, yellowish, pale olive-green, bluegreen or pinkish in clolour. Heterocytes have the same shape as the vegetative cells. Akinetes are short barrel-shaped or spherical.

Order Stigonematales

Filamentous forms. Cell division in one or more planes giving rise to genuine branching and multiseriate filaments. Vegetative cells possess the ability to make heterocytes and sometimes also akinetes.

In this guide toxigenic species belonging to Stigonematales are mentioned only in Chapter 2; Table 5.

2.2. Photo-Guide

2.2.1 Chroococcaies

Aphanocapsa delicatissima (W. et G.S. West) Kom.-Legn. & Cronb. 1994. Fig. 1.
Colonies are planktic, spherical or irregular, in diffuse mucilage with evenly-spread, spherical, minute cells. The cells are 0.5-1 μm in diameter, pale blue-green or grayish, without aerotopes.

A. delicatissima has probably a world-wide distribution in eutrophic waterbodies. The genus *Aphanocapsa* comprises about 30 well-described species.

Aphanothece clathrata W. et G.S. West 1906.
Fig 2.
Planktic, large clathrate colonies, initially spherical, later elongate and flat. Cells are fine, rod-like or very slightly spindle-shaped, pale blue-green or greyish, without aerotopes, regularily distributed in the mucilaginous sheath, (0.8)-1.5-3.5(4.5) μm long and 0.4-1(2) μm wide. Fragments of colonies break off easily and develop into new colonies.

Common in mesotrophic to eutrophic shallow lakes in the temperate region, but has also been found in tropical lakes.

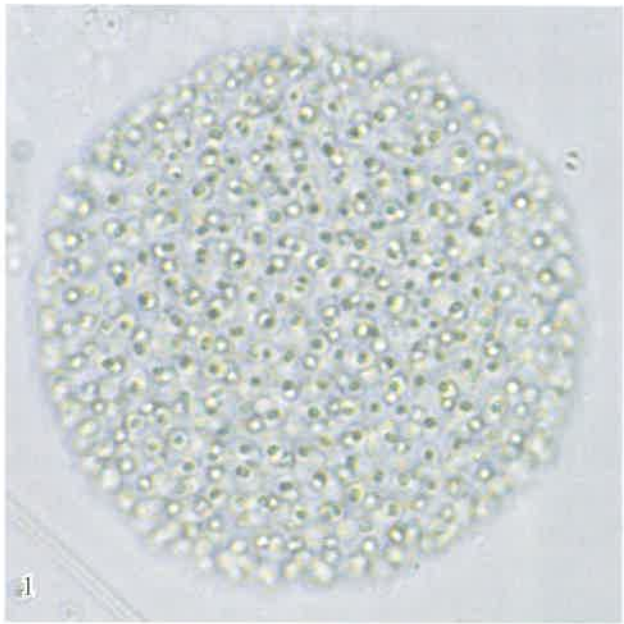
Aphanothece minutissima (W. West) Kom.-Legn. & Cronb. 1994. Fig. 3
Planktic, cloud-like, often clathrate colonies. Cells are oval without aerotopes, embedded in mucilage, pale blue-green to light green. The cells are 1-(2) μm long and 0.8 μm wide, evenly but not so densely packed in the colony as *A. bachmannii*.

Common in eutrophic waterbodies in the temperate zone. Probably overlooked or identified as *A. clathrata*, which, however, has longer cells.

The genus *Aphanothece* comprises about 25 well-described species from all parts of the world, but often in specific habitats.

Chroococcus limneticus Lemm. 1898.
Fig. 4.
Planktic colonies. Spherical or hemispherical cells in small groups, pale blue-green, without aerotopes, 6-9-12 μm in diameter embedded in colourless, homogeneous mucilage.

Distribution world-wide. Eutrophic. About 70 species described within the genus.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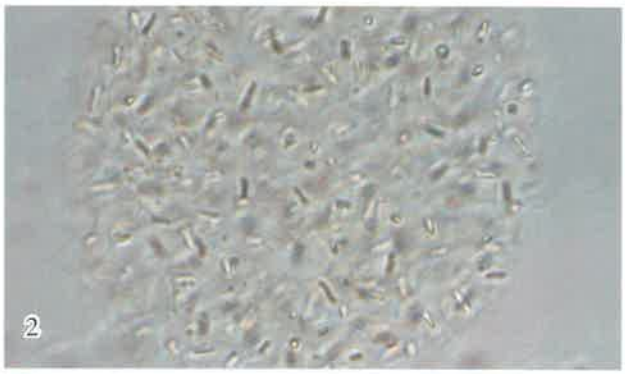
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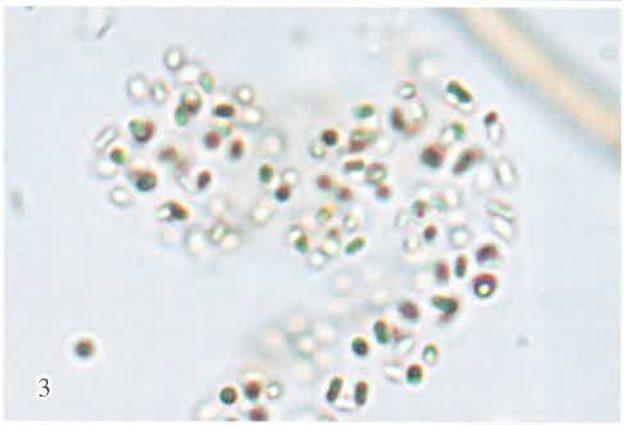
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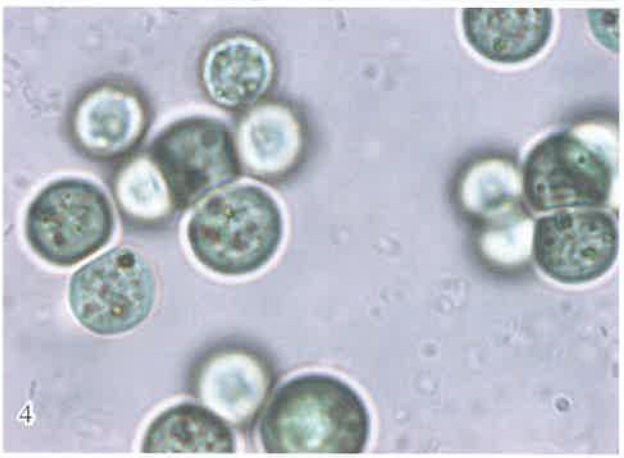
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Cyanodictyon imperfectum Cronb. & Weib. 1981.
Figs. 5-6.
Cells are spherical, minute, 0.5-0.8-1 μm in diameter, pale blue-green in colour, in pairs and assembled into loose filaments with 6-8 cells inside the sheath (Fig. 5). Between the cells ring-like Iron-precipitations are deposited and look like two black dots (Fig. 6). Cell division in only one plane.

C. imperfectum belongs to the picoplankton and has cosmopolitan distribution. It is common in eutrophic to mesotrophic lakes, also recorded in profusion in African lakes. Although it is very minute, it is easy to recognize because of the characteristic iron-rings and net-like colonies. Toxic properties are discussed.

The genus *Cyanodictyon* comprises 8 species.

Merismopedia marssonii Lemm. 1900.
Fig. 7.
Colonies are plate-like, quadrate, with up to 200 cells arranged in perpendicular rows. The cells are spherical to oval, hemispherical after cell division, which takes place in two planes. The cell dimensions are 1.6-2 x 0.8-1.5 μm. Mucilage is fine, colourless, diffuse. The cells have up to three aerotopes in the central part of the cell.

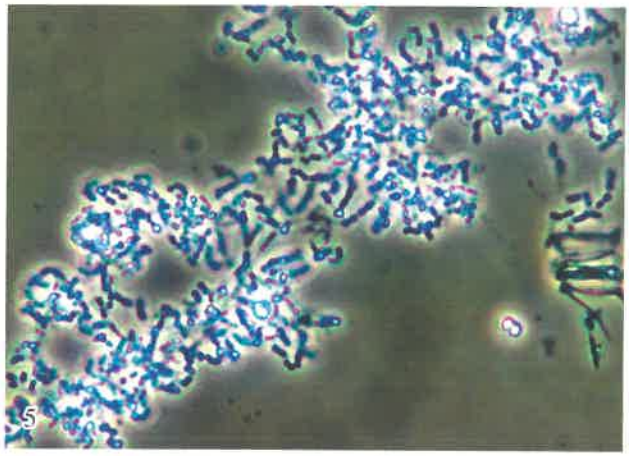
It is common in freshwater, metaphytic and benthic, often in the littoral zone of stagnant water. Common in Europe.

Merismopedia is characterized by the flat colonies with cells arranged in regular rows. It comprises at least 30-35 species.

Radiocystis geminata Skuja 1948.
Fig. 8.
Colonies planktic, spherical to elongate, composed of sub-colonies, 20-70 x 20-100 μm in size. Cells are spherical to slightly oval, pale blue-green, with aerotopes, often forming pairs, 1.7- 2.6 μm long and 2.5-2.8 μm wide. Cells are arranged in more or less regular rows, radiating from the centre of the colony.

A species developing in mesotrophic to slightly eutrophic lakes in the temperate zone. Similar populations exist in the tropical region, and may be the same species.

The genus *Radiocystis* comprises 5 species, two of which are tropical.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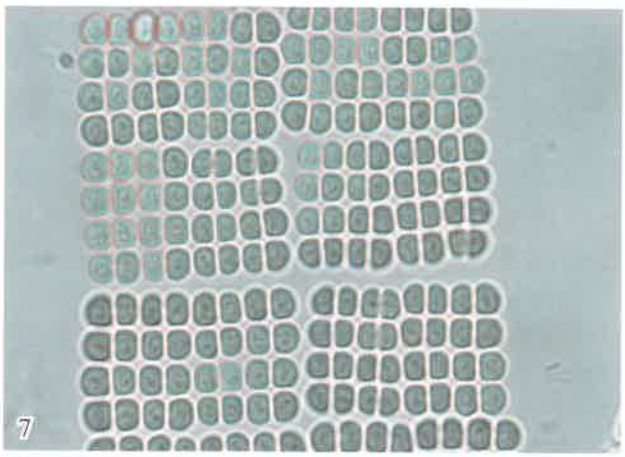
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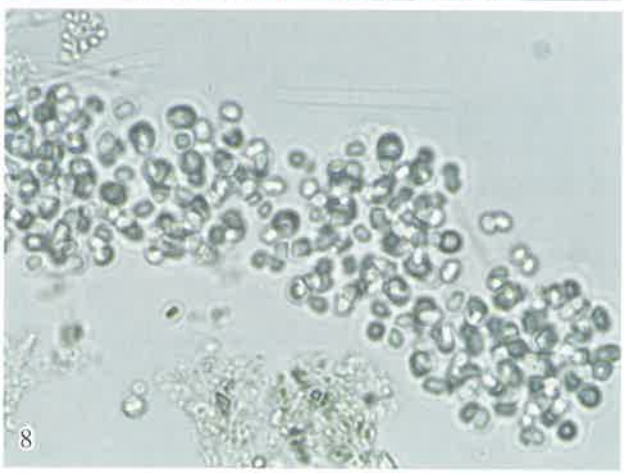
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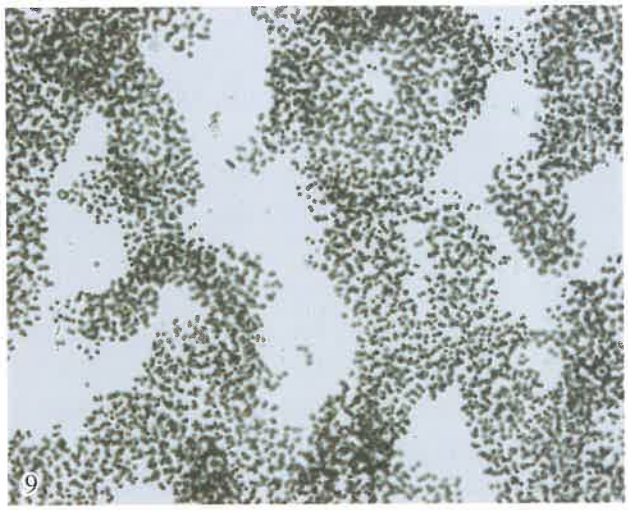
Microcystis aeruginosa (Kütz.) Kütz. 1846.
Fig. 9.
M. aeruginosa is a colony-forming cyanophyte with spherical cells. The colony is clathrate with the cells sparsely distributed in the colony. The cell has a bluish-green colour with many aerotopes. Cell diameter is 4-6(9.4) μm. Colourless mucilage sometimes forms a distinct, narrow margin around the cells. Colonies appear in many modifications depending on the seasons.

Occurrence: In fresh and brackish water, planktic in eutrophic waterbodies, sometimes making heavy waterblooms. Toxigenic species, producing cyanotoxin of the microcystin group.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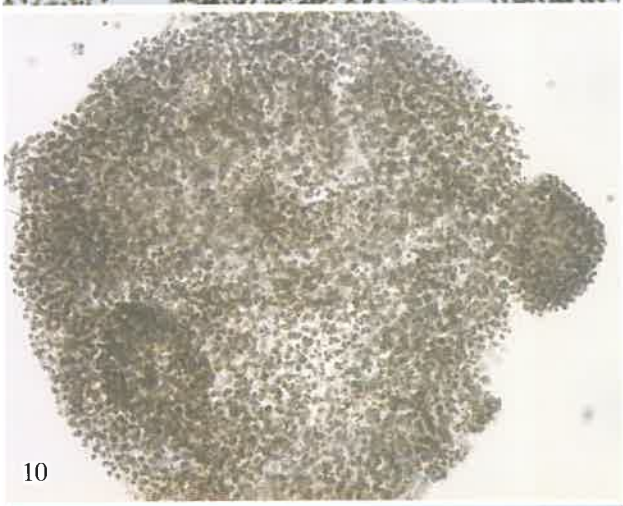
Microcystis flos-aquae (Wittr.) Kirchner 1898.
Fig. 10.
Colonies free-floating, spherical to irregular with only a thin layer of mucilage. Cells densely packed in the colony. The edge of the colony is smooth with thin mucilage. Cells are spherical, with aerotopes, 3-4.2-4.8 μm in diameter.

It appears in mesotrophic to eutrophic waterbodies, often forming waterblooms together with other cyanoprokaryotes. Distributed mainly in the temperate zone. Toxic properties are discussed.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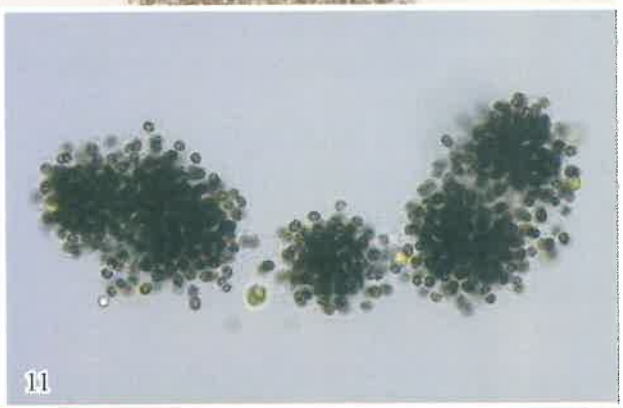
Microcystis botrys Teiling 1942.
Figs 11-12.
M. botrys forms more or less spherical colonies with irregularly, densely aggregated cells in mucilaginous sub-colonies (fig. 11). The mucilage surrounding the colonies is wide, colourless sometimes structured with gelatinous tubes radiating from the densely packed sub-colonies (Fig. 12). The cells are spherical, and 5-6 μm in diameter, containing many aerotopes.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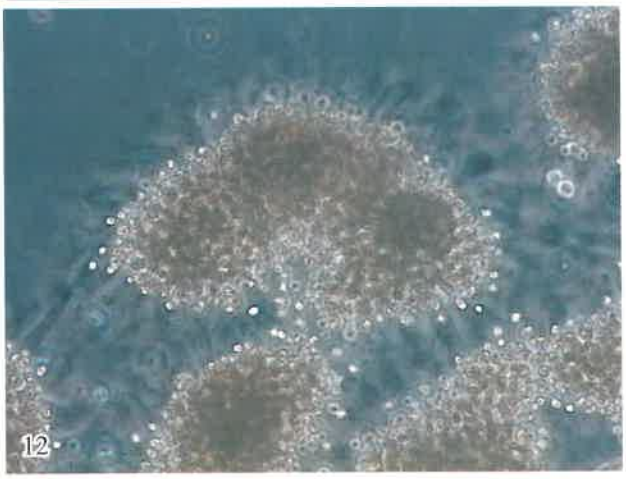
It appears planktic in eutrophic lakes and slightly brackish water. *M. botrys* was described from Sweden but has probably a cosmopolitan distribution. It is common in waterblooms together with different *Microcystis* species and has now been found in several African lakes. A toxigenic species with potent production of microcystins.

The genus *Microcystis* comprises about 25 different species (Table 1). Several *Microcystis* species exist, that have their main distribution in tropical and warm geographical areas.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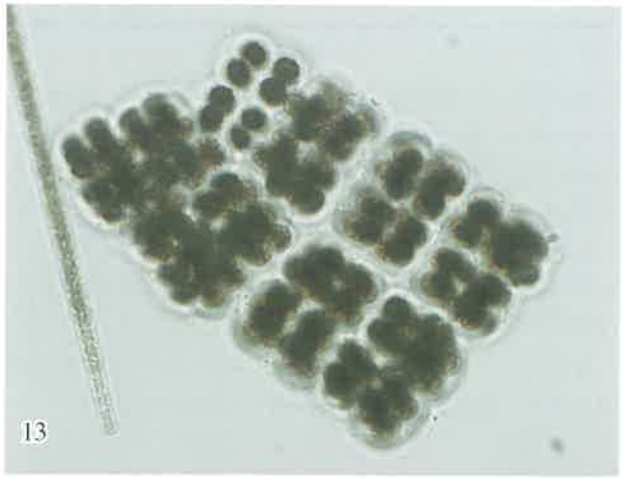
Microcystis viridis (A. Braun in Rabenhorst) Lemm. 1903. Fig. 13

M. viridis forms microscopic, free-floating, packet-like colonies, round to cubic, with colourless mucilage. The slime edge is undulating and more or less follows the cells in the sub-colonies. Cells are spherical mainly 3.5-7(8) μm wide, containing many aerotopes.

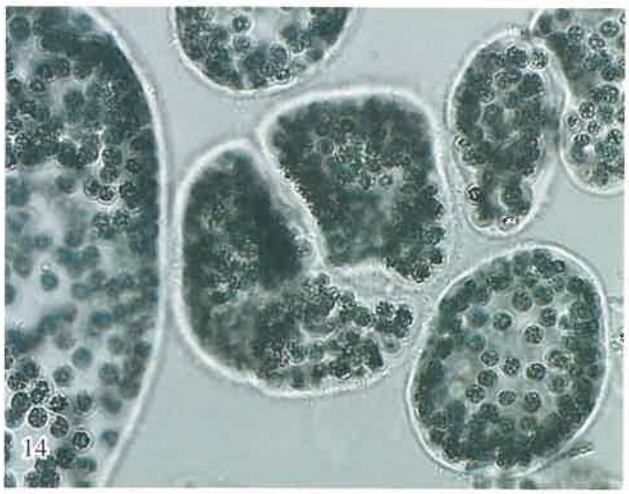
M. viridis is common in eutrophic inland waters, sometimes appears in waterblooms, world-wide distribution. A toxigenic species with production of microcystins.



Microcystis wesenbergii (Kom.) Kom. in Kondr. 1968b. Fig. 14

M. wesenbergii forms spherical to elongate, often lobate, clathrate colonies, sometimes composed of sub-colonies, distinct, refractive mucilage edge. The colonies can be up to 6 mm long. Cells more or less evenly spread in the colony, with many aerotopes, spherical, 4-7(8.5) μm in diameter.

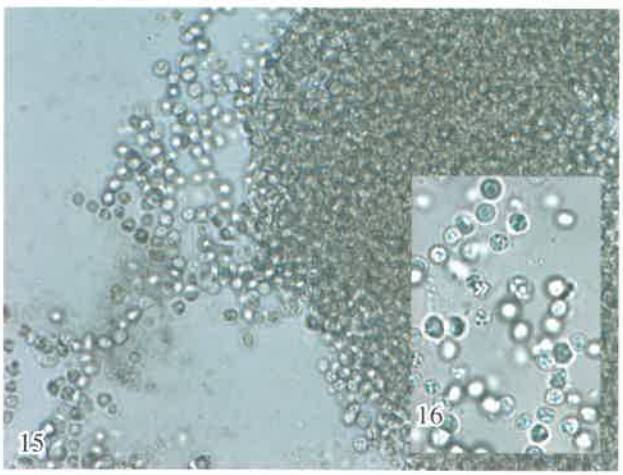
It is common in waterblooms with other *Microcystis* species, eutrophic with cosmopolitan distribution.



Microcystis panniformis Komárek *et al.* 2002. Figs. 15-16

Microscopic or macroscopic, planktic colonies. which are initially irregular, tightly aggregated in three-dimentional clusters, later flattened. The cells are densely and evenly distributed, mainly near the surface of the colony, yellowish, blue-green, olive-green to dark brown in colour. The colonies sometimes hollow. The margins of the colonies are irregular, not distinctly delimited or overlapping cells. Spherical cells (2.5)3-4.6(4.8) μm in diameter, containing aerotopes.

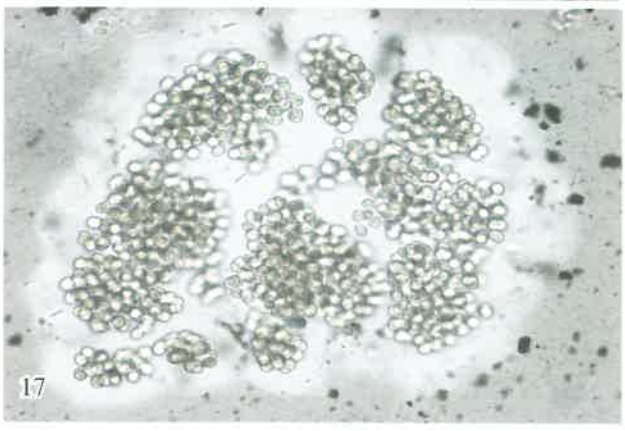
It has pantropical to tropical distribution. A toxigenic species with production of microcystins.



Microcystis novacekii (Kom.) Compère 1974. Fig. 17

Colonies lenticular, nearly spherical, composed of sub-colonies surrounded by thick mucilage with wavy edge. Cells spherical (2.4)3-5.5(6) μm in diameter.

In eutrophic freshwaters, in waterblooms, distributed in the tropical zone.



Snowella litoralis (Häyrén) Kom. & Hind. 1988.
Fig. 18
Colonies free-floating, small, spherical, mainly 20-40 μm in diameter. Cells spherical, after division hemispherical, remaining in pairs for some time, without aerotopes, pale blue-green in colour. Cells situated in the periphery of the colony at the end of a thin, thread-like, usually visible stalk. Cell dimensions 1.5-2.3-4 μm in diameter.

Planktic in mesotrophic lakes, in ponds also in brackish water. Cosmopolitan distribution.

Snowella lacustris (Chodat) Kom. & Hin. 1988.
Fig. 19
Colonies microscopic, more or less spherical, about 80 μm in diameter, sometimes compound. Cells obovoid, 2-4 x 1.5-3.5 μm, pale blue-green, without aerotopes, mostly distant from each other, connected with mucilaginous, branched stalks radiating from the centre of the colony.

Common in mesotrophic to eutrophic stagnant waters, lakes and ponds, also in the Baltic Sea, never in masses, probably world-wide distribution. A species shown to have hepatotoxic properties in mouse bioassay.

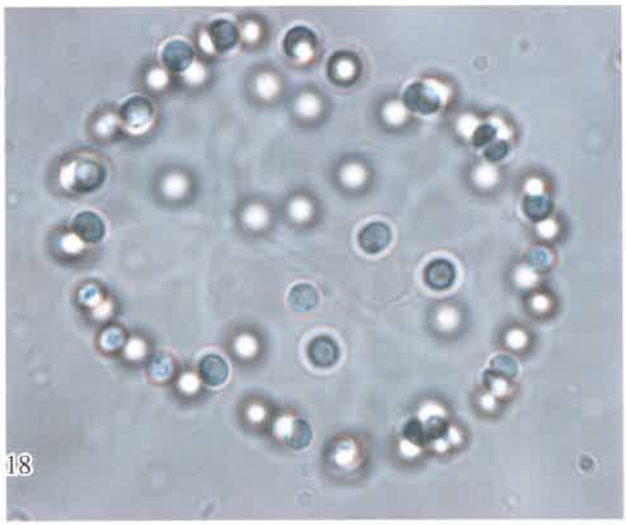
Snowella comprises about 7 species.

Woronichinia elorantae Kom. & Kom.-Legn. 1992.
Fig. 20
Planktic, solitary colonies usually oval, sometimes composed of sub-colonies, compact with radially, densely packed cells in a peripheral layer, 20-40 μm in size. The cells are located on thick, colourless mucilaginous stalks radiating from the centre of the colony, oval or widely obovate in pale green to blue-green in colour, without aerotopes, 2.5-3 x 1.6-2 μm in size.

Woronichinia karelica Kom. & Kom.-Legn. 1992.
Figs. 21-22
Resembles to *W. elorantae*, but with elongated obovate cells, 3-6 x 1.5-2 μm.

Both species common in plankton and meta-phyton of small mesotrophic to slightly eutrophic waterbodies, among waterplants. It has a temperate, northern distribution.

The genus comprises about 15 species.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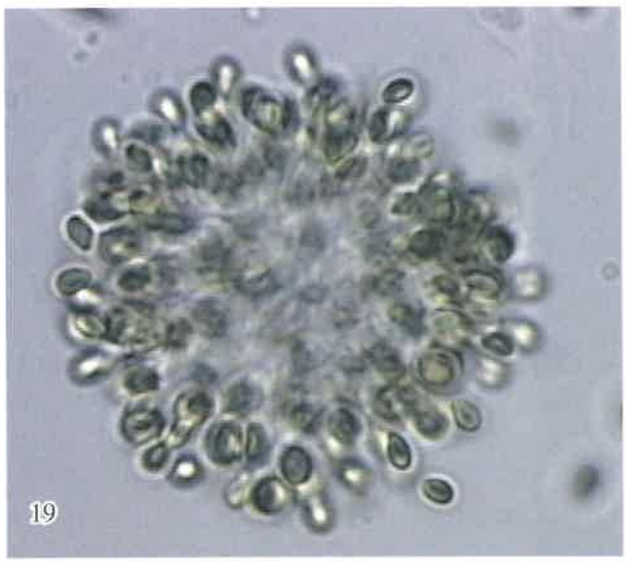
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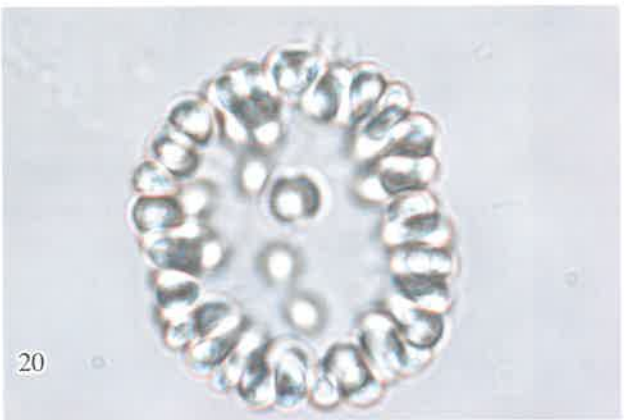
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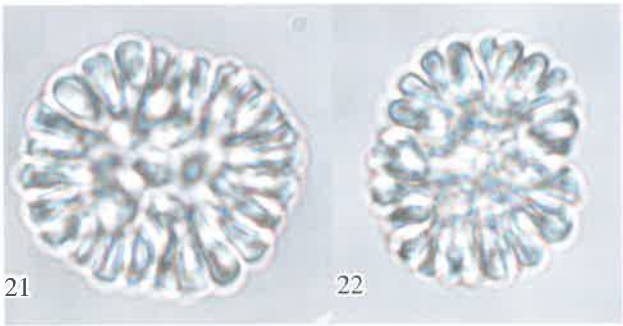
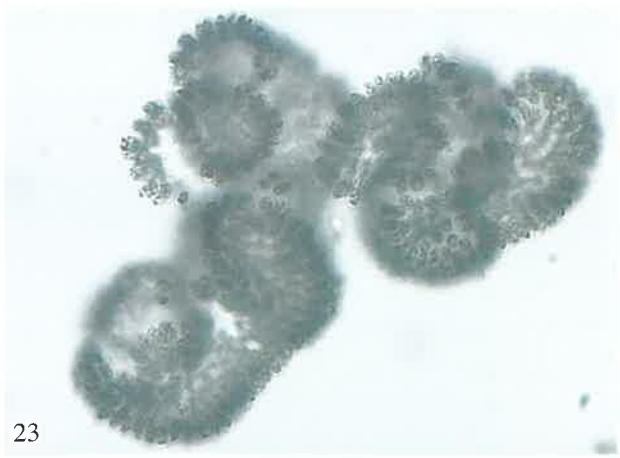

21 22

Woronichinia naegeliana (Unger) Elenk. 1933.
Fig. 23-24
The colonies are microscopic, spherical to kidney-shaped, sometimes lobate, colony size up to 180 μm. They are composed of radially arranged cells attached to unbranched mucilaginous stalks radiated from the centre of the colony. The cells are blue-green, ellipsoid to ovoid, with many aerotopes, dimensions 5-7 x 2.5-3.5(5) μm.

W. naegeliana is one of the most common planktic cyanophytes in the temperate zone. Rarely found in the tropics. A species shown to have neurotoxic and hepatotoxic properties in mouse bioassays.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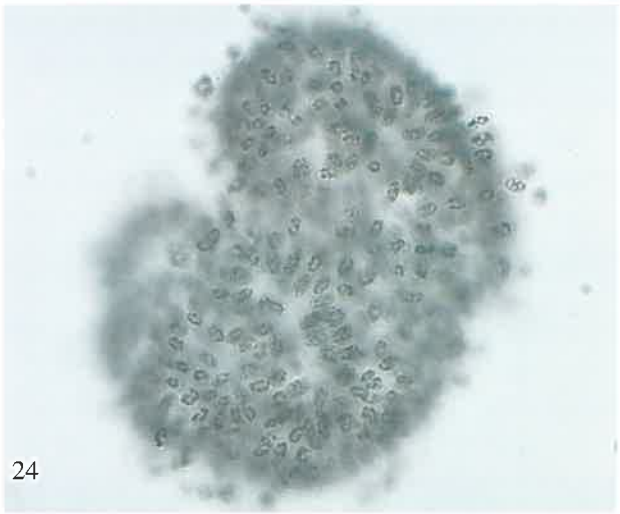
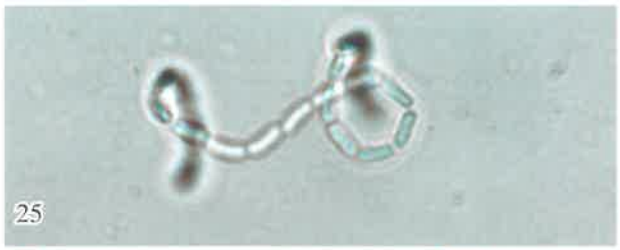

24

2.2.2 Oscillatoriales

Romeria simplex (Hind.) Hind. 1988. Fig. 25.
Trichomes free-living, solitatary or a few in irregular, mucilaginous assemblages. Trichomes more or less coiled, few-celled, 4-20 cells, easily fragmenting into small trichomes. Cells long, rod-shaped, slightly sigmoid or bent, in fine colourless mucilage, constricted at cross walls, 4-12 x 1.2-1.5 μm, pale blue-green.

Planktic in ponds and lakes, cosmopolitan. To date 19 species have been described (Komárek, 2001), but in most textbooks only 3-5 species are mentioned. *Romeria* has mostly been classified belonging to the Oscillatoriales. The arrangement of thylacoids are similar to the *Aphanothece-, Synechococcus*-types, and some forms of Pseudanabaenaceae. The systematic position must be revised.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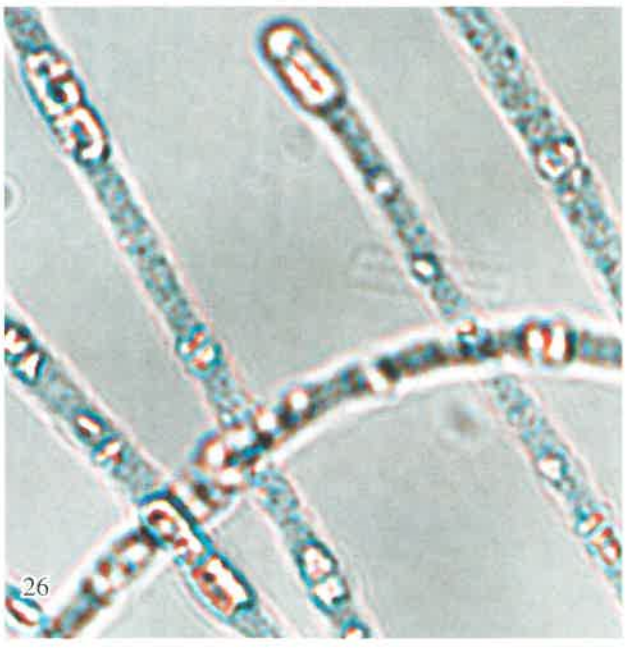
Limnothrix redekei (Van Goor) Meffert 1987.
Fig 26.
Planktic. Trichomes straight or slightly bent, not constricted at cross walls. The cells are long cylindrical, containing large aerotopes near the cross walls. The trichomes are grey-blue in colour, with a cell width of 1.2-2.5(3.5) μm and cell length of 6-14 μm.

It is common in meso- to eutrophic ponds and small lakes. *L. redekei* was recently found to be toxigenic producing microcystins (Gkelis *et al.*, 2005).

The genus *Limnothrix* comprises about 20 species, which are recorded mostly from the benthos. Only a few are planktic, mainly distributed in the temperate and northern regions.


26

Planktolyngbya circumcreta (G. S. West) Anagn. & Kom. 1988. Fig. 27.
Solitary, free-floating filaments, in narrow spirals, screw-like or irregularly twisted. The cells are cylindrical, mostly shorter than wide, 1-2 μm long and 1.8-2 μm wide, pale blue-green. Trichome enveloped in thin colourless sheath.

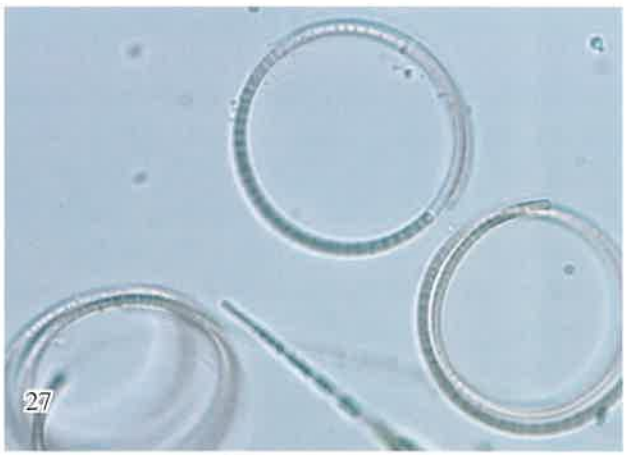
Planktolyngbya limnetica (Lemm.) Kom.- Legn. & Cronb. 1992. Fig. 28
Solitary, free-floating filaments, straight or slightly curved, cells cylindrical, up to 3-(5) times longer than wide, pale blue-grey 1.8-5 x 1-1.8 μm, without aerotopes. The trichome is enveloped in a fine, thin and colourless sheath.

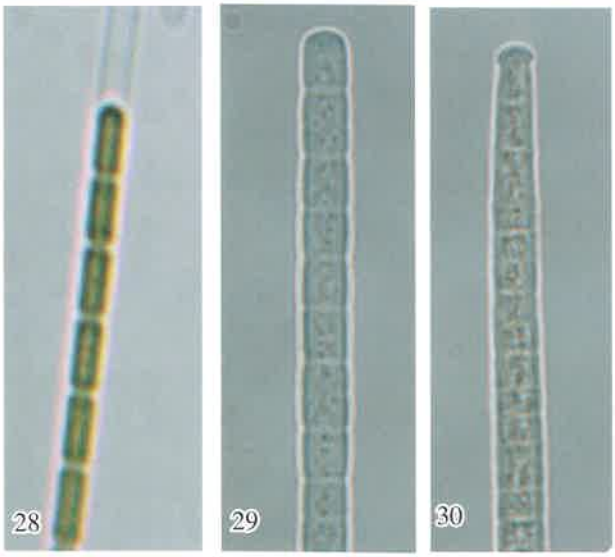
Both species are common in meso- to eutrophic waterbodies, cosmopolitan. *P. circumcreta* occurs frequently in the tropics. The genus *Planktolyngbya* comprises about 8 species.

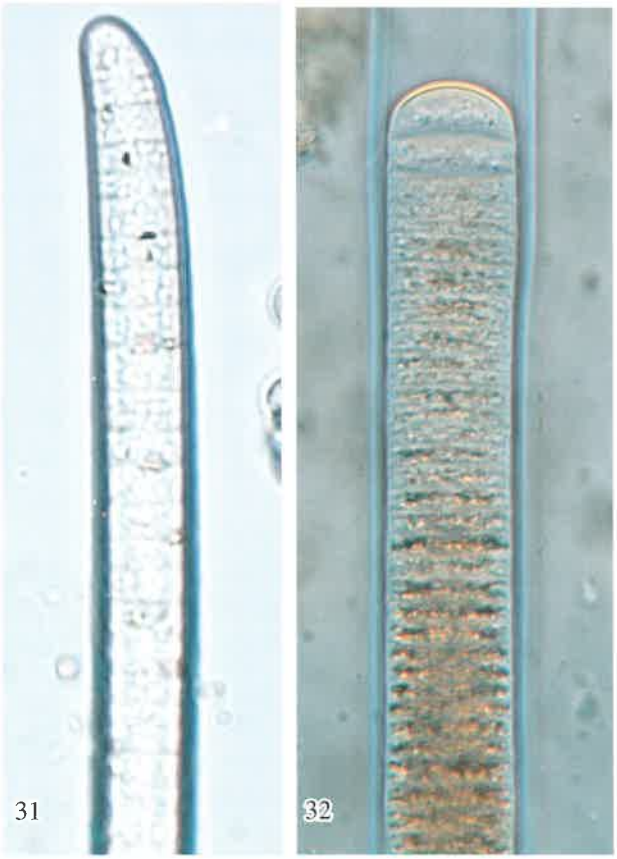
Planktothrix (Oscillatoria) agardhii (Gom.) Anagn. & Kom. 1988. Figs 29-30
Trichomes solitary, straight, blue-green in colour, 3.5-5 μm wide, not constricted at cross walls, towards the end slightly narrower, mostly bluntly pointed, sometimes with a calyptra (Fig 30). Cells short cylindrical, 3-4 μm long with granulation and aerotopes. Cosmopolitan, common in eutrophic waters. The genus *Planktothrix* have toxigenic species with production of hepato- and neurotoxins. The genus includes 12 species.

Planktothricoides raciborskii (Wolosz.) Suda &. M.M. Watanabe in Suda *et al.* 2002. Fig. 31.
Planktic. Trichomes solitary, without sheath, straight or slightly coiled, cylindrical, not constricted at cross walls, narrowed and slightly bent towards the ends, 9.0-9.5 μm wide. Cells cylindrical, mainly shorter than wide, 3.2-6.2 μm long, with aerotopes, grey to blue-green coloured. Common in the tropics.

Lyngbya birgei G. M. Smith 1916. Fig. 32.
Filaments free-floating. Trichomes within firm hyaline sheath, straight or slighly flexible. Cell shape disciform, width 18-23 μm and length 2-2.5 μm. Cell contents homogeneous, colour grey to olive green. Trichome ends rounded without calyptra. Metaphytic in North American lakes.





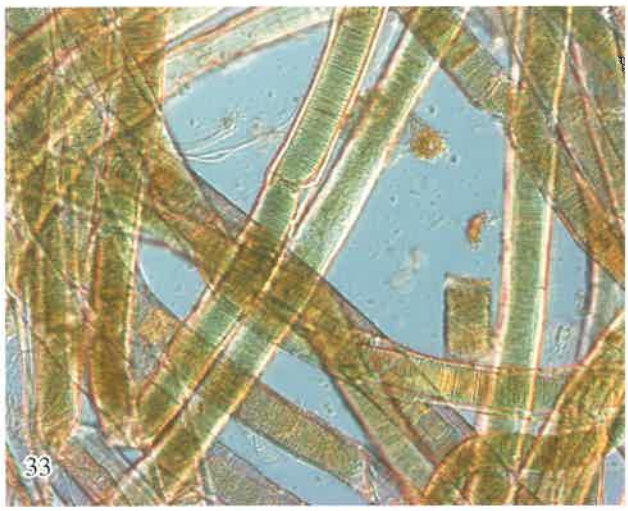


Lyngbya majuscula Harvey in Hooker 1833.
Figs 33-34.
Marine, benthic organism. Trichomes are straight, long and enclosed in a distinct, unbranched sheath. The sheath is 4 μm thick. Cells are about 12 μm in diameter and only 1-1.5 μm long. Total thickness of filament is about 20 μm. Trichomes are blue-green, brown-green or grey-purple, not constricted at cross walls, without granules. The end cells are rounded without calyptra.

It grows on rocks or on sediments in large mats, which can tear loose and be found free-floating. *L. majuscula* is toxigenic, producing the dermatotoxin lyngbyatoxin. Direct external contact causes irritation on human skin, known as the "swimmers itch".

L. majuscula is reported from the Pacific and Atlantic Oceans and has been a regular nuisance in the Brisbane area, Australia.

The genus *Lyngbya* comprises more than 60 confirmed species, and several have cosmopolitan distribution. Most species are benthic and grow in submersed mats on rocks and sediment surfaces. They develop in freshwater, brackish and marine waters. Also terrestrial and aerophytic species are described.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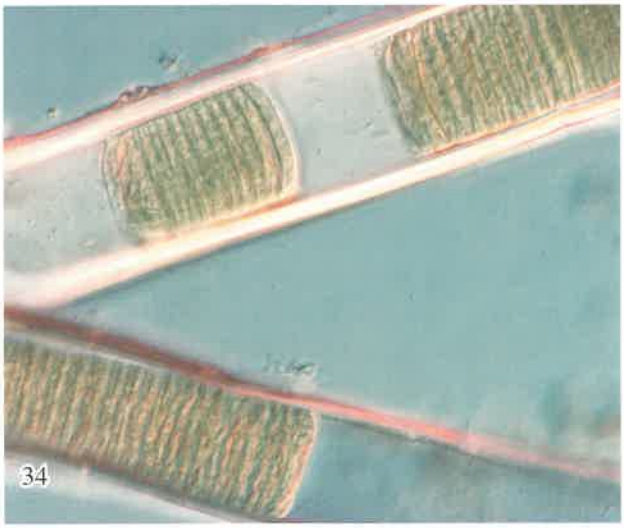
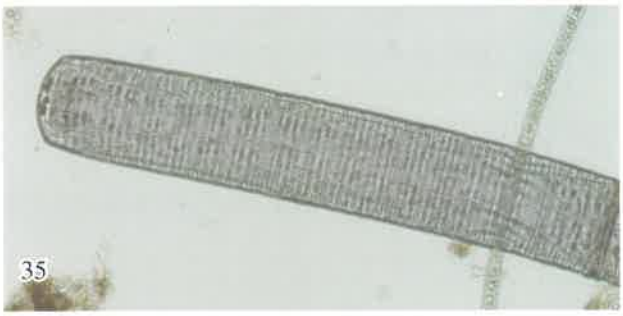

34

Oscillatoria kawamurae Negoro 1943.
Figs 35-36.
Trichomes straight, not bent, with uniformly parallel sides slightly attenuated at the apex, and hardly constricted at cross walls. End cells rounded or slightly capitate, without calyptra. Filaments are purple, brownish or light green in colour, with minute granules in the cells, and 3-4 large, reddish-brown aerotopes, centrally arrranged near the cross walls, forming a loose spiral along the whole length of the trichome. The cells are compressed, (56)65-66(78) μm wide, 5-12 μm long.

O. kawamurae was originally described from Manchuria, China (Negoro, 1943), but have recently been reported from Lake Biwa, Japan (Watanabe, 1996). It is common in lakes in southern Vietnam and formed blooms, in 1991 in the Nam Ngum Reservoir in Laos (Cronberg, unpublished). It is metaphytic and grows in mesotrophic to eutrophic lakes. So far reported only from Asia.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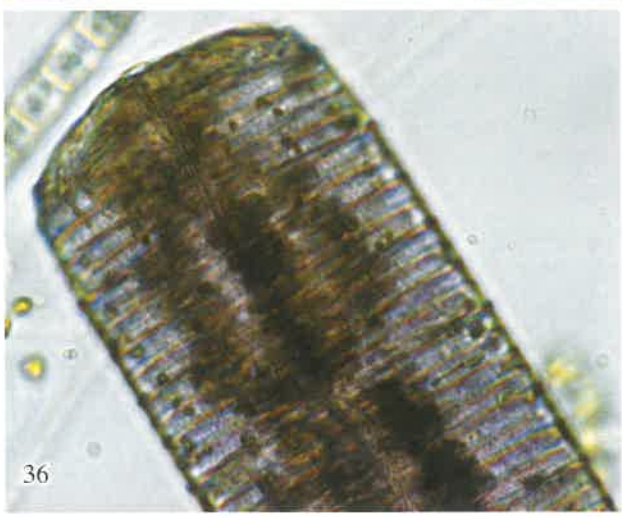

36

Oscillatoria limosa ***(C.*** Agardh 1812) Gomont 1892.
Figs 37-38.
Benthic organism. Trichomes growing in black or blue-green to brownish coloured submersed mats. The trichomes are straight or slightly wavy, blue-green to brown or olive green in colour, not constricted at cross walls, (11-)13-16(-22) wide and 2-5 μm long, with granules assembled at the cross wall. End cells are flat-rounded, sometimes with a thickened membrane.

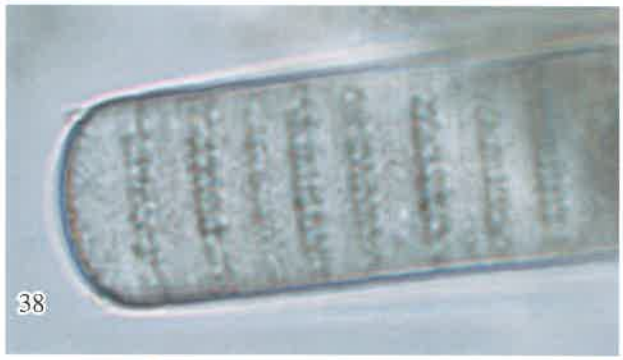
It develops in standing or slowly flowing fresh or slightly brackish, often polluted water. Flakes or trichomes loosen from the substrate and can be found free-floating. It grows also on moist mud or soil. Common, cosmoplitan species.

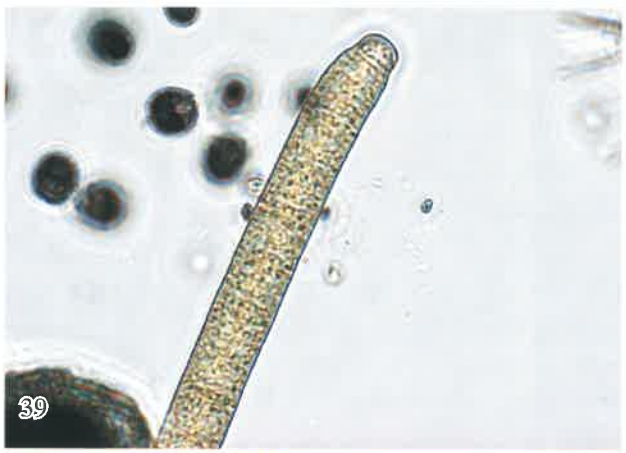
Oscillatoria princeps Vauch. ex Gomont 1892
Fig. 39.
Benthic organism. Trichomes blue-green, brownish, purple or reddish, mostly straight, not constricted at cross walls, without adjacent granules, 16-60 μm wide and 3.5-7 μm long, at apex lightly and abruptly bent, aerotopes often present in apical region. Cells up to 0.5 times as long as wide. End cells flatly rounded, slightly capitate, with or without a slightly thickened membrane. Trichomes form extensive mats, or are floating in stagnant or flowing, nutrient-rich freshwaters, cosmopolitan.

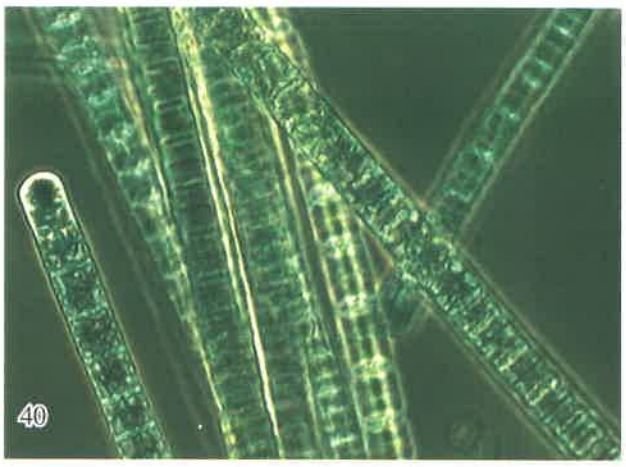
The genus *Oscillatoria* comprises about 70 widely distributed, mainly benthic species.

Tychonema bornetii (Zukal) Anagn. & Kom. 1988.
Fig. 40.
Benthic organism. Trichomes red-brown to brown-purple, rarely greenish, forming slimy tufts among waterplants. Trichomes straight or slightly bent, with or without mucilaginous sheaths, 12-16 μm wide. The cells are almost quadrangular, rarely longer than wide, not constricted at cross walls, more or less granular, often with keratomized chromatoplasma, without aerotopes. End cells are colourless, rounded or flat rounded, slightly capitate and with slightly thickened membrane.

It is mostly attached to waterplants, but can be free-floating in oligotrophic to mesotrophic freshwaters, probably cosmopolitan.

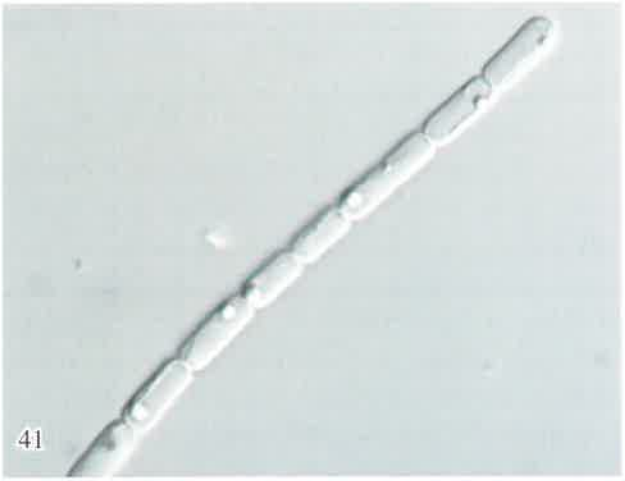






Pseudanabaena moniliformis Kom. & Kling 1991. Fig. 41.
Planktic. Trichomes solitary, straight or slightly arcuate, cylindrical without sheath. Filaments slightly coiled and constricted at cross walls, with up to 80 cells. The cells are cylindrical, always longer than wide. Cells are usually unequally long, 4-6-9 μm long and 1.5-2.5 μm wide, pale grey-blue in colour, slightly keritomized, often with aerotopes at the cell ends.

P. moniliformis is probably widely distributed in tropical and subtropical, more or less eutrophic waterbodies.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Pseudanabaena voronichinii Anagnostidis 2001. Fig. 42.
Trichomes solitary, short, straight or slightly curved, mostly without sheath. Cells are cylindrical, 2.5-8 x 1.0-2 μm. The terminal cells are rounded or conically, bluntly pointed. Trichomes contain 2-8 cells.

P. voronichinii grows endophytically in the mucilage of planktic colonies of *Microcystis, Gomphosphaeria, Chroococcus* and *Woronichinia*. Observed mainly in Northern Europe, probably more widely distributed. *P. mucicola* (Naum. & Hub.-Pest.) Schwabe is a similar species, but it has shorter and thicker trichomes than *P. voronichinii*.

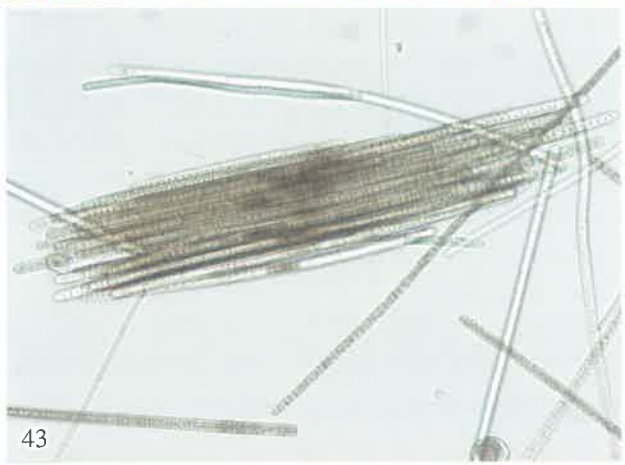
The genus *Pseudanabaena* comprises about 10 species.


42

Trichodesmium erythraeum (Ehrenb.) Gomont 1892. Figs 43-44.
Planktic. With straight, motile trichomes oriented parallel in bundles, which are embedded in mucilage. The cell width is 7-11 μm (rarely 21) and cell length 5.4-11μm. Most cells are shorter than wide, red in colour and with aerotopes. Although they have no heterocytes, *Trichodesmium* species can fix atmospheric nitrogen.

It is a marine cyanophyte and appears in blooms together with other *Trichodesmium* species. To date two species have been recorded to be toxigenic, *T. erythraeum* and *T. thiebautii*. Strains can produce neurotoxins and hepetotoxins.

The genus *Trichodesmium* comprises about 10 species, which have their main distribution in warm-temperate and tropical seas.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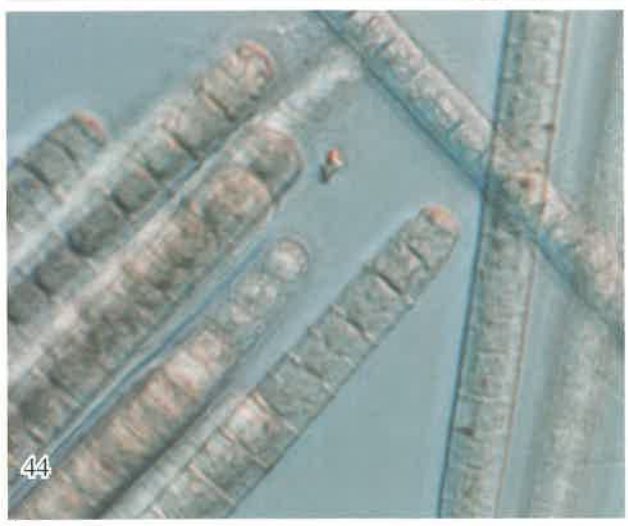
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Arthrospira fusiformis (Voronich.) Kom. & Lund 1990. Figs 45-46.
Planktic. Trichomes more or less screw-like. Cells have transverse walls with slight or no constrictions. Trichome ends are rounded or slightly narrowed, some-times with calyptra. Aerotopes are present. The trichomes are more or less densely coiled. The amplitude of the spirals is 10-50 μm, and the length in space between coils 0-80 μm. The trichome width is 3.4-6(9.5) μm. *A. fusiformis* appears in different forms, s-forms with long distances between the helices (Fig. 45) and h-forms with tight coils (Fig. 46). *A. fusiformis* is common in African soda lakes.

A few strains have been found to produce microcystins and anatoxin-a.

The genus *Arthrospira* comprises about 10 species, and has been found mainly in alkaline, brackish and saline waters in tropical and semi tropical regions.


45

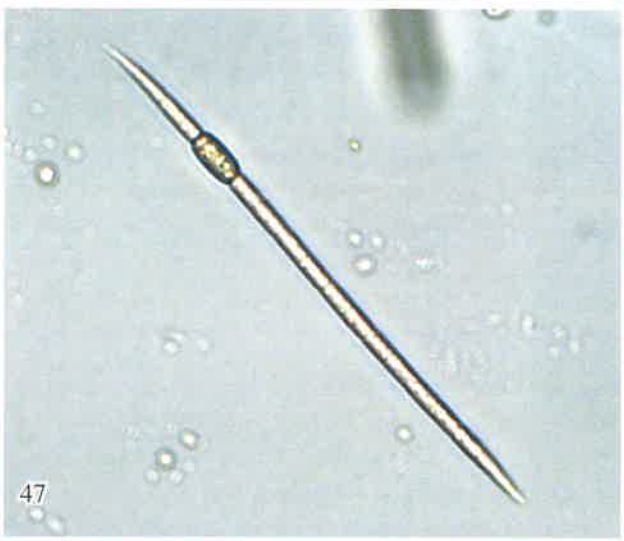

46

2.2.3 Nostocales

Raphidiopsis mediterranea Skuja 1937. Fig. 47.
Planktic. Trichomes straight or slightly bent, 40-200 μm long. Trichome ends are pointed to a very thin tip. Cells are constricted or only slighly constricted at cross walls, 1.5-2.5 μm wide and mostly 2-4 times longer than wide, blue-green to yellow-green, with aerotopes. The akinetes are intercalar, cylindrical to oval, situated close to the end of the trichome, containing granules, pale green, single or in pairs, dimensions 6.5-13 x 2.5-3 μm.

It appears in eutrophic waterbodies mainly in tropical or pantropical regions. Toxigenic species producing homoanatoxin-a och anatoxin-a.

Trichomes of *Aphanizomenon issatschenkoi* and *A. tropicale* without heterocytes may be incorrectly identified as *R. mediterranea* because of the similarity in appearance.


47

Raphidiopsis curvata Fritsch & Rich 1929.
Figs 48-49.
Planktic. Trichomes short, curved or spirally twisted, attenuated at one or both ends, not constricted at cross walls. Cells are cylindrical, 4.5 μm wide and 1.5-2 times as long as broad. Akinetes are barrel-shaped to cylindrical, intercalar, 10-13.5 μm long.

Raphidiopsis comprises 3 species mainly found in the tropics.


48

49

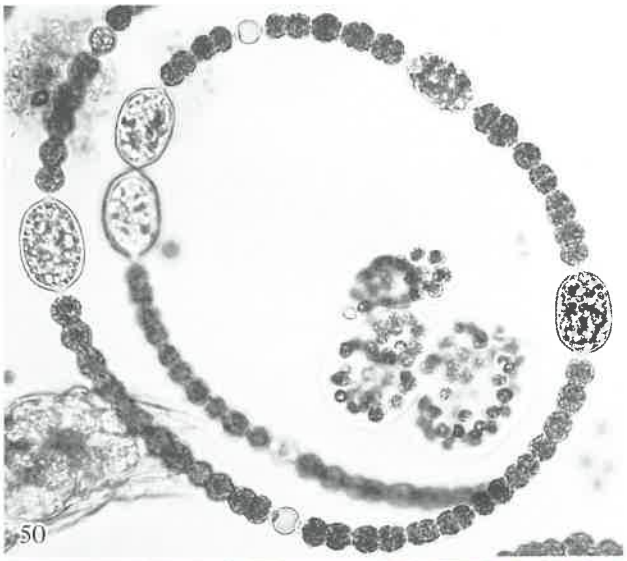
Anabaena
The genus *Anabaena* comprises more than 80 species distributed world wide.

Anabaena circinalis Rabenh. ex Born. et Flah. 1888. Fig. 50.
Planktic. Trichomes solitary, widely spirally coiled, 70-120 μm wide, sometimes forming small bundles of several trichomes joined together. The trichomes are mostly without mucilage, or have a very narrow diffluent sheath. Cells barrel-shaped to spherical, 7-9 μm long, 7-8-11 μm wide, with aerotopes and blue-green in colour. Heterocytes spherical, as wide as the vegetative cells. Akinetes are elongate oval to short cylindrical, sometimes conical at the ends, 20-28 x 15-21 μm. Toxigenic species, producing neurotoxins (anatoxin and saxitoxin). Common in eutrophic waterbodies in the temperate zone. Similar types, albeit without akinetes, occur in the tropics.

Anabaena crassa (Lemm.) Kom.-Legn. et Cronb. 1992. Fig. 51.
Planktic. Trichomes regularly spirally coiled, about 40-70 μm in diameter, 45-55 μm distant from each other, embedded in thick mucilage up to 20 μm wide. Cells barrel-shaped to spherical, on the cross-wall flattened, slightly shorter than wide, 9-12.5-15 μm wide, with aerotopes. Heterocytes spherical, often smaller than the vegetative cells, 10-17 μm in diameter. Akinetes widely ellipsoidal, distant from the heterocytes, sometimes in pairs, 15-28-35 μm long and 13-18-22 μm wide. It appears in water-blooms together with other cyanophytes. Common in the temperate region.

Anabaena spiroides Kleb. 1895. Fig 52.
Planktic. Trichomes solitary, ± screw-like twisted. Cells are barrel-shaped to spherical, 6.5-8 μm wide, with aerotopes. Heterocytes round, 6.5-10 μm in diameter. Akinetes widely oval, sometimes slightly curved, distant from heterocytes, solitary or in pairs, 17-21 x 10-14 μm. Trichomes surrounded by massive mucilage. Toxigenic species. Common in eutrophic lakes often in water-blooms. *A. spiroides* is not as wide-spread as *A. crassa*.

Anabaena flos-aquae Bréb. ex Born. et Flah. 1888. Fig. 53.
Planktic. Trichomes irregularly twisted, spirals of different widths in clusters. Cells round, 3.5-7 μm; heterocytes slightly oval, 5-6 μm in diam, with aerotopes; akinetes oval to cylindrical, 2-3 in a row, distant from heterocytes, 15-35 μm long. Trichomes without mucilage. Toxigenic species. Common in the temperate zone, but rare in the tropics.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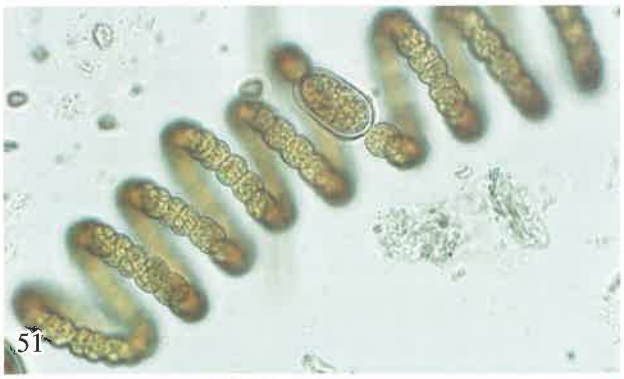
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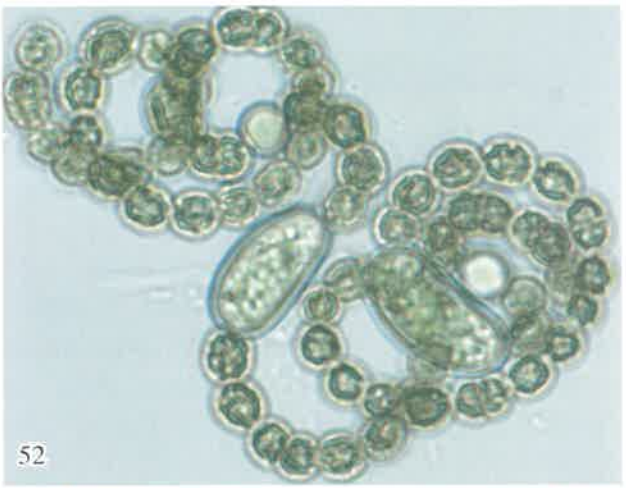
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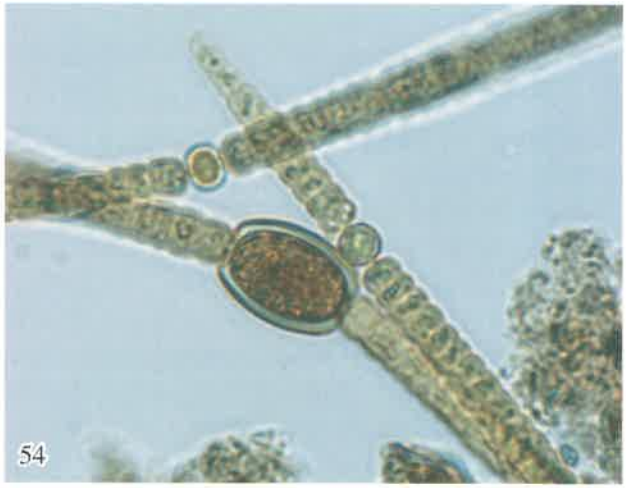
Anabaena bergii Ostenfeld 1908.
Fig. 54.
Planktic. Trichomes solitary, without mucilage, straight or slightly bent, slowly tapering to the end. The end cell is conical and bluntly pointed. Cells short, barrel-shaped, mostly shorter than the width of the trichome, 4-5 μm long and 6-7-8 μm wide, with many aerotopes. Heterocytes spherical, slightly compressed, 5-6 μm long and 6-7 μm wide. Akinetes spherical to oval, 13-32 x10-24 μm. Toxigenic species producing cylindrospermopsin.

It appears in brackish water probably most frequent in subtropical to tropical regions.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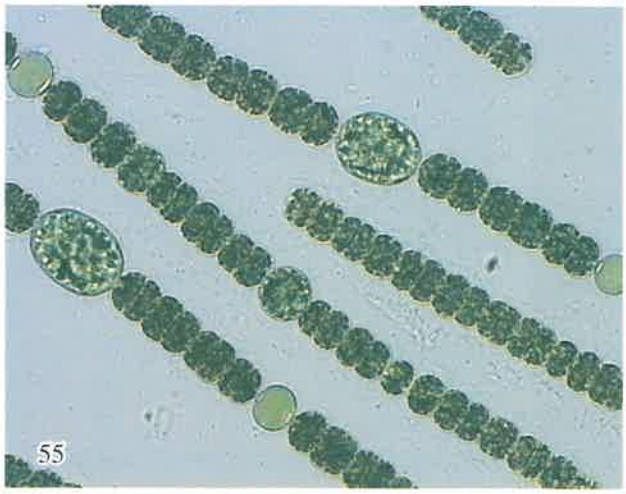
Anabaena viguieri Denis et Frémy 1923.
Fig. 55.
Planktic. Trichomes solitary, straight or slightly curved, slightly tapering to the ends. Cells more or less spherical to barrel-shaped, 4.2-6.4-7-2 μm long, 4.2-5.8-8.7 μm wide, with aerotopes. Heterocytes spherical, 3.5-5-6 μm in diameter. Akinetes widely oval to almost cylindrical, 9-18-25 x 10-13-15 μm, mainly solitary, distant from heterocytes.

It is common in eutrophic waterbodies, probably with cosmopolitan distribution.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Anabaena austro-africana Cronb. et Kom. 2004.
Fig. 56-57.
Planktic. Trichomes solitary, free-floating, straight or slightly curved, without mucilaginous envelopes, distinctly constricted at the cross walls, successively attenuated towards the ends, in the middle 4-6 μm wide. Cells spherical to barrel-shaped, isodiametric or slightly shorter than wide. Cells pale blue-green with small granules and aerotopes. Heterocytes develop singly, intercalary, approximatively equidistant from each other, spherical, 3.5-4-7.2 μm in diameter with hyaline content. Akinetes develop on both sides of the heterocytes, widely oval to almost spherical, with slightly granulated content, and brownish epispore, 10.6-14.8 x 9.2-11.2 μm.

It was recently described from southern Africa, but is probably common throughout the tropics.


56


57

Anabaena carmichaelii Cronb. et Kom. 2004.
Fig. 58.
Planktic. Trichomes solitary, straight or curved, without mucilaginous envelopes, constricted at cross walls, successively attenuated towards the ends, in the middle 3.5-3.8 μm wide. Cells cylindrical to barrel-shaped, isodiametric or longer than wide. Cells pale blue-green, with elongated aerotopes. Heterocytes several, up to 7 or more in the trichome. They develop intercalarly, singly, are spherical, 4 μm in diameter. Akinetes are solitary or in pairs, 2-4 cells distant from the heterocyte, oval to widely oval, 9-12 x 8-10 μm.

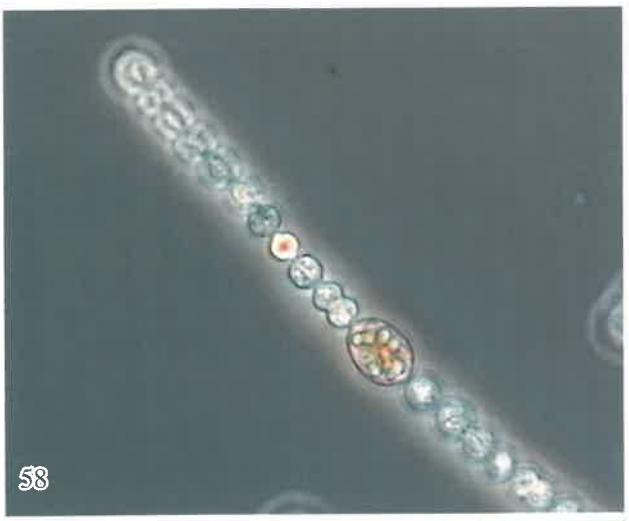
Recently described species from southern Africa, however also found in Laos. It is probably mainly distributed in the tropics.

Anabaena maxima Cronb. et Kom. 2004.
Fig. 59.
Planktic. Trichomes solitary, straight trichomes without mucilaginous envelopes, sometimes with 2-3 hyaline end cells. Trichome width is 9-11.4 μm. Cells distinctly barrel-shaped, usually isodiametric. Heterocytes develop at metameric positions, up to 9 in one trichome. Heterocytes are spherical or slightly compressed, up to 12 μm wide with hyaline content. Akinetes develop at short distance from heterocytes, large cylindrical to oval, with granular content, brownish epispore and asymmetric juncture, 16-43 x 13-20 μm.

This alga appeared together with *A. austro-africana* and *A. carmichaelii* forming blooms in mesotrophic to eutrophic waterbodies in Africa.

Anabaena smithii (Kom.) M. Watanabe 1992.
Fig. 60.
Planktic. Trichomes solitary, straight filaments embedded in homogeneous, diffluent mucilage. Cells compressed, barrel-shaped, shorter than wide, with aerotopes, 9-15 μm broad, 9-10 μm long. Heterocytes are spherical, somewhat smaller than the vegetative cells, 7-12 μm in diameter. akinetes spherical, 15-30 μm in diameter.

Anabaena solitaria Kleb. 1895. Fig 61.
Planktic. Trichomes, solitary, straight and embedded in wide, mucilaginous sheath. Cells spherical to ellipsoidal, with aerotopes, 6.5-8-12 x 6.5-7.5-10 μm. Heterocytes spherical to lemon-shaped, of the same size as the vegetative cells. Akinetes long cylindrical, wider than the filament, 20-45 x 10-12-16 μm. *A. smithii* and *A. solitaria* form water-blooms in the temperate region.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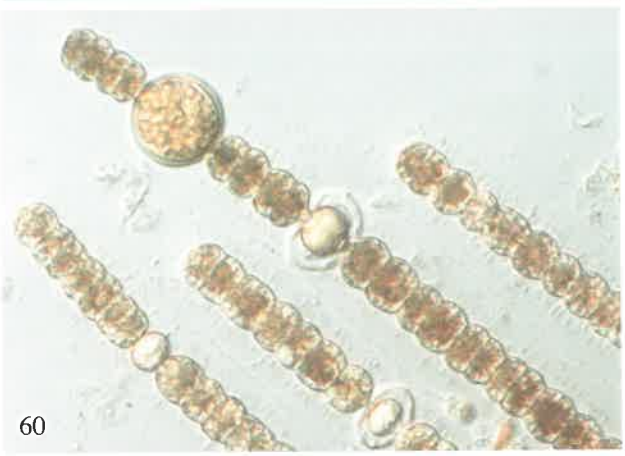
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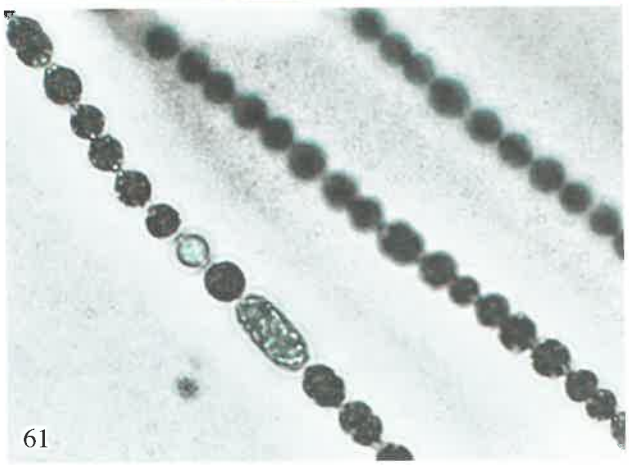
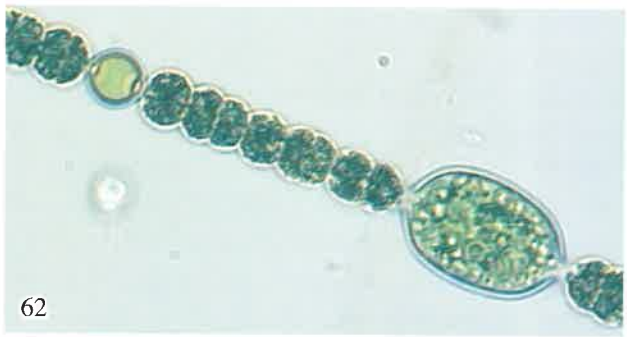

61

Anabaena planctonica Brunnth. 1903.
Fig. 62.
Planktic. Trichomes, solitary, straight, surrounded by a wide muclaginous sheath. Cells are spherical to barrel-shaped, often shorter than wide, 9-15 μm broad, up to 10 μm long with aerotopes. Heterocytes spherical, as wide as the vegetative cells. Akinetes oval to cylindrical, with broadly rounded to bluntly conical ends, sometimes hexagonal, 10-20 μm wide and 15-30 μm long. Toxigenic species producing neurotoxin (anatoxin-a).

Common in eutrophic lakes and reservoirs in the temperate zone.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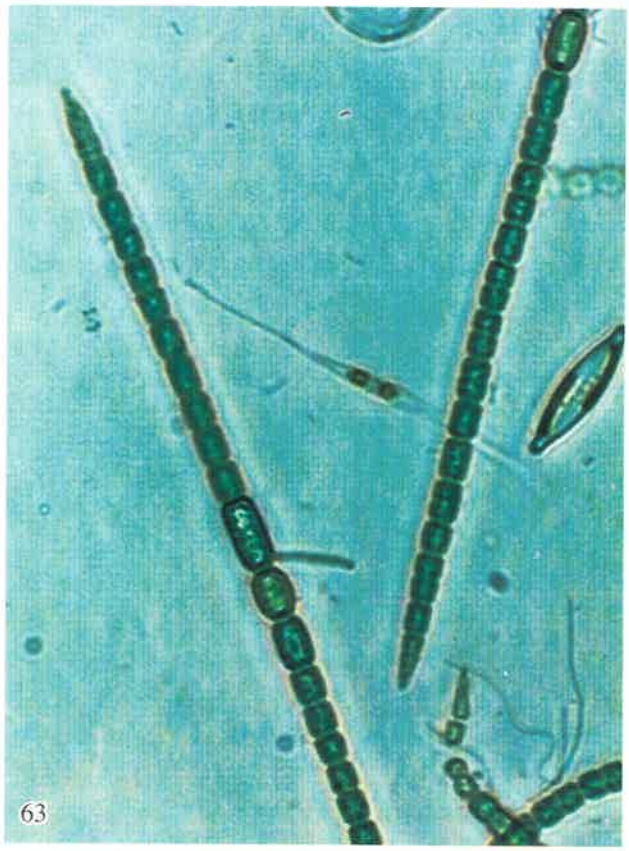
Anabaena torulosa (Carm.) Lagerh. 1883.
Figs 63-64.
Trichomes aggregated to form a thin, slimy, blue-green layer. Cells are barrel-shaped, 4.2-5 μm wide and 3-5 μm long with conical end cells. Heterocytes are cylindrical to almost spherical, 6 μm wide, 6-10 μm long. Akinetes rounded-cylindrical, in the middle often narrowed (Fig. 64), 7-12 μm wide and 10-24 μm long, smooth with light brown cell wall, 1-2 akinetes on both sides of the heterocyte.

It appears in standing fresh water, is also found in brackish water, attached to waterplants, described from the temperate zone, but reported also from the tropics, probably cosmopolitan.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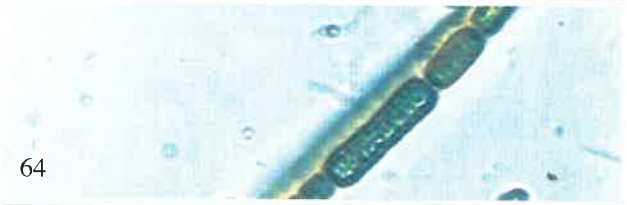
Anabaena reniformis (Lemm.) emend. Aptekarj 1927. Figs 65-66.
Planktic. Trichomes more or less spirally coiled, solitary or sometimes forming small clusters, distance between coils 16-17 μm, height of coils 13-15 μm. Cells are blue-green, spherical to oval, 4 μm wide, 7-8 μm long, with many aerotopes. Heterocytes nearly spherical, about 4 μm in diameter. Akinetes spherical on both sides of the heterocyte (fig. 65), 8.5-11 μm in diameter. Mature akinetes have a granular content, and brownish cell wall.

It is similar to *Anabaena spiroides*, but akinetes are adjacent to the heterocyte in *A. reniformis* and distant from the heterocyte in *A. spiroides*. *A. reniformis* has been reported only a few times, it might have been overlooked. However, it has been found in Europe, Africa and South America.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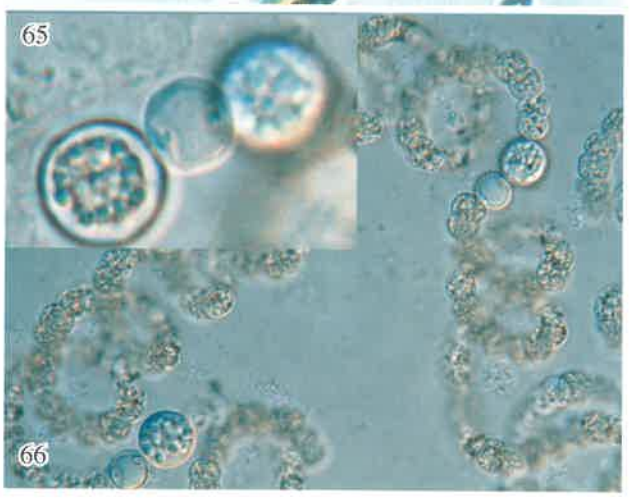
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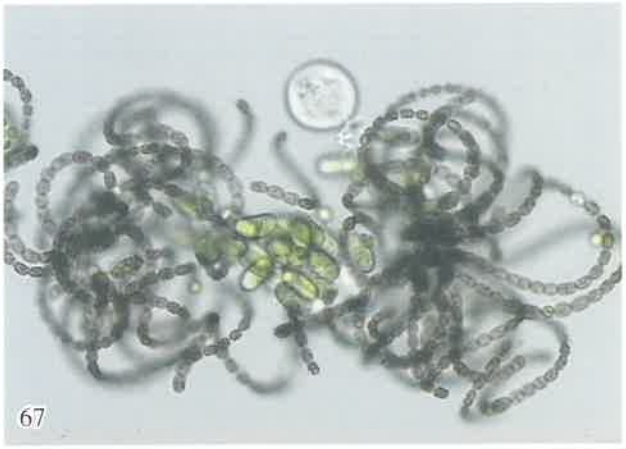
Anabaena lemmermannii var. ***minor*** (Uterm.) Kom.-Legn. 1988. Fig 67.
Planktic. Trichomes single or joined in large clusters, irregularly twisted. Cells are spherical to barrel-shaped, 3-6-8 μm long, 3-5-7 μm wide, with aerotopes. Akinetes at both sides of a single spherical or widely oval heterocyte. Heterocytes 4.5-5-6.2 μm long, 4-4.5-6 μm wide. Akinetes are long, a little bent, with rounded ends, 11-15-22 x 6-9 μm. Toxigenic species producing neurotoxin (anatoxin-a) and hepatotoxins (microcystins).

Common in eutrophic lakes in the temperate regions.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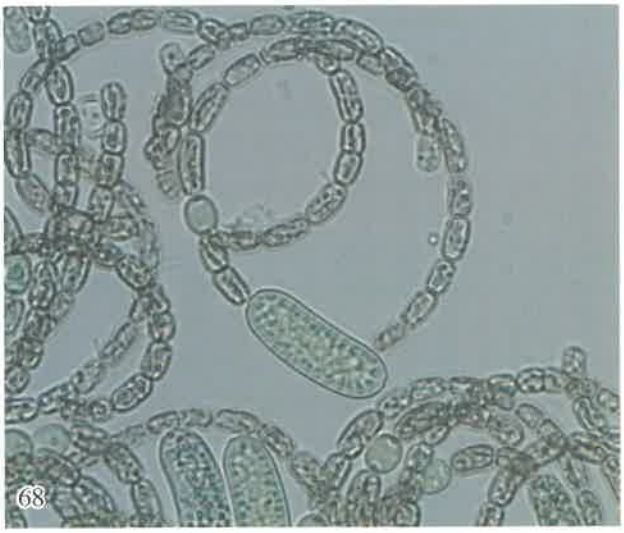
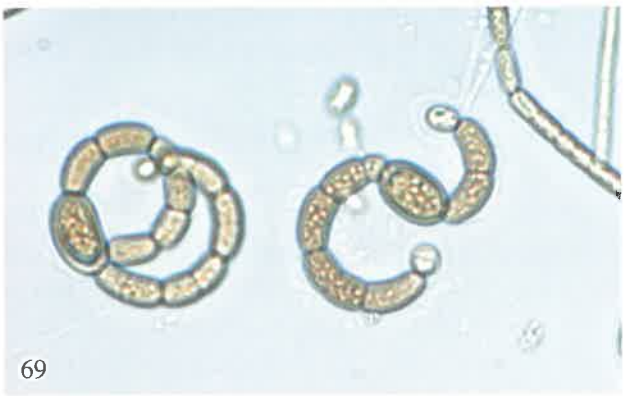
Anabaena mendotae Trelease 1889. Fig 68.
Planktic. Trichomes irregularly, spirally twisted in wide loops. Cells cylindrical mostly longer than wide, at the ends slightly rounded, 3-7-11 μm long, 2.5-4.5 μm wide with aerotopes. Heterocytes widely ellipsoidal, 54,4-7-11x4-7 μm, akinetes long cylindrical, sometimes slightly bent following the shape of the filament, distant from heterocytes, 16-30-40 x 4.5-5.5-7 μm.

Often occuring in slightly eutrophic water bodies in temperate regions.


68

Anabaenopsis elenkinii Miller 1923. Fig. 69.
Planktic. Trichomes solitary, spirally twisted, from 3/4-3 spirals per trichome. Cells ellipsoidal, 4.6-5.7 wide, one to two times longer than wide, with aerotopes. Terminal heterocytes spherical, 4.6-4.7 μm wide. Akinetes single, rarely in pairs, spherical to short ellipsoidal, 9.3-12 x 8.3-10.5 μm with smooth, uncoloured membrane.

In stagnant brackish or saline waters, common in the tropics.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Anabaenopsis circularis (G. S. West) Miller 1923. Fig. 70.
Planktic. Trichomes spirally twisted, number of spirals per trichome about 1-2 and 4.5-6 μm wide. Cells cylindrical to barrel-shaped, with aerotopes, 6.2-10.2 x 3.1-4.3 μm. Terminal heterocytes are spherical to oval, 4.7-6.3 x 4.7-5.9 μm. Akinetes are ellipsoidal, 10-14 x 6.2 μm.

It occurs sporadically in stagnant waters with higher salinity. Common in tropical regions. The genus comprises about 10 species.


70

Aphanizomenon

The genus *Aphanizomenon* comprises about 15 well-defined species. Most of these are more or less common in the temperate region, but certain species have a distribution limited to tropical regions (Table 3). Four species have been found toxigenic producing anatoxin, saxitoxin or cylindrospermopsin.

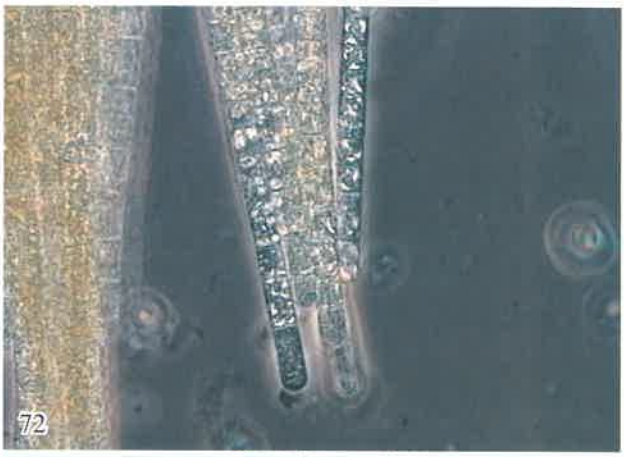
Aphanizomenon flos-aquae (Linné) Ralfs ex Born. et Flah. 1888.
Figs 71-72.
Planktic. Trichomes in parallel rows, forming macroscopic flakes, 2.5-9 mm, grass-blade or sickle-shaped. Filaments long, up to 1mm, straight with cylindrical cells, slightly constricted at cross walls. The end cells are long with plasmatic threads extending throughout the cell. Cells in the middle of the trichomes are 5-7 μm wide and 5-14 μm long. End cells are long, 10-19 μm, and of nearly the same width as the middle cells. Heterocytes cylindrical, 10-14(17) x 5-6.4 μm, intercalar, 1-2 per trichome, and distant from the akinetes. Long, cylindrical akinetes, 54-82 x 7-10 μm.

Toxigenic species producing neurotoxins (saxitoxin and neosaxitoxin).

Common in eutrophic lakes and ponds in the temperate zone. It occurs often together with large-sized daphnids.

Aphanizomenon yezoense M. Watanabe 1991.
Figs 73-74.
Planktic. Trichomes solitary or in short bundles, 100-180-330 μm long, slightly or not constricted at cross walls, end cells more or less attenuated. Cells in the middle of the trichome are cylindrical, 2.4-4 μm wide, 3.1-7.8 μm long. The terminal cells are elongated, cylindrical, with abrutly rounded ends, 2.8-4 μm in width, 11-29 in length. Heterocytes are cylindrical, 3.8-5.1 in width and 5.4-11.4 μm in length. Akinetes are long, cylindrical 4.7-7.3 μm in width and 31-49 in length. It has a maximum of one akinete per trichome. This species thrives in humic, mesotrophic lakes and ponds in the temperate zone.

The *Aphanizomenon* species described below are characterized by having solitary, thin, straight or slightly bent trichomes, without mucilage. Occurring in plankton in eutrophic to mesotrophic lakes or ponds.



Aphanizomenon capricorni Cronb. & Kom. 2004. Figs 75-77.
Planktic. Trichomes up to 200 μm long, constricted at cross walls, continually attenuated towards the ends. Cells cylindrical 3.3-7.4 μm long, 2-3.5 μm wide, pale blue-green in colour and with elongated aerotopes. End cells long, conical, sometimes hyaline, 5-10 x 1.5-2 μm. Mostly only one heterocyte per trichome, intercalarly and asymmetrically positioned, barrel-shaped to cylindrical, 3.8-5 x 2-3.5 μm. Akinetes develop on one or both sides of the heterocyte, oval, solitary or 2-3 in a row, 11-12 x 7-8 μm.

A. capricorne was recently described from southern Africa, also recorded from East Asia (Rajaniemi *et al.*, 2005a, 2005b; Cronberg, unpublished).

Aphanizomenon gracile (Lemm.) Lemm. 1907. Figs 78-80.
Planktic. Trichomes with elongated cells, slightly constricted at cross walls, with aerotopes, 2.6-3 μm wide, 3-6 μm long. Heterocytes oval 4-8 μm long and 3-5 μm wide. Akinetes long cylindrical with rounded ends, distant from the heterocytes, 4-6 μm wide and 8-15 μm long. End cells slightly narrower. Toxigenic species, producing neurotoxin (saxitoxin). Distributed in the temperate region, possibly also subtropical.

Aphanizomenon issatschenkoi (Usač.) Prošk.-Lavr.1962. Figs. 81-82.
Planktic. Trichomes with cylindrical cells, not or only slightly constricted at cross walls. Cells 3-4 μm wide, 6-9(15.9) μm long, pale blue-green to gray-blue. End cells colourless, thin, pointed. Heterocytes cylindrical to oval, 1(2-3) per tri-chome, 7-11 x 3-6 μm. Akinets cylindrical, 1-3 in a row and distant from heterocytes, 9-19 x 4-7 μm. Distributed mostly in the temperate zone.

Aphanizomenom skujae Kom.-Legn. & Cronb. 1992. Fig. 83.
Planktic. Trichomes with elongated cells, in the middle 1.7-2.1-2.6 μm wide, tapering towards the ends, 1-1.5μm wide. Cells cylindrical 4.2-6.6-16.8 μm long with many aerotopes. Heterocytes oval to cylindrical, 7.5-8.5-11.4 x 1.9-2.8-3 μm long. One to three akinetes, distant from heterocyte, cylindrical with round ends, 25-27-34 x 2.7-3.7 μm. Distributed in northern temperate zone.

Cylindrospermopsis raciborskii
Seenayya et Subba Raju 1972.
Figs 84-87.
Planktic. Trichomes solitary straight or slightly curved trichomes with lengths up to 200 μm. Cells are cylindrical, slightly constricted at the cross walls, 2.5-4 μm wide and 2.5-16 μm long. The terminal heterocytes are long and conical with dimensions 5-6 x 2-2.5 μm (Fig. 85). Akinetes are elongated oval and situated adjacent to the heterocyte or the terminal vegetative cell, 2.8-3.3 μm wide and 4.5-11(16) μm long.

Common in eutrophic waterbodies and can make heavy blooms. It is wide-spread in tropical and pantropical regions (Padisak, 1997). Several morphotypes of *C. raciborskii* exist. In habitats with a high N/P quotient it appears without heterocytes and can easily be mistaken for *Raphidiopsis mediterranea* (Table 4).

C. africana is a related species, but differs in having thinner and longer cells than *C. raciborskii*.

Toxigenic species, producing cylindrospermopsin.

Cylindrospermopsis curvispora M. Watanabe 1995.
Figs 88-89.
Planktic. Trichomes spirally or circularly coiled, forming usually 1.5-2 coils, without mucilaginous envelopes, cylindrical, slightly, but distinctly constricted at the cross walls, 2.9-3.9 μm wide, not attenuated at the ends. Cells cylindrical to indistinctly barrel-shaped, isodiametric, 3-7 μm long, with pale blue-green content, usually without visible aerotopes, but if present occuring only singly. Heterocytes at one or both trichome ends, spherical to slightly elongated, cylindrical or conically-rounded, 4-7 μm long and 2.5-4 μm wide, broadly rounded at the apex, with hyaline content. Akinetes develop in pairs (fig. 89), beside the apical heterocyte. Occasionally 1-2 vegetative cells can be found between the akinetes and the heterocyte. Cylindrical akinetes with widely rounded ends, sausage-shaped, 7.4-11 x 3.5-4.2μm. A potential toxigenic species.

This alga appears in eutrophic ponds and lakes, often with a high salinity, common in the tropics, e.g. in southern Africa. The genus comprises about 10 described species, all but *C. raciborskii* have been discovered recently.

Gloeotrichia echinulata (J. E. Smith) P. Richter 1894. Figs 90-91.
Planktic. Forming round, macroscopic colonies, 1-3(8) mm in diameter with radially arranged trichomes, which are provided with a sheath at the base. The trichomes have a terminal heterocyte at the base, and at the other end terminate in a hair-like filament. The vegetative cells are cylindrical and at the base, 5.5-10 μm, while the opposite hair-like cells are only 2 μm wide. The cylindrical akinetes are formed adjacent to the basal heterocyte and have rounded ends, 44-50 x 8-18 μm in size. The whole colony is embedded in mucilage and the cells have aerotopes. Toxigenic species, producing neurotoxins and hepatotoxins.

Gloeotrichia appears in eutrophic, stagnant water in the temperate region, but has also been recorded from tropical regions. The genus comprises 14-20 species.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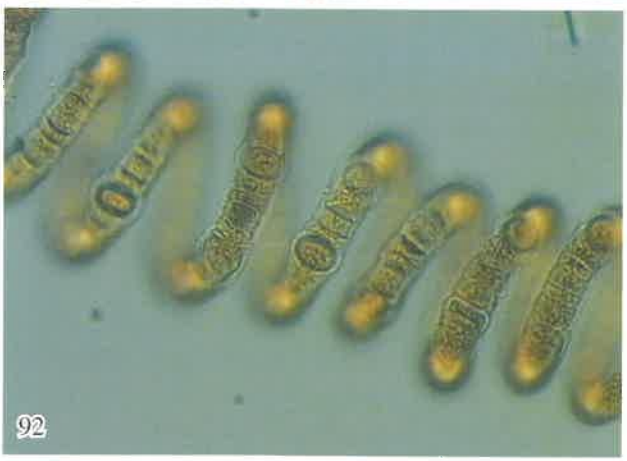
Nodularia spumigena Mertens ex Born. et Flah. 1886.
Figs 92-93.
Planktic. Trichomes are straight or spirally twisted, enclosed in a mucilaginous sheath. The vegetative cells are disc-shaped, constricted at the cross walls, 8-24 μm in width and, only about 3-5 μm long. The heterocytes are compressed and elliptical in shape, about 14 μm in diameter and 7 μm long. The akinetes are transversely oval, 6-11 x 10-12 μm with brownish cell walls, in series, rarely solitary or in pairs. It has aerotopes.

Nodularia spumigena thrives in brackish water e.g the Baltic Sea, forming dense waterblooms in the summer. It has a world-wide distribution. A toxigenic species, producing hepatotoxins (nodularin).

The genus *Nodularia* comprises seven species. Developing in plankton or attached to macroalgae or sediments. Mostly distributed in the temperate zone, but recorded also from subtropical regions.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2.2.5. Table 1 (1). Characteristic features of *Microcystis* species.

Species	Cell size (μm)	Aerotopes	Mucilage	Colonies, size (μm), shape	Comments
**M. aeruginosa* (Kützing) Kützing 1846	4–6	+	Diffluent	Colonies often clathrate, large, 600–900 μm long, cells in old colonies loosely spread	Brackish and fresh water, frequent, forms heavy water-blooms
**M. botrys* Teiling 1942	5–6	+	Radial, indistinct tube-like formations	Large, with sub-colonies, outer cells arranged in more or less radial rows	Frequent in water-blooms, probably often misinterpreted as *Microcystis aeruginosa*
**M. flos-aquae* (Wittrock) Kirchner 1898	(2.5) 3.5-4.8	+	Very narrow	Densely packed cells	Often associated with, and misidentified as, *Microcystis aeruginosa*
**M. ichthyoblabe* Kützing 1843	2–3.2	+	Wide, fine, diffluent	Spherical, ellipsoidal, composed of small colonies, easily broken up	Uncommon, causing fish kill
M. natans Lemmermann ex Skuja 1932	1.5–(2.23)	+	Diffluent, fine	40–200 μm, spherical to oval	Frequent, probably overlooked
M. firma (Kützing) Schmidle 1902	(0.8)2-3.7(4.8)	+	Indistinct mucilaginous sheath, which does not exceed the cell clusters	Spherical to more or less irregularly spherical, never clathrate, densely packed cells.	In stagnant fresh or brackish water. Known from Northern Europe, sporadic records from the tropics.
M. smithii Komárek et Anagnostidis 1995	3.2-5.6	+ With one or several brownish aerotopes in every cell	Mucilage fine, colourless, more or less distinctly delimited or diffuse, homogeneous without refractive margin.	Spherical or slightly irregular, later gelatinous, never clathrate, cells in a scattered arrangement.	Planktic in clear freshwater lakes. N. America, Argentine, N. Europe, Poland and N. Greece

2.2.5. Table 1 (2). Characteristic features of *Microcystis* species.

Species	Cell size (μm)	Aerotopes	Mucilage	Colonies, size (μm), shape	Comments
M. novacekii (Komárek) Compère 1974	(2.4)3-5.5(6)	+	Colourless or pale yellowish, sometimes irregular and slightly concentrically stratified, wide without refractive margin.	Microscopic almost spherical colonies that can grow to macroscopic size, composed of sub-colonies, lenticular with densely packed cells	Freshwater, planktic in eutrophic to mesotrophic lake, ponds, and reservoirs, can form water blooms, common in the tropics and occasional during summer in the temperate zone.
**M. viridis* (A. Braun in Rabenhorst) Lemmermann 1903	3.5–7(7.9)	+	Well defined, waved, more or less refractive	Small, packet-like colonies	Occurs often with other *Microcystis* species
**M. wesenbergii* (Komárek) Komárek in Kondrateva 1968	4–7-(8.5)	+	Well-defined, smooth, distinctly refractive	Ellipsoidal to irregular, sack-like	Sometimes forms monospecific water-blooms
**M. panniformis* Komárek, Komárková-Legnerová, SantÁnna, Azevedo et Senna 2002	(2.5)3-4.6(4.8)	+ Small dot-like aerotopes	Colourless, not extending beyond the cells, homogeneous, indistinctly visible, diffuse	Initially irregular tightly aggregated few celled, three-dimensional clusters of cells. Later more or less spherical or elongated with cells aggregated close to the surface of the colony. Colonies sometimes hollow, old colonies large, macroscopic	Widely distributed in eutrophic water reservoirs in Brazil, also found on Cuba, in Australia. Can form monospecific water blooms, probably toxic (Komárek *et al.* 2002)
M. protocystis Crow 1923	(3)3.5-6.5	+	Indistinct, colourless, homogeneous slime with diffuse margin. The slime widely overlaps the cells.	Colonies usually microscopic, irregular in outline, without distinct lobes and never with holes. Cells with individual mucilage, sparsely distributed in the colony, which easily disintegrates into solitary cells.	It occurs in mesotrophic to eutrophic reservoirs. It can form monospecific water blooms with high biomasses. It is widespread throughout the tropics, thus known from tropical Africa, Asia and America (Komárek *et al.* 2002).

*Toxigenic or harmful species.

From: Komárek and Anagnostidis (1999) and Komárek *et al.* (2002).

2.2.6. Table 2 (1). Characteristic features of *Anabaena* species. (*=Toxigenic or harmful species; ø: diameter; W: width; L: length; dimensions in μm.

Straight trichomes	Vegetative cells	Heterocytes	Akinetes	Trichomes	Mucilage layer	Habitat	References
A. planctonica Brunnthaler 1903	Short, barrel-shaped to spherical W 9-15 L up to 10	Spherical Ø 9-16	Ellipsoidal to cylindrical, distant W 9-21 L 15-30-35	Solitary, straight, long	+ Diffluent	Eutrophic	Geitler (1932); Komárková-Legnerová & Eloranta (1992)
A. smithii (Komárek) Watanabe 1991	Short, compressed, barrel-shaped to spherical W 9-15 L 9-10	Spherical Ø 7-12	Spherical, sometimes slightly angular Ø (12)15-30	Solitary, straight	+ Diffluent	Highly eutrophic, water blooms common	Watanabe (1991)
A. solitaria Klebahn 1895	Spherical to ellipsoidal W 6.5-7.5-10 L 6.5-8-12	Spherical to lemon-shaped. Of the same size as vegetative cells	Long, cylindrical wider than the filament W 10-12-16 L 20-45	Solitary, straight	+ wide mucilagenous sheath	Eutrophic, forming water blooms in temperate region	Komárková-Legnerová & Eloranta (1992
**A. macrospora* Klebahn 1895	Spherical to lemon-shaped Ø 5-6.5 or [5-7 x 6-9]	Spherical Ø 5-7	Single, seldom in pairs, separated, widely ellipsoidal, epispore bursts at excentric line, polyhedral form 17-19 x 20-26	Solitary, Straight, sometimes bent	–	Eutrophic, Water-blooms	Komárková-Legnerová & Eloranta (1992)
A. viguieri Denis et Frémy 1923	Spherical or slightly barrel-shaped W 5.5-7.5 L 4-6	Spherical or shorter than wide W 5-7 L 4-5.5	Widely oval to almost cylindrical W 9-11 L 15-24	Straight or slightly curved	–	Eutrophic, not in masses, common	Komárková-Legnerová & Eloranta (1992)
**A. bergii* Ostenfeld 1908	Spherical or short, barrel-shaped, W 8 L <8	Spherical to slightly compressed Ø 10	Spherical to oval, distant from heterocytes, brownish W 20 L 24	Solitary, tapering to ends, end cells conical, bluntly pointed	–	Eutrophic, grows at higher conductivity, brackish water	Ostenfeld (1908); Couté & Preisig (1978)

2.2.6. Table 2 (2). Characteristic features of *Anabaena* species (*= Toxigenic or harmful species; ø: diameter; W: width; L: length; dimensions in μm).

Straight trichomes	Vegetative cells	Heterocytes	Akinetes	Trichomes	Mucilage layer	Habitat	References
A. torulosa (Carm.) Lagerheim 1883.	Barrel-shaped, W 4.2-5 L 3-5	Cylindrical to almost spherical W 6 L 6-10	Rounded-cylindrical, in the middle often narrowed W 7-12 L10 24	Straight with conical end cells	Trichomes aggregate to form thin slimy blue-green layer	Appears in standing fresh and brackish water, attached to water plants, in temperate zone, and in the tropics, cosmopolitan	Lagerheim (1883) Huber-Pestalozzi 1938.
A. austro-africana Cronberg et Komárek 2004	Spherical to barrel-shaped, isodiametric, or slightly shorter than wide W 4-6	Spherical Ø 3.5-4-7.2	Almost spherical, develop on both sides of the heterocyte W 9.2-11.2 L 10.6-14.8	Straight or slightly curved, attenuated towards the ends	–	Mesotrophic lakes or ponds. Recently described from southern Africa, probably wider distribution	Cronberg & Komárek (2004)
A. carmichaelii Cronberg et Komárek 2004.	Cylindrical to barrel shaped, isodiametric or longer than wide W 3.5/3.8	Spherical, up 7 or more heterocytes per trichome, Ø 4	Cylindrical or oval or widely oval, solitary or in pairs, 2-4 cells distant from heterocytes, W 8-10 L 9-12	Straight or curved, slightly attenuated towards the ends	+ With mucilaginous sheaths,	Mesotrophic lakes or ponds. Described from Africa but have also been found in Laos and India.	Cronberg & Komárek (2004)
A. maxima Cronberg et Komárek 2004.	Cells distinctly barrel-shaped, usually isodiametric, W 9-11.4	Spherical or slightly shorter than wide, slightly wider than vegetative cells, Ø up to 12	Cylindrical to oval, with granular content, brownish epispore and asymmetric juncture. W 13.20.4 L 16-43	Solitary, long, straight, curved or irregular waved.	– Clearly constricted at cross walls	Planktic in large lakes in Southern Africa, forming weak water blooms. Also recorded from Brazil.	Cronberg & Komárek (2004)

2.2.6. Table 2 (3). Characteristic features of *Anabaena* species (*=Toxigenic or harmful species; ø: diameter; W: width; L: length; dimensions in μm.

Coiled trichomes	Vegetative cells	Heterocytes	Akinetes	Trichomes	Mucilage layer	Habitat	References
A. crassa (Lemmermann) Komárková-Legnerová et Cronberg 1992	Spherical to barrel-shaped, slightly shorter than wide Ø 11-12.5-15	Spherical Ø 10-17	Widely ellipsoidal, distant from heterocytes W 13-22-25 L 15-28-35	Regularly spirally twisted	+ Dense, wide	Eutrophic	Komárková-Legnerová & Cronberg (1992)
**A. circinalis* Rabenhorst ex Bornet et Flahault 1888	Spherical to barrel-shaped Ø 8-11-14	Spherical to widely ellipsoidal Ø 9-12	Elongated, ellipsoidal to cylindrical W 15-21 L 20-28	Wide regular spirals	- (+) Narrow, diffluent	Eutrophic	Komárek (1958)
**A. spiroides* Klebahn 1895	Spherical to widely ellipsoidal, sometimes barrel-shaped Ø 7-8-8.6	Spherical Ø 7-9	Widely to elongated ellipsoid, slightly curved, distant from heterocytes W 10-14-15 L 17-22	Solitary, regularly or irregularly coiled	–	Water-blooms common	Komárková-Legnerová & Eloranta (1992)
A. compacta (Nygaard) Hickel 1985	Spherical Ø 4-5	Spherical Ø 3-5	Widely ellipsoidal W 6-7 L 7.7-10	Solitary, regularly coiled 200-300 μm 20-30 spirals	+ Narrow	Eutrophic	Hickel (1985)
**A. flos-aquae* Brébisson ex Bornet et Flahault 1888	Spherical Ø 4-5	Spherical to slightly ellipsoidal Ø 4-5	Ellipsoidal to cylindrical, slightly arcuate, apart from heterocytes, one to three in a row W 5.5-13 L (13)-15-35	Irregularly, rarely regularly twisted, forming clusters	–	Eutrophic	Starmach (1966); Komárková-Legnerová & Eloranta (1992)
**A. lemmermannii* var. *lemmermannii* P. Richter 1903	Elongated cylindrical with rounded ends W 3-4 L 4-8-12	Shortly ellipsoidal or ovoid, disposed between two akinetes W 4-6 L 5-7	Elongated cylindrical with rounded ends, straight or slightly bent, next to heterocyte, star-like disposed W 6-7-10 L 15-23-29	Irregularly loosely twisted, clusters	+ Weak, diffluent	Mesotrophic to oligotrophic	Komárková (1988)

2.2.6. Table 2 (4). Characteristic features of *Anabaena* species (*=Toxigenic or harmful species; ø: diameter; W: width; L: length; dimensions in μm).

Coiled trichomes	Vegetative cells	Heterocytes	Akinetes	Trichomes	Mucilage layer	Habitat	References
*A. *lemmermannii* var *minor* (Utermöhl) Komárková-Legnerová 1988	Spherical to shortly barrel-shaped Ø 3-4.5-(9)	Spherical to oval, disposed between two akinetes, Ø 4-6	Elongated cylindrical with rounded ends, straight or slightly bent, next to heterocyte, star-like disposed W 6-9 L 11-22	Individual or loosely irregularly twisted	+ Weak, diffluent	Eutrophic lakes, humic character, frequent in Europe	Komárková (1988)
*A. *mendotae* Trelease 1889	Cylindrical, longer than wide W 2.5-4 L 6-10	Widely oval to cylindrical W 4-7 L 5.4-7-11	Long cylindrical, slightly bent W 5.5-7 L 16-30-40	Irregularly twisted in loops, individual large circles	–	Slightly eutrophic lakes	Komárková-Legnerová & Eloranta (1992)
A. perturbata var. *tumida* (Nygaard) Cronberg et Komárek 1992	Spherical Ø 6-7.5-9	Spherical Ø 5.5-6	Wide kidney-shaped W 10-13.5 L 18-23	Coiled circular	–	Eutrophic, scarce	Cronberg & Komárek (1992)
*A. *farciminiformis* Cronberg et Komárková-Legnerová 1988	Kidney-shaped W 4.3-4.8-5.7 L 5.9-17.1	Spherical to ellipsoidal W 6.4-8.6 L 7.1-10	Cylindrical, arcuate, rounded ends W 7.1-8.6 L 22.2-30	Twisted irregular spirals	–	Eutrophic	Cronberg and Komárková (1988); Willén & Mattson (1997)
A. bituri Cronberg et Komárek 2004	Spherical, isodiametric, all cells morphologi-cal the same Ø 3.5-3.8	Spherical, solitary, intercalary, slightly wider than vegetative cells.	Cylindrical, slightly curved, abruptly rounded ends, distant from heterocytes, W 4.5-5 L 9-10	Mostly solitary, tightly irregularly twisted, screw-like	–	Planktic in small and large water bodies, forming part in water blooms. Known to date only from Zimbabwe	Cronberg & Komárek (2004)
A. nygaardii Cronberg et Komárek 2004	Cells spherical, shorter than wide, with aerotopes, Ø 5.5-6	Spherical, solitary, intercalary, Ø 6.5-7	Widely oval, sometimes slightly asymmetric, solitary, with smooth colourless epispore, W 10-11 L 16-16.5	Tightly coiled, with irregular spiralled with up to five coils in loose spiral.	– Invisible mucilaginous	Common in eutrophic, water bodies, forming water blooms. To date only found in Africa	Cronberg & Komárek (2004)
A. reniformis (Lemmermann) emend. Aptekarj 1927	Spherical to oval W 4 L 7-8	Nearly spherical to oval, Ø 4	Spherical on both sides of the heterocytes, Ø 8.5-11	More or less spirally coiled, sometimes forming small clusters	–	Found in small slightly saline water bodies in northern Germany and also recorded from Brazil	Lemmermann 1898 Aptekarj 1927

2.2.7. Table 3. Characteristic features of *Aphanizomenon* species (dimensions in μm).

Species	Vegetative cells	Terminal cells	Heterocytes	Akinetes	Trichomes	Habitat	References
**A. flos-aquae* Ralfs ex Bornet et Flahault 1886							
Diacritic features	Cylindrical	Hyaline, cylindrical, rounded ends	Cylindrical	Elongated cylindrical	Form fascicles, cell width the same over the whole trichome, constrictions at the cross walls	Polluted and eutrophic water also brackish water	Komárek & Kovácik (1989)
Diameter	(4.6) 5–6.8 (7.8)	(4) 4.3-5 (6.4)	5-6.4	7.1-10			
Length	(5) 5.7-12.1 (13.6)	(7.1)10.7-18.6 (24.3)	10-14.3 (24.3)	(42.8) 54.3-82.8 (88.5)	bundles 80-150-2000.		
**A. flos-auae var. klebahnii* Elenkin 1909							
Diacritic features	Cylindrical	Hyaline, cylindrical, rounded ends	Elliptic	Elongated cylindrical	Form spindle-like bundles	Eutrophic water bodies also brackish water	Komárek & Kovácik (1989)
Diameter	4.3-5.5 (5.7)	2.8-4.5 (5)	3.1-5 (5.7)	(5.5) 7.1-8.6 (9.3)			
Length	(4.3) 5.7-10.7 (11.4)	(7.1) 8.6-15.7 (17.1)	(6.4) 7.1-10.7 (11.4)	(30) 35.7-47.1 (54.3)	bundles up to 500		
**A. gracile* (Lemmermann) Lemmermann 1907							
Diacritic features	Barrel-shaped	Narrowing, not hyaline	Round to cylindrical	Cylindrical with cup-like structures at the ends	Solitary	Eutrophic, also brackish water	Watanabe (1991) Pereira *et al.* (2004)
Diameter	(2.3) 2.6-3.1 (3.7)	1.7-2.1 (2.8)	3.4-4.9 (5.9)	(2.9) 3.9-5.9 (6.4)			
Length	(2.6) 2.8-6.4 (7.1)	(3.6) 4.3-6.4 (7.4)	3.9-7.8 (9.8)	(6.4) 7.8-14.7(16.7)	20-30-60		
A. skujae Komárková-Legnerová and Cronberg 1992							
Diacritic features	Cylindrical	Narrow, elongated with ends bluntly pointed	Oval to cylindrical	Cylindrical with rounded ends	Solitary	Eutrophic	Komárková-Legnerová & Cronberg (1992)
Diameter	1.7-2.1-2.6	1-1.5	1.9-2.8-3	2.7-3.6-3.7			
Length	4.2-6.6-16.8		7.5-8.5-11.4	25-27-34 (40)	Up to 300		
A. yezoense Watanabe 1991							
Diacritic features	Cylindrical	Elongated, cylindrical, with abruptly rounded ends	Cylindrical	Long, cylindrical	Solitary or in bundles, not constricted at cross walls	Mesotrophic	Watanabe (1991)
Diameter	2.7-4.0	2.8-4.0	3.8-5.1	4.7-7.3			
Length	3.1-7.8	10.9-28.8	5.4-11.4	31.2-48.9	100-180-330		
A. issatschenkoi (Usačev) Proškina-Lavrenko 1962							
Diacritic features	Oval	Pointed end cells	Oval to cylindrical	Oval to cylindrical	Solitary	Eutrophic water bodies	Watanabe (1991)
Diameter	2.6-3.5-4.4	1.2-1.9-2.9	3.3-4.1-6.2	4.3-5.6-7.0			
Length	5.5-8.8-15.9	8.7-22.6-34.8	7.0-8.8-10.9	9.3-14.1-18.7	180-600		
**A. ovalisporum* Forti 1911							
Diacritic features	Cylindrical	Rounded	Oval	Oval to barrel-shaped	Solitary, constricted at cross walls	Mesotrophic	Forti (1911) Banker *et al.* (1997)
Diameter	4-5	3-5	5-7	10-12-14			
Length	6-7	25-30	8-12	8-14-20	500-1500		
A. capricorni Cronberg et Komárek 2004							
Diacritic features	Cylindrical	Long, conical	Barrel-shaped to cylindrical	Oval, on one or both sides of the heterocyte	Solitary, constricted at cross walls	Eutrophic to mesotrophic water bodies. Africa, East Asia	Cronberg & Komárek (2004)
Diameter	2-3.5	1.5-2	2-3.5	7-8	Up to 200		
Length	3.3-7.4	5-10	3.8-5	11-12			
A. tropicale Horecká et Komárek 1979							
Diacritic features	Cylindrical	Elongated, conically narrowed to a sharp point	Long, cylindrical	Oval, on both sides of the heterocyte	Solitary, long, slightly flexuous, not constricted at cross walls	Eutrophic species, appears with *Anabaena* and *Microcystis* species, tropical distribution	Horecká & Komárek (1979)
Diameter	2.6-3.5		4-5	4-7			
Length	up to 11.8			9-14			

2.2.8. Table 4. Diacritical features of *Aphanizomenon capricorne, A. issatschenkoi, A. tropicale, Cylindrispermopsis raciborskii and Raphidiopsis mediterranea* (dimensions in μm).

	Species	***Aphanizomenon capricorni***	***Aphanizomenon issatschenkoi***	***Aphanizomenon tropicale***	***Cylindrospermopsis raciborskii***	***Raphidiopsis mediterranea***
Trichomes	Length	200	7-160-600	-	250	40-163
	Form	Straight or slightly bent	Straight or slightly bent	Long, slightly flexuous	Straight or rarely coiled	
Cells	Width	2-3.5	1.5-4	2.6-3.5	(1.8)2.5-4	2-2.5
	Length	3.3-7.4	4-8(12)	3.1-1.8	2.5-16	4-10
	Form	Cylindrical, constrictions at cross walls	Cylindrical, not or slightly constricted at cross walls	Long, cylindrical, constricted at cross walls	Long cylindrical, no constrictions at cross walls	Cylindrical, no constrictions at cross walls
Heterocytes	Width	2-3.5	(1.5)2-3	2.6-3.5	2-2.5	-
	Length	3.5-8	6-10	7.4-11.2	5-7	-
	Position	Intercalar	Intercalar	Intercalar	Terminal	None
	Form	Barrel-shaped to cylindrical	Cylindrical	Spherical to oval	Conical at the end	-
Akinetes	Width	7-8	2-4.5	4-7	2.5-4	2.5-3
	Length	11-12	6-10	9-14	8-15	6,5-13
	Position	Both sides of heterocyte	Distant from heterocytes	Both sides of heterocyte	Next to terminal cells	Next to terminal cells
	Number	1-2	2-3 in chain	1-2	1-(2)	1-(2)
	Form	Oval	Cylindrical	Oval to cylindrical	Oval to cylindrical	Cylindrical
End cells	Form of end cell	Elongated, with apices narrowly conical, but not to a point	1-4 end cells, attenuated, tapered end cells, at the apices pointed	Attenuated with elongated conical and pointed, hyaline end cells.	Attenuated rounded	Attenuated pointed
References		Cronberg & Komárek (2004)	Proškina-Lavrenko & Makorova (1968)	Horecká & Komárek (1979)	Cronberg & Komárek (2004)	Skuja (1937)

2.2.9. Table 5 (1). Toxigenic cyanobacteria from marine, brackish and freshwaters.

Species	Toxin or toxic effect	Habitat	References
Chroococcales			
Coelosphaerium kuetzingianum NÄGELI	neurotoxins, hepatotoxins, mouse-test	Freshwater	Fitch *et al.* 1934
Cyanobium bacillare (BUTCHER) KOMÁREK; SYN. *Synechococcus bacillare* BUTCHER	microcystins	Brackish water	Cronberg *et al* 2003
Microcystis aeruginosa KÜTZING	microcystins	Freshwater	Carmichael *et al.* 1988
Microcystis botrys TEILING	microcystins	Freshwater	Henriksen 1996
Microcystis ichthyoblabe KÜTZING	microcystins	Brackish and freshwater	Sabour *et al.* 2002
Microcystis viridis (A. BR.) LEMMERMANN	microcystins	–	Watanabe *et al.* 1988
Microcystis wesenbergii KOMÁREK	microcystins	Freshwater	Yasuno *et al.* 1995
Snowella lacustris (CHODAT) KOMÁREK; SYN. *Gomphospaeria lacustris* CHODAT	hepatotoxins, mouse-test	Freshwater	Gorham et Carmichael 1988
Synechococcus sp. NÄGELI	hemolytic toxin	Brackish/marine	Mitsui *et al.* 1987
Synechocystis sp. SAUVAGEAU	microcystins	Wastewater pond	Oudra *et al.* 1998; Odebrecht *et al.* (2002).
Woronichinia naegeliana (UNGER) ELENKIN; SYN. *Gomphospaeria naegeliana* (UNGER) LEMMERMANN	neurotoxins, hepatotoxins, mouse-test	Freshwater	Berg *et al.* 1986
Oscillatoriales			
Arthrospira fusiformis (VORONICHIN) KOMÁREK et LUND	microcystins, anatoxin-a	Alkaline-saline lakes	Ballot *et al.* 2004
Limnothrix redekei van GOOR	microcystins	Freshwater	Gkelis *et al.* 2001
Lyngbya aerugineo-coerulea (KÜTZ.) GOMONT	neurotoxins, hepatotoxic	Feshwater	Teneva *et al.*, 2003
Lyngbya majuscula HARVEY	neurotoxins, lyngbyatoxin-a, debromoaplysiatoxin	Marine/tropical water	Osborne *et al.* 2001
Lyngbya wollei (FARLOW ex GOM.) CARMICHAEL *et al.*	PSP-toxins	Freshwater	Carmichael *et al.* 1988; Carmichael *et al.* 1997
Oscillatoira acutissima KUFFERATH	dermatoxin	Freshwater	Barchi *et al.* 1983
Planktothrix agardhii (GOM.) ANAGN. & KOM.; SYN. *Oscillatoria agardhii* GOM.	microcystins, anatoxin	Freshwater	Sivonen *et al.* 1989
Planktothrix rubescens (DeCANDOLLE ex GOMONT) ANAGN. & KOM.; SYN. *Oscillatoria rubescens* DeCANDOLLE	microcystins	Freshwater	Skulberg and Skulberg 1985, Sivonen *et al.* 1989, Ericsson 1990

2.2.9. Table 5 (2). Toxigenic cyanobacteria from marine, brackish and freshwaters.

Species	Toxin or toxic effect	Habitat	References
Planktothrix isothrix (Skuja) Komárek & Komárková 2004; SYN. *Planktothrix mougeotii* (Bory ex Gomont) ANAGN. & KOM. 1988; *Oscillatoria agardhii* var. *isothrix* SKUJA 1948	microcystins, anatoxin	Freshwater	Carmichael 1988
Planktothrix planctonica (ELENK.) ANAGN. - KOM. SYN. *Oscillatoria ornata* f. *planctonica* ELENK.	microcystins	Freshwater	Nogueira et Vasconcelos 2001
Planktothrix perornata (SKUJA) ANAGN. - KOM. SYN. *Oscillatoria perornata* SKUJA	microcystins	Freshwater/tropical	Nogueira et Vasconcelos 2001
Phormidium formosum (BORY) KOM. & ANAGN. SYN. *Oscillatoria formosa* BORY	microcystins, homoanatoxin-a	Freshwater	Skulberg *et al.* 1992; Steffensen *et al.* 2001
Oscillatoria nigro-viridis THWAITES	debromoaplysiatoxin, oscillatoxin-a	Marine water	Mynderse *et al.* 1977
Pseudnanbaena catenata LAUTERBORN	anatoxin	Freshwater	Gorham *et al.* 1982
Trichodesmium erythraenum (EHRENB.) GOM.	neurotoxins, microcystins	Marine water	Hawser et Codd 1992; Shaw *et al.* 2001; Janson *et al.* 1995
Trichodesmium thiebautii GOM. ex GOM.	neurotoxins, microcystins	Marine water	Hawser et Codd 1992; Shaw *et al.* 2001; Janson *et al.* 1995
Nostocales			
Anabaena circinalis RABENH.; SYN. *Anabaena hassalii* (KÜTZING) WITTROCK	anatoxins, PSP-toxins	Freshwater	Sivonen *et al.* 1989; Humpage *et al.* 1994
Anabaena flos-aquae (LYNGBYE) BRÉBISSON	anatoxin-a	Freshwater	Carmichael et Mahmood 1984
Anabaena macrospora KLEBAHN	anatoxin-a	Freshwater	Park *et al.* 1993
Anabaena mendotae TRELEASI	anatoxin-a	Freshwater	Rapala *et al.* 1993
Anabaena lemmermannii P. RICHTER	anatoxin-a(s)	Freshwater	Henriksen 1996
Anabaena planctonica BRUNNTAHL	anatoxin-a	Freshwater	Park *et al.* 1993
Anabaena spiroides KLEB.; SYN. *Anabaena spiroides* var. *contracta* KLEB.	anatoxin-a	Freshwater	Park *et al.* 1993
Anabaena variabilis KÜTZING	anatoxin	Freshwater/marine water	Andrijuk *et al.* 1975
Anabaenopsis milleri WORONICHIN	anatoxin	Freshwater	Lanaras *et al.* 1989
Aphanizomenon gracile LEMM.	PSP-toxins	Brackish/freshwater	Pereira *et al* 2001
Aphanizomenon flos-aquae (LINNEUS) RALFS	anatoxin, PSP-toxin	Freshwater	Rapala *et al.* 1993, Ikawa *et al.* 1982

2.2.9. Table 5 (3). Toxigenic cyanobacteria from marine, brackish and freshwaters.

Species	Toxin or toxic effect	Habitat	References
Aphanizmenon ovalisporum FORTI	cylindrospermopsin	Freshwater	Banker *et al.* 1997
Cylindrospermum sp. KÜTZING	neurotoxins	Freshwater	Sivonen *et al.* 1989
Cylindrospermopsis raciborskii (WOLOSZYNSKA) SEENAYA & SUBBA RAJU	cylindrospermopsin	Freshwater	Hawkins *et al.* 1985
Gloeotrichia echinulata (J. E. SMITH) P. RICHTER	neurotoxins, hepatotoxic	Freshwater	Ingram et Prescott 1954
Hormothamnion enteromorphoides GRUNOW	hepatotoxins,	Marine/tropical water	Gerwick *et al.* 1986
Nodularia spumigena MERTENS	nodularin	Brackish water	Runnegar *et al.* 1988, Carmichael *et al.*, 1988
Nostoc linckia (ROTH) BORNET & FLAHAULT	microcystin	Brackish/fresh water	Ransom *et al.* 1978
Nostoc paludosum KÜTZING	microcystins	Freshwater	Andrijuk *et al.* 1975
Nostoc rivulare KÜTZING	microcystins	Freshwater	Davidson 1959
Nostoc zetterstedtii ARESCHOUG	microcystins	Freshwater	Mills et Wyatt 1974
Nostoc sp.	microcystins	Fresh water	Beattie *et al.* 1998
Nostoc sp.	microcystins	Freshwater	Sivonen *et al.* 1990, 1992
Raphidiopsis mediterranea Skuja	homoantoxin-a, anatoxin.a	Freshwater	Namikoshi *et al.* 2003
Stigonematales			
Fisheriella epiphytica GHOSE	neurotoxins, hepatotoxic	Eptiphytic/tropical	Ransom *et al.* 1978
Hapalosiphon hibernicus W. et G. S. WEST	microcystins	Freshwater	Prinsep *et al* 1992
Hapalosiphon fontinalis (AGARDH) BORNET	neurotoxins, hepatotoxic	Freshwater	Moore *et al.* 1984
Schizothrix calcicola (agardh) GOM.	contact dermatitis	Freshwater	Mynderse 1977
Scytonema mirabile (DILLW.) BORN.	antimicrobial	Freshwater	Dow & Swoboda 2000
Scytonema ocellatum LYNGBYE ex BORNET & FLAHAULT	scytophycin	Epiphytic	Patterson et Bolis 1984
Scytonema pseudohofmannii BHARADWAJA	scytophycin	Epiphitic/tropical	Moore *et al.* 1986, Carmichael *et al.* 1990
Tolypothrix byssoidea (HASS.) KIRCHNER	cytotoxin	Epiphytic/cosmopolitan	Barchi *et al.* 1983
Umezakia natans HARADA 1994	cylindrospermopsin	Freshwater	Harada *et al.* 1994

3. Cyanotoxins and their effects

Background

In the past few decades, a large number of reports of mass developments of toxic cyanobacteria have appeared in national and international publications (Fig. 1). However, toxic blooms of cyanobacteria have been reported for over a century. In 1878 the first acute cyanotoxin poisoning of domestic animals was described by the Australian naturalist and chemist George Francis (1878).

The toxins produced by cyanobacteria are a diverse group of phycotoxins, both from the chemical and the toxicological point of view. They cause acute and possibly chronic health problems in humans and fatal poisoning in animals (Skulberg *et al.*, 1984; Carmichael, 1992, 1994).

The majority of cyanotoxins can be regarded as secondary metabolites i.e. compounds that are not involved in the organism´s primary metabolism (Carmichael, 1992; Lukac and Aegerter, 1993). Mechanisms of cyanobacterial toxicity are very diverse and range from hepatotoxic, neurotoxic, cytotoxic, dermatotoxic and pyrogenic effects to the general inhibition of protein synthesis.

Methods for the detection and analysis of cyanobacterial toxins are summarised by among others Chorus and Bartram (1999) Codd *et al.* (2001) Meriluoto and Codd (2005) Nicholson and Shaw (2001).

The neurotoxins

Neurotoxins are produced by several cyanobacterial genera, for example *Aphanizomenon, Planktothrix, Oscillatoria* and *Trichodesmium* (Carmichael, 1992, 1994; Keevil, 1991). The major neurotoxins are anatoxin-a, homoanatoxin-a, anatoxin-a(s), saxitoxin and neosaxitoxin (aphanotoxins I and II).

a) Anatoxin-a

Anatoxin-a is produced by *Anabaena, Oscillatoria, Aphanizomenon, Cylindrospermum* and *Phormidium* (Gugger *et al.*, 2005). The toxin is a low molecular weight, secondary amine and is a structural analogue of cocaine and

Fig. 1. A bloom of cyanobacteria in the recreational Lake Finjasjön, Sweden. (Photo: *Cronberg*).

the neurotransmitter acetylcholine. The nerve synapsis is the primary target organ in mammals. There is no antidote to anatoxin-a (Carmichael, 1994). Signs of poisoning are staggering, muscle fasciculations, gasping and convulsions. Death by respiratory failure occurs within minutes to a few hours depending on species and dose (Carmichael, 1992, 1994; Hunter, 1995; Keevil, 1991; Repavich *et al.*, 1990). The intraperitoneal LD_{50} for mice is 50-250 μg kg^{-1} body weight, with a survival time of 4-7 minutes (Carmichael and Gorham, 1978; Carmichael and Biggs, 1978; Kluge and Szinicz, 2005).

b) Homoanatoxin-a

This toxin has been purified from *Oscillatoria formosa* (*Phormidium formosum*) and *Raphidiopsis mediterranea* and characterised as a secondary amine alkaloid, methylene-anatoxin-a (Skulberg *et al.*, 1992; Namikoshi *et al.*, 2004). Just as anatoxin–a, it is a potent neuromuscular blocking agent with an intraperitoneal LD_{50} in mice of 250 μg kg^{-1} body weight. Lethal doses of homoanatoxin-a lead to severe body paralysis, convulsions and death by respiratory failure (Aas *et al.* 1996; Lilleheil *et al.*, 1997).

c) Anatoxin-a(s)

Anatoxin-a(s), is produced by strains of *Anabaena flos-aquae* (Mahmood and Carmichael, 1986a, 1987; Matsunaga *et al.*, 1989), and by strains of *Anabaena lemmermannii* (Henriksen *et al.*, 1997). The *s* in anatoxin-a (s) denotes salivation in vertebrates. Symptoms are similar to those of anatoxin-a with the addition of ataxia, diarrhoea, hypersalivation, and tremors. Anatoxin-a(s) is chemically different from anatoxin-a. It is a naturally occurring phosphate ester of a cyclic N-hydroxyguanine and functions as an acetylcholinesterase inhibitor similarly to the organophosphate insecticides such as malathion and parathion (Carmichael, 1992, 1994). Even though anatoxin-a(s) differs structurally from the synthetic organophosphate, its lethal power, just

Fig. 2. Mass development of *Cylindrospermopsis raciborskii* in Lake Kariba.
(Aerial photo: *Ian Games*).

like that of the pesticides, stems from its ability to inhibit acetylcholinesterase. The intraperitoneal LD_{50} for mice is 20 $\mu g\ kg^{-1}$, i. e., it is ten times more lethal than anatoxin-a (Carmichael *et al.*, 1990).

d) Saxitoxins

The saxitoxins are a group of carbamate alkaloid neurotoxins that interfere with neurotransmission by blocking the neuron sodium channels across the axon membrane. The presence of saxitoxin has been established in *Aphanizomenon flos-aquae, Aphanizomenon gracile, Anabaena circinalis, Cylindrospermopsis raciborskii* and *Lyngbya wollei* (Mahmood and Carmichael, 1986b; Molica *et al.*, 2002; Pereira *et al.*, 2004). These toxins cause symptoms such as irregular breathing, loss of co-ordination, twitching and death by respiratory failure (Carmichael 1992, 1994; Hunter, 1995; Keevil, 1991). The intraperitoneal LD_{50} for mice is 10–30 $\mu g\ kg^{-1}$ (Codd *et al.*, 2005; Kluge and Szinicz, 2005). The saxitoxins are also associated with a number of marine dinoflagellates e.g. *Alexandrium* spp. and *Pyrodinium bahamense* (Zingone and Enevoldsen, 2000) which are responsible for paralytic shellfish poisoning in humans and massive deaths of marine animals associated with "red tides" in coastal waters world-wide.

Hepatotoxins

a) Microcystins and nodularins

The major cyanotoxins causing death and illness in animals are the cyclic peptide hepatotoxins (liver toxins) (Carmichael 1992; Codd and Beattie, 1991; Keevil, I991, 1994; Sivonen, 1990).

Microcystins, monocyclic heptapetides, were named after the first organism found to produce them, *Microcystis aeruginosa* (Eriksson *et al.*,1988; Sivonen *et al.*, 1992; Carmichael *et al.*, 1988), but later studies showed their occurrence also in other cyanobacterial genera. Microcystins have been identified in bloom-forming species of *Anabaena, Microcystis, Planktothrix, Oscillatoria, Nostoc, Synechocystis* and *Anabaenopsis* (Carmichael, 1992, 1994; Codd and Beattie, 1991; Keevil, 1991; Luukkainen *et al.*, 1993, 1994, Oudra *et al.*, 2000). More than 70 structural variants are known in cyanobacteria (Codd *et al.*, 2004).

The monocyclic pentapeptide nodularin is structurally and toxicologically similar to microcystin. Nodularin, of which six variants are known, has been isolated from the brackish water cyanobacterium *Nodularia spumigena* (Fig. 3) (Rinehart *et al.*, 1988; Codd and Beattie, 1991; Codd *et al.*, 2004; Falconer, 1993; Hunter, 1995a, Keevil, 1991; Luukkainen *et al.*, 1993).

The symptoms of poisoning by cyanobacterial hepatotoxins in laboratory mice include diarrhoea, pallor of the mucous membranes, vomiting, weakness, anorexia and death (Carmichael, 1992, 1994; Hunter, 1995; Keevil, 1991). Death occurs within 1-2 h due to intrahepatic haemorrhage - liver necrosis and the disintegration of the architecture of the liver i.e. hepatic parenchyma and hypovolaemic shock (Carmichael, 1992, 1994; Hunter, 1995; Keevil, 1991; Repavich *et al.*, 1990).

The weight of the liver of the mouse may increase up to 100% as a consequence of internal haemorrhaging (Carmichael, 1992, 1994; Keevil, 1991).

Microcystins act similarly to another group of cyclic peptides, the thermostable hepatotoxic heptapeptides "phallotoxins" (e.g. phalloidin) of the poisonous mushroom *Amanita phalloides*, "the green death cap" (Lukac and Aegerter, 1993, Keevil, 1991; Wieland and Faulstich, 1978).

Microcystins enter the body from the intestine, probably via the bile acid transporter system (Ito *et al.*, 2000). From the blood, microcystins then bind quite specifically to liver tissue where they form adducts with serine protein phosphatases 1 and 2A (Craig *et al.*, 1996; Yoshida *et al.*, 1998) resulting in the inhibition of these important enzymes (Honkanen *et al.*, 1990, MacKintosh *et al.*, 1990). These enzymes are vital to various cellular processes such as cell growth and tumour suppression and are therefore possible potent cancer promoters (Carmichael, 1992, 1994; Falconer and Yeung, 1992; Luukkainen *et al.*, 1993, 1994; MacKintosh *et al.*, 1990; Runnegar *et al.*, 1995). Microcystin-LR is an extremely potent tumour promoter in laboratory animals (Falconer, 1991; Nishiwaki-Matsushima *et al.*,

1992) and is the most potent liver carcinogen yet characterised. Microcystins and nodularins have a high acute toxicity with LD_{50}'s of 36-122 μg kg^{-1} in mice and rats i.p. or i.v. (Dawson, 1998; Sivonen *et al.*, 1989).

Most of the existing data concerning the toxic effects of cyanobacteria on living organisms is based on studies using animal experimental systems, namely the mouse bioassay. Unfortunately the cyanotoxins interaction with plants is a neglected field. It is noteworthy that most species of plants - both dicotelydonous and monocotelydonous - are sensitve to microcystins. Microcystin-LR e.g. prevents the light induced activation of sucrose phosphatase synthetase via modulating the inhibition of protein phosphatase. A biotest system using the mustard plant () has been developed for studies of cyanotoxins and plant interactions (Kos *et al.*, 1995). Some cyanotoxins have a role as allelochemicals, and may influence ecosystems in a broader ecological perspective (Nagle and Inderjit, 2002), but that is outside the scope of this manual.

Microcystins influence the growth and photosynthetic capacity of water plants. In a study in which axenic plants of duckweed, *Lemna minor* L. were cultivated with different concentrations of Microcystin-RR (Weiss *et al.*, 2000) the highest concentrations (3 and 5 mg l^{-1}) decreased the formation of fronds after three days of treatment. Dwarfism, malformed fronds were recorded among the plants grown at 5 mg l^{-1} and some of these also showed chlorosis. The maximum electron transport rate, an indicator of photosynthetic capacity, was reduced up to 16 % compared to the values obtained from control plants. Moreover, chlorophyll a, b and the total amount of carotenoids were reduced by more than 50%.

Endotoxins (lipopolysaccharides - LPS)

Endotoxin was first described by Richard Pfeiffer (1892) as a heat-stable, cell-associated compound from *Vibrio cholerae* that induces toxic reactions

Fig. 3. *Nodularia spumigena* in profuse occurrence on the coast of the Swedish island of Öland, 2003. The poisoning and deaths of a number of dogs along the Baltic coast have been attributed to mass developments of this hepatotoxic cyanobacterium (Edler *et al.*, 1985). (Aerial photo: *Forssblad*).

in guinea pigs. The role of endotoxins is prominent during severe infection, trauma, and shock. Endotoxins stimulate monocytes/macrophages to produce cytokines such as IL-1, TNF-alpha, IL-6 and IL-10. When present in the bloodstream, endotoxins cause various pathophysiological reactions such as fever, leukopenia, tachycardia, tachypnea, hypotension, disseminated intravascular coagulation, and multi-organ failure (Ulmer, 1997).

In common with gram-negative bacteria, cyanobacteria form lipopolysaccharides as an important part of their outer cell envelope, but unlike the former, cyanobacterial lipopolysaccharides lack phosphate in the lipid A core (Keleti and Sykora, 1982).

Endotoxins have been isolated from a number of common, bloom-forming cyanobacteria such as *Anacystis nidulans* (=*Synechococcus* PCC73109 KM), *Microcystis aeruginosa*, *Anabaena flos-aquae* UTEX 1444, *Anabaena cylindrica, Oscillatoria tenuis* (=*Phormidium tenue*) and *Oscillatoria brevis* (= *Phormidium breve*) (Weise *et al*, 1970; Martin *et al*, 1989; Keleti and Sykora, 1982).

Cytotoxins

a) Cylindrospermopsin

The substance is a sulphated-guanidinium alkaloid with 5-substituted-2,4-dioxypyrimidine (uracil) moiety (Banker *et al.*, 1997; Harada *et al.*, 1994; Ohtani *et al.*, 1992). This hepatotoxin has been isolated from several cyanobacterial species as *Cylindrospermopsis raciborskii* (Fig. 2) (Ohtani *et al.*, 1992), *Umezakia natans* (Harada *et al.*, 1994), and *Aphanizomenon ovalisporum* in Israel (Banker *et al.*, 1997; Shaw *et al.*, 1999) and Australia (Smith *et al.*, 1998). It has been demonstrated that cylindrospermopsin causes both liver and renal damage in mice (Falconer *et al.*, 1999). Cylindrospermopsin has a LD_{50} for mice i. p. of 200 $\mu g\ kg^{-1}$ (Banker *et al.*, 2001). Pathological changes in livers exposed to cylindrospermopsin include inhibition of protein synthesis, fat droplet accumulation, membrane proliferation, and cell death. Exposure of mice to cylindrospermopsin at subacute concentrations, via drinking water, resulted in the development of abnormal red blood cells, known as acanthocytes (Reisner *et al.*, 2004).

b) Dermatotoxins

Lyngbyatoxin-A, aplysiatoxin and debromoaplysiatoxin are alkaloids produced by the brackish water and marine cyanobacterium *Lyngbya majuscula*.

Aplysiatoxin and debromoaplysiatoxin are structurally identical except that debromoaplysiatoxin does not have a bromine on the aromatic ring. Both toxins cause dermatitis in animals and humans. Aplysiatoxin may, unlike debromoaplysiatoxin, increase cell transformation and stimulate DNA synthesis in vitro (Solomon and Stoughton, 1978). The production of debromoaplysiatoxin has also been demonstrated in the cyanobacteria *Schizothrix calcicola* and *Oscillatoria nigro-viridis* (Moore, 1981, 1984).

Lyngbyatoxin-A is structurally related to teleocidin B, a toxic compound associated with *Streptomyces*. It was described from Hawaii by Cardellina *et al.*, (1979) as a vesicatory and inflammatory substance isolated from *Lyngbya majuscula*. Lyngbyatoxin A and aplysiatoxin may promote tumor formation by binding to the same receptor site to activate protein kinase C (Fujiki *et al.*, 1981).

Poisoning incidents of animals

A large number of cases of animal deaths, particularly cattle, sheep and dogs, have been reported from many parts of the world (Carmichael, 1992; Lukac and Aegerter, 1993; Luukainen *et al.*, 1993, 1994; Skulberg, 2005).

In contrast to pathogenic bacteria, the cyanobacteria do not proliferate within the body after uptake, only in the aquatic environment before uptake. The cyanotoxins are normally contained within the cells and are only released in substantial amounts on cell lysis.

The floating cyanobacteria can be concentrated by winds and currents to form a scum on shores (Carmichael 1994). Most poisoning incidents are, thus, connected with bloom-forming planktic species of cyanobacteria such as *Microcystis, Anabaena, Aphanizomenon* and *Nodularia* (Fig. 3).

Benthic cyanobacteria may, however, also cause

Fig. 4. Net sampling of cyanobacteria (Lake Naivasha, Kenya). (Photo: *Annadotter*).

health hazards. These cyanobacteria may grow on bottom sediments in waters which are sufficiently clear to allow light penetration to these surfaces.

In 1990, in the clear Loch Insh, Scotland, several dogs died after ingesting a neurotoxic benthic *Oscillatoria* scum (Codd and Beattie, 1991; Gunn *et al.*, 1992). Two of the dogs returning from a walk on the loch shore, died within ten minutes of the onset of clinical symptoms. A few days later another dog died after drinking from the lake. The dog showed cyanosis, rigors, limb twitching and hypersalivation and died within thirty minutes of the onset of illness. Flecks of algal material detached from the sediment were driven inland by wind and wave action. Unlike the planktic cyanobacteria, toxic algal material from the lake bottom is likely to become apparent only when it accumulates on the shore.

In Switzerland, cattle died from ingestion of benthic *Oscillatoria limosa* after drinking from pristine mountain lakes (Mez *et al.*, 1997).

Marine cyanobacteria are involved in poisoning of sea animals. Antagonistic reactions and antibiosis are described in connection with mass development of *Trichodesmium* spp. Death of fish, oysters and crabs have been reported in tropical and warm temperate waters. Bloom samples from Caribbean waters were reported highly neurotoxic to mice, leading to severe convulsions and death within 2-30 minutes by respiratory arrest (Chidambaram and Unny, 1944; Nagabhushanam, 1967; Chellum and Alagarswami, 1978; Hawser and Codd, 1992).

The frequency of toxic blooms

How frequently do toxic cyanobacterial blooms occur in the natural environment?

In a study of cyanobacterial blooms in Finnish fresh and coastal waters, 44 % (83 out of 188) of the blooms were found to be lethally toxic by mouse bioassay (Sivonen *et al.*, 1990). Liver-toxic blooms were almost twice as common as neurotoxic ones.

In an investigation of more than one hundred freshwater sites in the United Kingdom, approximately 67 % of the cyanobacterial blooms were toxic (Codd and Beattie, 1991).

In a study of 331 lakes with water blooms in Sweden (Willén and Mattsson, 1997) 156

(47%) lakes showed toxicity by mouse bioassay. Hepatotoxic samples were about six times more common than neurotoxic ones.

Based on the results of cyanotoxin surveys from six countries – Norway, Denmark, Portugal, Germany, the Czech Republic and Korea - Chorus (2001) suggested a number of generalizations on cyanotoxin occurrence and concentration ranges. Hepatotoxins were found more frequently than cyanobacterial neurotoxins, but there seems to exist a geographic difference in dominance of microcystin-producing taxa. In southern Norway, almost 50% of the water bodies with hepatotoxic cyanobacteria were dominated by *Anabaena* spp. whereas *Microcystis* spp. and *Planktothrix* each dominated in only 25 % of the lakes. In the study from Denmark, microcystins occurred most frequently in samples with a mixture of several cyanobacterial taxa, followed by samples dominated by *Microcystis*. In Germany, *Planktothrix* was the most common dominant taxon in samples containing microcystins, closely followed by *Microcystis*. In the Czech Republic, Portugal, and Korea, *Microcystis* spp. were the dominant microcystin producers in more than 67-75% of the samples studied.

It was further observed, based on all six studies, that most populations of *Microcystis* and *Planktothrix,* but only about half of the *Anabaena*-populations, contained microcystins or showed hepatotoxicity. Toxicity, not accounted for by the currently known cyanotoxins, was frequently observed in the studies, especially in surveys in which both bioassays and chemical analysis were utilised. Several of the studies reported samples with neither hepatotoxicity nor neurotoxicity but with a pronounced, protracted toxic effect causing death of bioassay mice 4-24 h after injection, with no direct observable signs of organ damage (Skulberg *et al.*, 1994).

Endotoxins connected with phytoplankton were investigated in water samples from 21 water bodies in Kenya (Fig. 4), Zimbabwe, Tanzania, Uganda and Sweden (Annadotter *et al.*, 2005). Endotoxins in the samples, dominated by eukaryotic phytoplankton, ranged between 4 and 110 Endotoxin Units (EU) ml^{-1}, wheras endotoxins in the cyanobacteria-dominated samples varied between 110 and 16 000 EU ml^{-1}.

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4. Human health effects of cyanobacteria. Some selected case studies

Background

There are large numbers of documented incidents of livestock poisoning after consuming water with toxic cyanobacteria. However, information on harmful exposure of humans to cyanotoxins is limited worldwide. This is probably due to the fact that cases of illness are not sufficiently rapidly ascribed to cyanotoxins as a potential cause, and thus no relevant investigations have been undertaken to confirm or invalidate it.

Exposure to toxic cyanobacteria via drinking water is a key concern for human health, regarding both acute and chronic effects. In some geographical areas, cyanobacteria may mass-develop in water resources over extended periods of time (Hitzfeld *et al.*, 2000; Huszar *et al.*, 2000). A number of water-borne outbreaks of illness, associated with the occurrence of cyanobacteria in water reservoirs, have been reported from different parts of the world.

Cyanotoxins in drinking water - health injuries

a) Early reported outbreaks of illness associated with cyanobacteria.

In 1931, cyanobacteria in the Ohio River were pointed out as the cause of a number of outbreaks of gastrointestinal illness in several towns along the river. Infectious agents that could explain the outbreaks were not detected (Tisdale, 1931).

b) Water-borne illness among infants in Harare.

1960-1965, severe outbreaks of a gastro-intestional disorder occurred annually among young children in Harare, Zimbabwe (Zilberg, 1966). No pathogens were identified that could explain the disorders. The illness occurred only in those parts of the town that received their water from the eutrophic Lake Chivero, and at that time of the year when cyanobacteria mass-developed in the drinking water reservoir. A retrospective study discovered a close relationship between the number of colonies of the cyanobacteria *Microcystis aeruginosa* and *Anabaena flos-aquae* and the number of gastrointestinal cases (Marshall, 1991).

c) An outbreak of hepatoenteritis on Palm Island, Australia.

In 1979, 39 children and 10 adults fell ill after consuming water which probably contained the cyanobacterium *Cylindrospermopsis raciborskii* (Byth, 1980). This illness occurred in an Aboriginal community on Palm Island, Australia, and was later related to contaminations in the drinking water reservoir Solomon Dam. Some days before the outbreak, an cyanobacterial bloom was observed in the dam, which was treated with copper sulphate as an algicide (Bourke *et al.*, 1983). The reported symptoms for the illness were headache, vomiting, painful liver enlargement, bloody diarrhoea and anorexia. These symptoms and the results of blood and urine analyses indicated acute hepatoenteritis together with marked renal damage.

More than 50 % of the patients required intravenous treatment, and several individuals suffered hypovolaemic-acidotic shock (Byth, 1980). All patients survived and recovered.

The blooming organism, *Cylindrospermopsis raciborskii*, was isolated from the Solomon Dam and cultured in the laboratory. Intraperitoneal injection of a lysate of the cyanobacteria into mice resulted in a toxic response characterized by diarrhoea, anorexia and irregular respiration, and death at certain doses (Hawkins *et al.*, 1985). Successive studies via biotest with another *Cylindrospermopsis raciborskii* strain have established liver, kidney and small intestine haemorrhage and lung congestion, with histopathology revealing adrenal cortex necrosis, liver centrilobular necrosis and renal tubular epithelium damage (Hawkins *et al.*, 1985, 1997).

d) Fatal human cases during a water-borne outbreak in Bahia, Brazil.

In 1988, a gastro-intestinal outbreak ravaged in connection with cyanobacteria contaminated water in the Paulo Afonso region of Bahia State,

Fig. 1. An extensive bloom of cyanobacteria in a drinking water reservoir (Lake Vombsjön, Lund, Sweden). (Photo: *Cronberg*).

Brazil. Some 2000 humans, of whom 88 died, were reported to be affected. Despite extensive bacteriological, virological and toxicological investigations, no infectious agent or toxin was found in blood or faecal samples from the patients. *Microcystis* and *Anabaena*, which were present in large numbers in the public water supply, were, finally pointed out as the agents responsible (Teixera *et al.*, 1993). Also patients who had been drinking only boiled water fell ill.

e) An outbreak of gastroenteritis caused by insufficiently treated tapwater, Taalintehtaan, Finland.

In 1989, the concentration of microcystins was measured during an outbreak of acute gastrointestinal illness in Taalintehtaan, Finland (Lepistö *et al.*, 1993) The cause of illness was associated with a mass development of a toxic strain of *Planktothrix (Oscillatoria) agardhii* in the raw water supply. Filaments of *P. agardhii* and 0.1-0.5 μg microcystin l^{-1} were registered in the drinking water.

f) An outbreak of gastroenteritis caused by cyanobacteria, South Sweden

In 1994, gastroenteritis befell 121 persons in three South Swedish villages (Annadotter *et al.*, 2001). The illness took place similtaneous with a contamination of the municipal drinking water with polluted water from the River Kävlingeån, the outlet of the eutrophic Lake Vombsjön (Fig. 1).

Clinical examination of the patients did not detect any pathogenic agents that could explain the sudden outbreak of gastroenteritis. This circumstance lead to that the investigators turned their attention to the content of *Planktothrix (Oscillatoria) agardhii* in the river. HPLC-analysis of the phytoplankton in Lake Vombsjön six days before the outbreak showed a concentration of microcystins about 1 μg l^{-1} in cyanobacteria containing water from the lake. The content of toxic cyanobacteria in Lake Vombsjön, the lack of secondary cases, the fact that some persons fell ill from having consumed only boiled, drinking water, as well as the fact that also cats and dogs got sick, indicated that the outbreak was caused by cyanotoxins.

Just as was the case in the above mentioned Finnish outbreak of gastroenterites, the concentrations of microcystins were lower than

the WHO (1998) guideline level. This suggests that other bioactive substances, particularly lipopolysaccharide endotoxins, were contributing to this illness caused by cyanobacteria.

Poisoning incidents during haemodialysis

a) A pyrogenic outbreak at a dialysis centre in Washington D. C., 1975.

Pyrogenic reactions in 23 out of 79 patients were reported at a haemodialysis centre in Washington D. C. (Hindman, 1975). The symptoms commenced between 80 min and 4 h after dialysis. Infection was excluded as a diagnosis. High levels of endotoxins were detected in the dialysis fluid. Only low density of gram-negative bacteria were present in the dialysis system, and could not have caused the high endotoxin content. The dialysis fluid was prepared from the tap water, in which high endotoxin levels were measured. The endotoxin-contamination and pyrogeenicityof the tap water was explained by a water bloom in the raw water source, the Potomac river.

b) Outbreak of hepatitis among dialysis patients in Caruaru, Brazil.

In 1996, 131 renal dialysis patients fell ill after having been provided with contaminated dialysate at a haemodialysis centre in Caruaru, in the state of Penambuco, Brazil. One hundred patients developed acute liver failure and 76 of these patients died (Pouria *et al.*, 1998, Jochimsen *et al.*, 1998; Carmichael *et al.*, 2001). The symptoms reported among the patients include malaise, weakness, vomiting, nausea, upper abdominal discomfort, dizziness, fever, headaches, vertigo, tinnitus, massive tender hepatomegaly and jaundice. In some severe cases also blindness and grand mal convulsions were reported. Laboratory tests showed elevated contents of serum hepatic enzymes, alkaline phosphatases, bilirubin and triglycerides. Biopsy/necropsy revealed acute toxic hepatitis involving necrosis, apoptosis and neutrophil infiltration.

The dialysate was prepared from tap water taken from a reservoir containing species of the cyanobacteria genera *Microcystis, Anabaena, Aphanizomenon* and *Oscillatoria* (Pouria *et al.*, 1998). Microcystins and cylindrospermopsin were identified in the water used by the dialysis clinic, and in serum and liver tissue from patients (Carmichael *et al.*, 2001).

It was evident that the patients had suffered serious enterohepatic and neurological trauma, with the hepatotoxicity displaying many similaritites to experimental animals injected with microcystin-LR (Ressom *et al.*, 1994). Calculations based on liver concentrations and exposure volumes indicated that the water used for dialysis treatments had contained ca. 19.5 μg microcystin l^{-1}. This concentration is 20 times the levels set by the WHO (1998) as a guideline for drinking water.

In a trial with one patient whose serum microcystin level was approximately 100 ng ml^{-1}, haemoperfusion was effective in reducing this level (Pouria *et al.*, 1998).

These incidents demonstrate the need for special surveillance of the water supply which are utilized by haemodialysis clinics. Intravenous exposure involves contact with more than 100 L of water several times per week. This exposure route provides a dramatically higher relevant dose than oral uptake through water ingestion.

An influenza-like syndrome connected with inhalation of aerosol

A transient flu-like syndrome with fever, malaise, muscle pain, tightness of the chest and symptoms in the respiratory tract were observed 1.5 – 6 hours after taking a shower or tub bath (Fig. 2). This was observed at four locations in Scandinavia (Muittari *et al.*, 1980a and b; Atterholm *et al.*, 1978), in Homa Bay, Kenya (Annadotter *et al.*, 2000) and in Harare, Zimbabwe (Annadotter *et al.*, 2005).

A relationship was registered between the occurrence of the syndrome and mass developments of cyanobacteria and/or break-down of cyanobacterial blooms in the raw water sources (Annadotter, 1993; Annadotter *et al.*, 2005).

During a situation in Harare, when people reported symptoms after baths, high levels of endotoxins, 60-250 Endotoxin Units (EU) ml^{-1} were detected in the tap water (Annadotter *et al.*, 2005). Cyanobacteria occurred both in

Fig. 2. Influenza-like symptoms have been associated with indoor bathing in water with high levels of endotoxins. (Photo: *Forssblad*).

the Harare raw water reservoir and in the tap water. The physiological reaction was triggered by the inhalation of aerosols, and was similar to symptoms in experimental studies in which humans inhaled pure endotoxins (Thorn and Rylander, 1998).

Maternal milk reduces the harmful effect of endotoxins

In developing countries, clean water for human consumption may be scarce. People may have no other choice than to use surface water, even during periods when cyanobacteria are densely present in the water. In towns and villages where water-pipes are used, the scarcity of water induces many people to store water in open or covered tanks on the roof of the house. In this stagnant water, often exposed to the sun, microalgae and bacteria proliferate. Deterioration of the water quality is a consequence due to the high levels of endotoxins. Small children in particular, with an insufficient immune defence, should be protected from exposure to endotoxins in drinking and bath water.

A lower risk of infections in the gut and urinary tract has been noted in breast-fed, compared to bottle-fed, infants (Hanson, 1998; Pisacane *et al.*, 1992). These observations might be explained by recent findings by Håversen *et al.*, (2002). They reported that lactoferrin, a glycoprotein present in high concentrations in human milk, plays a role in host defence mechanisms by down-regulating the endotoxin (LPS)-induced cytokine production in human blood monocytes. Thus, orally administered lactoferrin, which the suckling child obtains via the maternal milk, has the ability to reduce infection and inflammation in the urinary tract, and to mediate anti-inflammatory activity in the colon. The suckling child may benefit from the dual anti-microbial and anti-inflammatory property of lactoferrin, especially during the colonization and expansion of the intestinal microflora. Differences exist in the predominating microbial species in the intestine of breast-fed infants compared to bottle-fed infants. Breast-fed infants had lower counts of clostridia and enterobacteria than bottle-fed children (Balmer and Wharton, 1989; Lundequist *et al.*, 1985; Yoshioka *et al.*, 1983). When water with high levels of endotoxins is used for consumption as well as for bathing, breast-feeding may be an important protective factor for infants.

The role of cyanobacteria in cholera epidemiology

In the middle of the 19th century, the English doctor John Snow discovered a link between cholera outbreaks and contaminated water

Fig. 3. In developing countries, poor people may not have access to drinking water of good quality. Water from this small river, at Embu, Kenya, blooming with toxin-producing cyanobacteria in March 2001, was the only water source for the families living nearby. Some days earlier, hundreds of humans, mostly children, died at the hospital of Embu after having drunk insufficiently treated municipal water originating from this river (Bengtsson & Mosén 2002; Codd *et al.*, 2005). (Photo: *Annadotter*).

supplies. Some decades later, the causative agent of cholera, *Vibrio cholerae*, was discovered by Koch (1884).

In the end of the 20th century, cholera epidemics became a worldwide health problem (Codeço, 2001).

Cholera is an acute disease of the intestine, caused by the toxin-producing bacterium *Vibrio cholerae* (Lee, 2001). Processes in the mucosal epithelium of the gastrointestinal tract cause an acute watery diarrhoea and vomiting, which result in water loss and severe dehydration. The mortality from cholera may be as high as 50% of the exposed if left untreated. Cholera has become endemic in many geographical areas and is a recurring problem in others. Since 1991, about 120 countries have reported indigenous cases of cholera.

Even though the biology of cholera is one of the best investigated among infectious diseases, much remains still to be elucidated (Lee, 2001). Cholera is more complex and durable than was earlier appreciated. *Vibrio cholerae* may exist permanently within the environment, rather than living only for a few days outside the human intestine, as previously thought. The reservoir of cholera remained a mystery until Colwell *et al.*, (1977) hypothesized that *V. cholerae* belonged to the natural flora of estuaries and brackish water. In 1994, Colwell and Huq, showed that toxigenic *V. cholera* can survive in aquatic environments for months to years. The water environment may play an important role in the emergence of new strains of toxigenic *V. cholerae* (Faruque *et al.*, 1998) and in the seasonality of cholera outbreaks (Islam *et al.*, 1994b; Colwell and Huq, 1994).

Islam *et al.*, (1990; 1991; 1994a, b & c; 2004) demonstrated that species of the genera *Anabaena, Hapalosiphon* and *Nostoc* can act as long-term reservoirs of *V. cholerae* 01 and O139. The bacterium lives symbiotically in the mucilaginous sheath of the cyanobacteria (Fig.5). Islam *et al.*, (1994b) suggested that the cyanobacterium benefited from the carbon dioxide produced by *V. cholerae*. The pathogen used oxygen and nutrients produced by the cyanobacterium.

Fig. 4. People from a village at the outlet of Lake Pequenos Libombo, Mozambique. The water in this river is used for cooking, drinking, swimming and washing clothes. When the photo was taken, September 2002, the phytoplankton community was dominated by microcystin-producing *Microcystis*. (Photo: *Annadotter*).

The coexistence between *V. cholerae* and *Anabaena* sp. was established in field samples from a pond in Bangladesh (Islam *et al.*, 1994c). In the same pond, *V. cholerae* was never found associated with any other microalgae (e.g. *Euglena* sp. and *Phacus* sp.).

Chronic exposure to cyanobacteria in drinking water

The incidence of primary liver cancer is high in South Eastern China. Tao *et al.*, (1991a and b) found a positive correlation between the incidence of primary liver cancer (PLC) and the use of water from ditches and ponds as drinking water. Microcystins in the drinking water were suggested to be partly responsible for the elevated frequency of PLC (Yu, 1995). In areas where the water supply came from deep wells, the incidence of liver cancer was lower than in regions where the drinking water was supplied from surface waters. The mortality rates due to PLC was c. 100 deaths per 100 000 for people drinking surface waters compared to <20 deaths per 100 000 for people drinking ground water. After improvement of the water quality by water purfication and a change from the use of surface water to ground water, the mortality of PLC decreased (Yu, 1995). Ueno *et al.*, (1996) investigated microcystins in 989 different water samples from pond and ditch water, river water and shallow well water. Seventeen percent of the pond/ditch water, 32 % of the river water and 4% of the shallow well water contained microcystins, cyanotoxins were not detected in deep well water. The mean concentration of microcystins was 101 pg ml^{-1} for pond/ditch water, respectively160 for river water and 68 pg ml^{-1} for shallow ground water wells. Ueno *et al.*, (1996) calculated that people living in regions with a reported high PLC incidence ingest 0.19 pg microcystin per day during the four summer months in their 40-50 year life span.

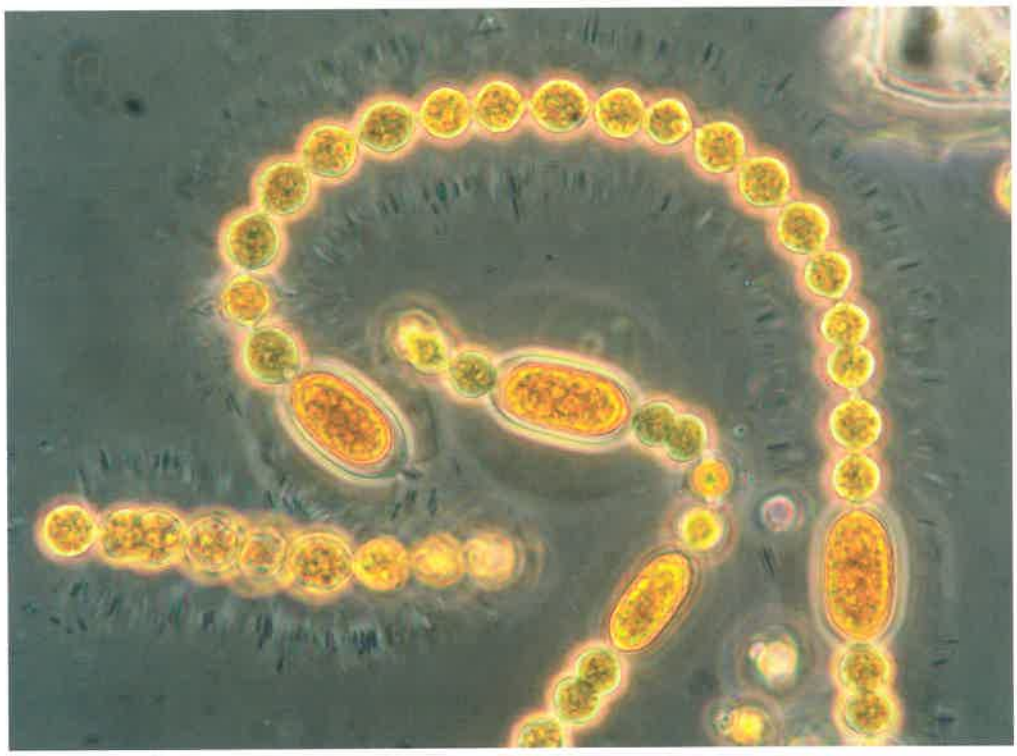

Fig. 5. Bacteria growing in the mucilage surrounding trichomes of *Anabaena solitaria*. (Photo: *Cronberg*).

The high PLC incidence in such areas may result from a coexposure to the potent liver carcinogen aflatoxin B_1 in food or to the hepatitis B virus.

A recent study by Zhou *et al.*, (2000) indicated that, the incidence of colorectal cancer in China correlated positively with the use of drinking water from rivers and ponds containing microcystins.

Food poisoning by toxins from *Lyngbya majuscula*

The ingestion of meat from the marine turtles *Eretmochelys imbricata* and *Chelonya mydas*, has been implicated in human poisonings (Hashimoto *et al.*, 1976; Champetier *et al.*, 1998).

Between 1933 and 1967 in Japan, 200 persons were poisoned and eight young children died. In the period 1993-1996, in Madagascar, 414 persons were affected, of which 29 cases were fatal. Characteristic symptoms include changes such as burning sensation of the tongue and the oral mucus membrane, difficulties in swallowing, salivation, papule of the tongue, acute gastritis, weakness, tachycardia, headache, dizziness, fever and bad breath.

The meat of the marine turtle *C. mydas* was found to contain lyngbyatoxin A (Yasumoto, 1998). The cyanotoxin is produced by *Lyngbya majuscula* which grow on macroalgae in turn being consumed by the turtles.

Ito *et al.*, (2002) studied histopathological changes induced in mice by lyngbyatoxin-A. They found that the toxin damaged the capillaries of the villi in the small intestine. Immature mice were more sensitive than mature ones, and died of bleeding from the small intestines. Exposed to sublethal doses, erosion in the stomach, small intestine, cecum, and large intestine, as well as inflammation of the lung were observed. Intraperitoneal lethal dose of lyngbyatoxin-A in immature mice was 250 $\mu g\ kg^{-1}$.

Dermatitis caused by toxins from *Lyngbya majuscula*

A severe contact eruption, a "seaweed itch" or *dermatitis escharotica* (WHO 1984), caused by direct irritation by *Lyngbya majuscula* has been reported from Hawaii, Japan and Vietnam by, among others, Banner (1959); Grauer and Arnold (1961) and Serdula *et al.* (1982). The symptoms are described as a dermatitis, similar to a burn.

The dermatitis appeared mostly in the genital, perineum and perianal areas. The initial symptoms commenced 4-20 h after contact with water, were characterized as erythema and a burning sensation, and resulted in the formation of blisters and desquamation causing deep sores which lasted 2-12 days (Grauer and Arnold, 1961).

The length of time in the water was not associated with the severity of the dermatitis. The symptoms occurred in bathers who had been swimming from 2 min to 4 h, and the rash occurred both on exposed areas of the body and on those areas covered by the swimsuit. Rinsing in freshwater after bathing has been reported to reduce the symptoms in some (Grauer and Arnold, 1961) but not all cases (Sedula *et al.*, 1982). Aplysiatoxin and debromoaplysiatoxin have been extracted from *Lyngbya majuscula* where people experienced the eruption effects.

Exposure to *L. majuscula* has also been associated with eye and respiratory irritation, even without direct contact with the water. In Japan, people walking along the shore where *L. majuscula* is present in the water have been affected by aerosolised *L. majuscula*. The affected subjects developed inflamed and watering eyes, conjunctivitis and facial rash (Hashimoto, 1979).

A comprehensive review of the toxins of *L. majuscula* and their human and ecological health effects has been presented by Osborne *et al.* (2001).

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5. Purification of Water with Toxigenic Cyanobacteria

Background

At the end of the 20th century, toxic blooms of cyanobacteria were observed with increasing frequency in drinking water reservoirs in almost every part of the world (Annadotter *et al.*, 1999 ; 2005; Hoeger *et al.*, 2005; Molica *et al.*, 2004; Pereira *et al.*, 2004; Skulberg et al. 1984; Svrcek and Smith, 2004; Viera *et al.*, 2003).

A consequence of cyanobacterial mass developments in raw water reservoirs is the risk of harmful health effects on humans, increased running costs and the need for supplementary treatment techniques at the water works.

The water purification plants are required to produce drinking water of acceptable quality according to relevant drinking water guidelines. These guidelines address chemical and hygenic parameters such as concentrations of substances relevant to health, and sensoric properties (e.g. smell and taste) as quality directives for the producers of drinking water.

It has become increasingly clear that almost every water supply system depending on drinking water from surface water, has or will encounter problems with toxigenic cyanobacteria due to their ubiquitous distribution (Hoeger *et al.*, 2005; Molica *et al.*, 2005; Viera *et al.*, 2003).

Drinking-water guideline for safe practice exists so far for one cyanotoxin (microcystin-LR). However, toxicologists are progessing in their defining guidelines for the number of relevant toxins (Chorus and Bartram, 1999). WHO expert committees have evaluated the literature on microcystin toxicology and proposed 1.0 μg microcystin-LR equivalents l^{-1} as consensus safe level for life-long consumption (WHO, 1998). Several countries, which historically have problems with cyanobacteria in drinking water reservoirs have particular guidelines for content of cyanobacterial toxins (Hoeger *et al.*, 2005; Jurczak et al., 2005).

Fig. 1. A bloom of *Microcystis* in the drinking water reservoir Lake Vombsjön, Southern Sweden. (Photo: *Annadotter*).

This chapter will focus on cyanobacteria and their toxins in drinking water treatment.

Most studies have examined the elimination of cyanobacteria and their toxins only in laboratory or pilot-scale experiments. Only a few studies have addressed the efficiency of full-scale drinking water purification plants, using different treatment processes, in the removal of cyanotoxins from water.

An important aspect in the evaluation of water treatment processes is the removal efficiency of both cell-bound and dissolved cyanobacterial toxins. Cyanotoxins can be released during water treatment processes as a result of mechanical and chemical stress factors (Schmidt *et al.,* 2002).

Prefiltration

Microstrainers ca be used in the initial process in water treatment with the purpose of removing phytoplankton and zooplankton. Mouchet and Bonnélye (1998), found that the removal rate for two cyanobacterial species varied between 40 and 70 %. Small *Microcystis*-colonies and single cells were poorly trapped (occasionally <10%) by the microstrainer. Annadotter (1993, 1995) investigated the removal rate of phytoplankton by the microstrainers (45 and 55 μm respectively) in two different water works. Both studies were carried out during periods of cyanobacterial mass developments in the raw water reservoirs (Fig.1 and 2). In the 45 μm microstrainer, the average removal rate was 25% while 33 % was removed by the 55 μm microstrainer.

Coagulation/flocculation and dissolved air flotation

In the coagulation/flocculation process at a water purification plant, small suspended particles (e. g. phytoplankton) aggregate into larger particles when chemical coagulants such as aluminium- or ferric-salts are added. A number of researchers have investigated the purification effect of coagulation/flocculation on cyanotoxins (Hoffman, 1976; Keijola *et al.*, 1988, Himberg *et al.*, 1989, James and Fawell, 1991). Even though cyanobacterial cells were relatively efficiently removed by coagulation/flocculation, none of the studies could report a total elimination by the

Fig. 2. The infiltration ponds at Hässleholm Water Works, Southern Sweden, have been clogged by *Microcystis botrys,* being present in the raw water. (Photo: *Annadotter*).

treatment of cyanotoxins .

Bernhardt and Clasen (1991) found that flocculation with aluminium sulphate and ferric chloride efficiently reduced the concentration of large diatoms. It was more difficult to eliminate small phytoplankton (<2 μm) and some filamentous cyanobacteria e.g. species of *Oscillatoria*. The removal of phytoplankton by coagulation and flocculation ranged from 50 to 99.9 %.

After flocculation, the suspended particles can be removed by sedimentation. Another method is based on dissolved air flotation, whereby the suspended particles become lighter by being joined with small air bubbles. These aggregates rise to the surface and can be skimmed off.

In water purification plants using dissolved air flotation, different cyanobacteria respond differently due to their special physical properties. Drikas and Hrudey (1994) studied the removal of species of the genera *Microcystis, Anabaena* and *Planktothrix* in a water purification plant in Belgium. They reported the removal of *Anabaena* sp. to be 90-100%, the removal of *Microcystis* was 40-80% while the removal of *Planktothrix* sp. was only 30%.

Rapid filtration

Rapid filtration can be used after flocculation/ coagulation to eliminate the flocs. However, this treatment step is not effective in removing cyanobacteria (Annadotter, 1993, 1995; Lepistö *et al.*, 1994; Steffensen and Nicholson, 1994). Rapid filters are regularly cleaned by backwashing. If the backwashing is not carried out frequently enough, cyanobacteria on the filter may decompose and toxins be released into the water (Chorus and Bartram, 1999).

Slow sand filtration

Sand filters are considered to be a relatively efficient way to purify water. The upward facing-surfaces of the sand grain complex are regarded as microscopic sedimentation beds which contribute to the reduction of taste, odour and turbidity in the water. In the sand filtration process, particles in the water are capturd by the sand grains. Particles accumulate primarily in the upper part of the sand filter where the freeing of suspended impurities are most active. In time, a biological layer develops in the upper layer of the filter. The biological community comprises usually of protozoa, rotifers, bacteria and micro algae that cooperate in a food-web decomposing the organic material in the water.

Hard, durable and angular grains are recommended for effective water filtration, and should not contain organic matter, loam or clay (Hardenbergh, 1946). In a slow sand filter, the grains should be fairly similar in size, approximately 0.10-0.90 mm. Too coarse sand will increase the flow rate and let suspended solids and bacteria pass through the cavties between the sand grains. On the other hand, by using too fine sand, the sand filter may clog and the flow rate diminish inopertunely.

In research with slow sand filters and raw water mixed with freeze-dried toxic cyanobacteria, Keijola *et al.*, (1988) reported some elimination of cyanotoxins. However, slow sand filtration is an uncertain method since different sand filters have unlike biological communities and their ability to remove cyanobacteria and toxins will vary.

Experiments with microcystin-containing water in full-scale slow sand filters were performed by Grützmacher *et al.*, (2002). A high removal rate was reported for both cell-bound and dissolved microcystins. The elimination potential for microcystins decreased with falling temperature because of the retarded ed biodegradation activity.

Activated carbon can also be incorporated in slow sand filters, a technique called sand-GAC sandwiching (Hrudey *et al.*, 1999).

A slow sand filter designed to be used intermittently on a single household level has been designed by David Manz, Canada. The filter is called the Biosand household water filter.

In February-March, 2000, extensive floods devastated large parts of Mozambique and serious consequences for the quantity and quality of the drinking water. The BioSand household water filter was introduced by aid organizations after the flood (Fig.3). The efficiency of the BioSand household filter in removing microcystins and lipopolysaccharide endotoxins was tested by

Fig. 3. The Biosand household water filter was introduced by aid organizations after the flood in Mozambique, 2000. (Photo: *Annadotter*).

Bojcevska and Jergil (2003). The removal efficiency was also evaluated when a layer of charcoal and granulated active carbon (GAC) was incorporated in the sandfilter. During the first three weeks operation, concentrations of endotoxins increased after filtration through the ordinary sandfilter and the containing charcoal, whereas the mean removal rate was positive through the sandfilter with GAC. From the fifth week, all three types of filter showed a positive removal rate. Microcystins concentrations (0.6-6.8 $\mu g\ l^{-1}$) were decreased to 0.2 $\mu g\ l^{-1}$ or lower after filtration through all the three different sandfilters. The removal of microcystins were most efficient in the sandfilter with GAC, although the difference were small between the three types of sandfilters.

Chlorination

Lahti & Hiisvirta (1992) reported that chlorination caused lysis of cyanobacterial cells at a chlorine dose of 1 mg l^{-1}. As a consequence, the cyanotoxins were liberated into the water. Consequently, prechlorination should be avoided if cyanobacterial toxins is a risk factor. Once released, dissolved cyanotoxins are not generally decomposed by chlorination (Hoffmann, 1976; Keijola *et al.*, 1988; Himberg *et al.*, 1989; Carlile, 1994). In the referred studies, the removal of cyanotoxins by chlorination seemed to depend on the concentration of chlorine, pH, and on the chloride compounds used. Himberg *et al.*, (1989) showed that sodium hypochlorite (1 mg $Cl_2\ l^{-1}$, contact time 10-15 min) was not effective to decompose microcystins. However, conflicting results are reported by Tsuji *et al.*, (1997) who found that microcystins easily were destroyed by chlorination with sodium hypochlorite, and emphasized that the decomposition depended on the applied free chlorine dose.

Calcium hypochlorite and aqueous solution of chlorine (1 mg $Cl_2\ l^{-1}$, 30 min contact time) with low pH removed microcystins or nodularins by more than 95% wheras sodium hypochlorite or chloramine at the same dose achieved 40-80 % removal at the most (Nicholson *et al.*, 1993, 1994). A minimum residual of 0.5 mg free chlorine l^{-1} should be present after half an hour's contact time in order to decompose the cyclic peptides completely (Nicholson and Rositano, 1997).

The neurotoxins anatoxin-a and saxitoxins were not decomposed with chlorine doses exceeding a 30-min chlorine demand or by changes in pH (Nicholson *et al.*, 1993; Carlile, 1994). Cylindrospermopsin (20-24 $\mu g\ l^{-1}$) was decomposed by 4 mg chlorine l^{-1} at pH 7.2-7.4 (Nicholson *et al.*, 1993).

Noxious organic chlorine compounds in drinking water may, however, be an undesired result of the addition of chlorine during periods with mass developments of cyanobacteria in raw water resources (Wardlaw *et al.*, 1991).

Ozonation

Ozonation has during recent years been used primarily to remove colour (humic compounds) and odour. In ozonation water treatment processes, ozone and OH radicals work as oxidizing agents (Staehelin and Hoigné, 1985). Ozone is a strong

oxidizing agent which can break down humic substances to smaller molecules, as well as oxidizing iron and manganese. During ozonation, the toxic microcystin-molecule is transformed to a non-toxic cyclic peptide without the amino acid Adda (Dahlem, 1989).

Elimination of microcystins by using ozone has been shown to be an efficient purification method (Keijola *et al.*, 1988; Himberg *et al.*, 1989; Fawell *et al.*, 1993). Keijola *et al.* (1988) showed, in laboratory experiments, that 100% of microcystins were eliminated at an ozone dose of 1.0 mg l^{-1}. A lower ozone dose resulted in a lower rate of reduction. Another laboratory study (James *et al.*, 1994) reported that dissolved microcystin was eliminated by 99 % at ozone doses > 2.4 mg l^{-1}. An experiment at a pilot plant showed that dissolved microcystins (about 50 μg l^{-1}) were completely eliminated at an ozone dose of 2 mg l^{-1} (Kaas *et al.*, 1997).

A rapid elimination of microcystins was reported in Australian studies (Nicholson *et al.*, 1993; Rositano *et al.*, 1998; Hoeger *et al.*, 1999). It was shown that a high concentration (800 μg l^{-1}) of microcystin-LR was oxidized to below the HPLC detection limit by 0.2 mg ozone l^{-1} within minutes. Nodularin (88 μg l^{-1}) was also destroyed very rapidly (15 s) with a concentrations of 0.05 mg ozone l^{-1} (Rositano *et al.*, 1998).

Hart *et al.*, (1997) showed that high ozone doses were necessary for treatment of water containing dissolved organic carbon (DOC). At ozone doses of 0.6 mg l^{-1}, the ozone broke down the DOC, but the decomposition of microcystin was small. Not until the DOC, was eliminated, did the ozone efficiently attacted the microcystin. At ozone doses of 0.6-1.3 mg l^{-1}, the effect of the ozone was only lysis of the cells. First at ozone doses of 2 mg l^{-1}, was the extracellular toxin destroyed.

Photochemical degradation

Microcystins are rather stable in natural sunlight (Tsuji *et al.*, 1994), but they can be decomposed by ultraviolet (UV) light. Croll and Hart (1996) showed that UV light could decomposed microcystin-LR at extremely high doses; 20 000 mWs cm^{-2}. In drinking water purification, a normal UV-dose is 30 mWs cm^{-2}. Although UV light destroys microcystins, its practical applicability to water treatment is limited due to the high irradiation intensities required (Drikas, 1994).

Activated carbon

Activated carbon is, in general, efficient for the removal of dissolved cyanotoxins, while cyanobacterial cells are not efficiently removed by activated carbon filters.

A number of laboratory and pilot studies have shown that high doses of powdered activated carbon (PAC), could eliminate cyanotoxins efficiently (Hoffman, 1976; , Falconer *et al.*, 1983a,b, 1989; Keijola *et al.*, 1988; Himberg *et al.*, 1985; Donati *et al.*, 1993; Hart and Stott, 1993). The size of the PAC doses used in these studies were considerably higher than normal doses applied in water works.

Activated carbon filters have pores (cavities, empty spaces) of different sizes and they are classified as micropore (12 nm), mesopore (25 nm) and macropore (50 nm). A study of different kinds of PAC, with very high initial concentrations of microcystins (2 500 μg l^{-1}) showed that the adsorption capacity correlated positively with the mesopore volume of carbon particles (Donati *et al.*, 1994). Other important factors that had an affect on the removal of microcystins were the contact time, and the competition for adsorption sites, with other organic molecules in the water.

Donati *et al.*, (1994) found that the quantity of doses was criticial for the removal of cyanotoxins when using powdered activated carbon. However, the removal ability of an activated carbon filter can be seriously impaired by the formation of a biofilm (Falconer *et al.*, 1983a, b; Lambert *et al.*, 1996), Gram-negative bacteria developing in the biofilm may increase the levels of lipopolysaccharide endotoxins in water after passing through the activated carbon filter. In a study of the water works in Windhoek (Namiba) and Cape Flats (South Africa), the levels of endotoxins were found to have increased after the activated carbon treatment (Burger *et al.*, 1989). Adsorption trials with activated carbon, produced from wood made of the pan-tropical tree *Moringa oleifera,* have

been reported by Warhurst *et al.*, (1997). They found, in batch experiments, that this low-cost, activated carbon removed microcystin-LR in quantitative significant amounts.

Experience with operating water works

a) Microcystins

The removal of microcystins after conventional water treatment and granulated activated carbon (GAC) filtration was investigated at Ferintosh water works, Canada (Lambert *et al.*, 1996). Initially, the concentration of microcystin-LR was 2.9 $\mu g\ l^{-1}$, but increased to 3.1 $\mu g\ l^{-1}$ after chemical precipitation and sedimentation. After filtration, the concentration decreased to 1.2 $\mu g\ l^{-1}$, and further to 0.5 after GAC filtration. The concentration was still 0.5 μg microcystin-LR l^{-1} after the final step with chlorination. Thus, only about 50 % of the microcystins that entered the GAC filter was eliminated.

The effect on microcystin of conventional water treatment with addition of PAC was investigated in Camrose water works, Canada (Lambert *et al.*, 1996). For a period of five weeks, the microcystin concentration in the raw water as well as in the potable water was monitored. Microcystin concentrations in the raw water was 0.15-0.87 $\mu g\ l^{-1}$, while the concentration in the treated water varied between 0.1 and 0.2 microcystin-LR equivalents l^{-1}. The elimination varied between 7 and 90 % and the average result was 48%.

The removal of phytoplankton was investigated in four Finnish water works where different treatment methods are in use (Lepistö *et al.*, 1994), namely rapid sand filtration and disinfection, and rapid sand filtration combined with chemical flocculation and disinfection. The reduction of the total phytoplankton biomass varied between 76 and 99.9 %, while the reduction of the cyanobacterial biomass varied between 14 and 99.9 %. The filamentous cyanobacteria *Planktothrix (Oscillatoria) agardhii* passed easily through the water treatment process. The reduction of phytoplankton was less efficient at the water works with only rapid sand filtration and disinfection compared to the water works with chemical flocculation. Phytoplankton and cyanobacterial cells could also pass through activated carbon filters.

1994-2002 microcystin in raw water and potable water was investigated at Vomb water works (Sweden) with artificial ground water recharge. The raw water was taken from a

Fig. 4. Cyanobacterial scum in an infiltration pond at Vomb water works, Southern Sweden. The drinking water is treated with artificial ground water recharge. Cyanobacterial cells were present in the final water. (Photo: *Cronberg*).

lake with mass developments of microcystin-producing cyanobacteria. Intact cells of eukaryotic phytoplankton and cyanobacteria were constantly present in the consumers drinking water (Cronberg *et al.*, 1996, 1997; Annadotter *et al.*, 2005). Particle bound microcystins, up to 0.5 $\mu g\ l^{-1}$, were occasionally detected in the drinking water.

In 2002 and 2003, the elimination of microcystins was investigated in two Polish water works, which supply drinking water to the city of Lodz from Sulejow Reservoir (Jurczak *et al.*, 2005). The consecutive steps of pre-oxidation, coagulation, sand filtration, ozonation and chlorination used in the water purification showed effective elimination of microcystins by the treatment process. Microcystin concentrations in the raw water varied from 6.7 $\mu g\ l^{-1}$ to below detection limit (0.01 $\mu g\ l^{-1}$). The concentration of microcystins in the water after purification was below the detection limit on all sampling occasions.

Hoeger *et al.*, (2005) made a comparative investigation on the efficiency in a Swiss and a German water work studying the elimination of microcystins. The results showed that the toxin concentration in the raw water ranged from below 1.0 μg microcystin-LR equiv. l^{-1} to more than 8.0 $\mu g\ l^{-1}$ in raw water. However, all samples after water purification were lower than 1.0 $\mu g\ l^{-1}$.

b) Endotoxins

The ability to remove endotoxins from feed water was studied in two African reclamation plants; Windhoek (Namibia) and Cape Flats (South Africa) by Burger *et al.* (1989). These two plants used different disinfection methods.

Ozonation was used for disinfection prior to active-carbon column treatment in the Cape Flats plant. This plant was more effective for endotoxin removal than the Windhoek plant in which only breakpoint chlorination steps were used. An increase in the level of endotoxins after active carbon column treatment occurred at both plants. Breakpoint chlorination, which was used in both plants, was shown to remove endotoxin when undertaken before as well as after sand filtration. The removal was, however, more effective when carried out before sand filtration.

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6. Cyanobacteria and Cyanotoxins - Effect on Fish and Seafood

Background

Extensive experience have shown that aquatic animals - wild or in cultures - may be exposed to toxigenic microorganisms (Falconer 1993). These events may result in injury to health and economic loss. Cyanotoxins imply a potential health risk for marine and freshwater farmed fish. Measurements of cyanotoxins in seafood is required particularly for crustaceans and shellfish harvested from water with high cyanobacteria concentrations.

Effects of toxic cyanobacteria on fish

Mass developments of cyanobacteria may have direct and indirect harmful effects on fish. Fish may be killed through oxygen depletion due to elevated respiration during the hours of darkness, or due to a rapid bloom die-off. When an algal bloom is decomposed, the oxygen requirement is increased and the concentration of ammonia in the water is enhanced (Pillay, 1992).

Fish may be exposed to cyanotoxins by oral uptake and through the surface tissues (epithelium). Cell-bound toxins enter the fish through the gastro-intestinal tract. Free toxins, liberated due to senescence and lysis of cells and dissolved in water, are taken up through the gills or the skin (Tencalla *et al.*, 1994). However, oral uptake is considered to be a more important exposure route than via the surface tissues. Therefore, fish, which feed primarily on phytoplankton, may be more influenced by cyanobacteria than fish feeding on other kinds of food.

Fish in the early stages of life are considered to be generally more sensitive to toxins than juvenile and adult fish (Oberemm, 2001). Some factors to explain this include their thin epithelial layers, their large body surface in relation to their body volume, a high metabolic rate, and moreover that early tissue and organ developmental processes can easily be disturbed.

Fig. 1. Mass development of *Microcystis* spp. and *Cylindrospermopsis raciborskii* at a commercial fish farm in Kariba, Zimbabwe. (Photo: *Cronberg*).

Field and laboratory cases

a) Brown trout

A mass kill of brown trout, *Salmo trutta*, coincided with a heavy bloom of microcystin-producing *Anabaena flos-aquae* in the Scottish Loch Leven (Rodger *et al.*, 1994). Histopathological investigations of moribund fish indicated that the cause of death was due to damage to the liver and gills. Consult also section c).

b) Common carp

Carbis *et al.*, (1997) reported the influence of microcystins on common carp (*Cyprinus carpio*) from the Australian Lake Mokoan. Blooms of hepatotoxic *Microcystis aeruginosa* were present for four consecutive months. They found that carp suffered from pinpoint necrosis on the gills, epithelial ballooning, folded lamellar tips, exfoliation of the lamellar epithelium, atrophy of hepatocytes and impaired hepatocyte function with elevated aspartate aminotransferase activity and serum bilirubin concentrations.

Based on experiments with intra-peritoneal injections of microcystin-LR in common carp, Råbergh *et al.*, (1991) found severe liver damage as well as degeneration of the tubules and glomeruli.

c) Tilapia

Tilapia, a herbivorous fish, is an important cultured fish in tropical and sub-tropical parts of the world. The absence of feeding and growth among cultured tilapia, *Oreochromis niloticus,* in Zimbabwe, was linked with the dominance and mass development of *Microcystis aeruginosa* (Annadotter, 1995) whereas tilapia, at the same fish-farm, grew well in ponds without bloom-forming cyanobacteria.

In laboratory experiments, Beveridge *et al.*, (1993) discovered that tilapia was able to select between toxic and non-toxic *M. aeruginosa*. The grazing of toxic *M. aeruginosa* was significantly lower compared to their grazing of non-toxic *M. aeruginosa*.

Experiments on tilapia and trout have shown that dissolved microcystin-LR can cause gill damage (Garcia, 1989; Gaete *et al.*, 1994; Bury *et al.*, 1996), which can, however, also be caused by the high pH associated with mass development of cyanobacteria and the high ammonia concentrations due to the break-down of cyanobacteria (Dow and Swoboda, 2000).

d) Atlantic salmon

A progressive damage to the liver of Atlantic salmon smolts (*Salmo salar*), a syndrome named Net Pen Liver Disease (NPLD), occurred in open-water net pens in coastal waters of British Columbia and Washington State, USA (Anderson *et al.*, 1993). The disease was caused by an unidentified toxigenic organism producing microcystin.

e) Crayfish

Mass mortality of cultured signal crayfish, *Pacifastacus leniusculus*, in connection with an intense mass development of benthic *Oscillatoria sancta* (Kützing) Gomont was reported by Liråas *et al.* (1998). In the course of the mass kill, the levels of oxygen, nitrite and ammonia did not reveal concentrations lethal to crayfish, whereas mouse bioassay indicated the presence of hepatotoxins in the cyanobacteria. Based on sampling of crayfish from one of these ponds, the feed consumption of cyanobacteria by the signal crayfish was demonstrated (Liråas *et al.*, 1998). The benthic cyanobacterium *Oscillatoria sancta* was observed in 97% (n=32) of the examined stomachs.

f) Penaeid shrimps

The mass development of benthic and planktic species of Oscillatoriales coincided with mass kills of farmed penaeid shrimps, *Penaeus monodon,* in Australian prawn farms (Smith, 1996). Experiments, in which sterile extracts of the benthic Oscillatoriales were injected into the shrimps, caused mortalities, in turn caused by a water-soluble, heat-labile neurotoxin (Smith, 1996).

Bioaccumulation

a) Crayfish

Microcystins were accumulated in the hepatopancreas of signal crayfish (*Pacifastacus leniusculus*) following a 14-day feeding trial with

hepatotoxic *Planktothrix (Oscillatoria) agardhii*, (Liråas *et al.*, 1998). However, the cyanobacteria did not appear to have any negative impact on the crayfish. At the end of the experiment, no differences in glucose concentration and relative wet weight of the hepatopancreas were found between crayfish fed on toxic respectively non-toxic *P. agardhii* and the controls (fed crayfish pellets).

b) Mussels

The freshwater mussel *Anodonta cygnea* has the capacity to accumulate microcystins in the hepatopancreas without any apparent impact on the health of the mussel (Eriksson *et al.*, 1989).

In order to study uptake and depuration of microcystin-LR, mussels (*Mytilus galloprovincialis*) were exposed to hepatotoxic *Microcystis aeruginosa* for 16 days. They were found to contain a maximum of 10.5 μg of cyanotoxin per g dry mussel weight after ten days (Vasconcelos, 1995). Microcystins were already detectable after two days of exposure, and 24.1-54.8% of the toxin administered was taken up. When the mussels were fed non-toxic phytoplankton, a 50 % decrease of the accumulated toxin occurred within two days. 96% of the toxin that was taken up was found in the digestive gland and stomach, while the rest of the toxin was present in the gills, muscle, foot and other organs.

Falconer *et al.*, (1992) showed the accumulation of nodularin in mussels in an Australian estuary. Data on the dynamics of the accumulation of cylindrospermopsin by the freshwater mussel *Anodonta cygnea* were presented by Saker *et al.*, (2004). They showed experimentally that mussels could accumulate up to 2.5 μg cylindrospermopsin /g tissue dry weight. At the end of a 2-week accumulation period, the concentration of cylindrospermopsin varied in different parts of the body. Most of the toxin was detected in the haemolymph (68%) while the rest was found in the viscera (23%), foot and gonad (8%) and mantle (1%). Fifty percent of the toxin remained in tissues after a depuration period of two weeks. The bioaccumulation of saxitoxins, produced by the cyanobacterium *Anabaena circinalis* has been demonstrated in freshwater mussel (Shumway *et al.*, 1995)

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7. Epilogue

The modern taxonomic classification of cyanobacteria summarizes biochemical, cytological, morphological, physiological, molecular and ecological data when new genera or species are established. Recently, molecular investigations of various species belonging to the genera *Anabaena* and *Aphanizomenon* have been performed (Rajaniemi *et al.*, 2005a, 2005b). It was shown by 16S rRNA gene sequencing of *Aphanizomenon issatschenkoi* strains, that they clustered in a third subgroup, different from the *Aphanizomenon flos-aquae-* and *Anabaena*-groups. After statistical analysis of morphological and molecular characteristics a new generic name, *Cuspidothrix,* was established for *Aphanizomenon issatschenko*i, and related species (Rajaniemi *et al.*, 2005b).

Several species taken up in this manual have thus been reviewed, and been given new names. We keep the old names here in the manual, as these are the names mostly found in the conventional literature on cyanobacteria. Below we list the new names with their synonyms.

New combinations:

Cuspidothrix issatschenkoi (Usačev) Rajaniemi *et al.*, 2005.
Syn: *Aphanizomenon issatschenkoi* Usačev 1938.

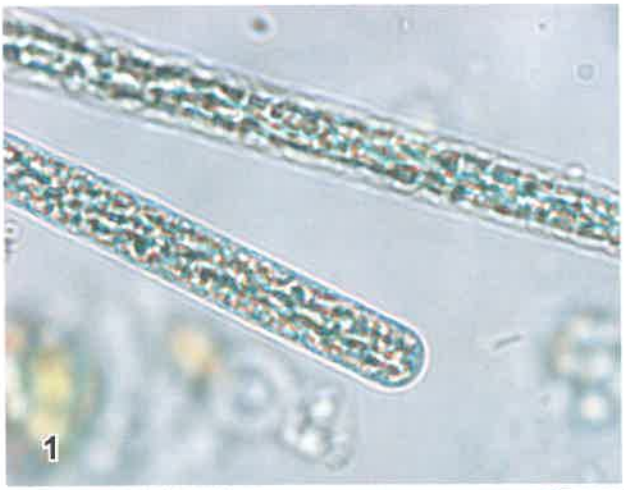
Cuspidothrix capricorni (Cronberg et Komárek) Rajaniemi *et al.*, 2005.
Syn: *Aphanizomenon capricorni* Cronberg et Komárek 2004.
For more information on these two species see Chapter 2 in this manual.

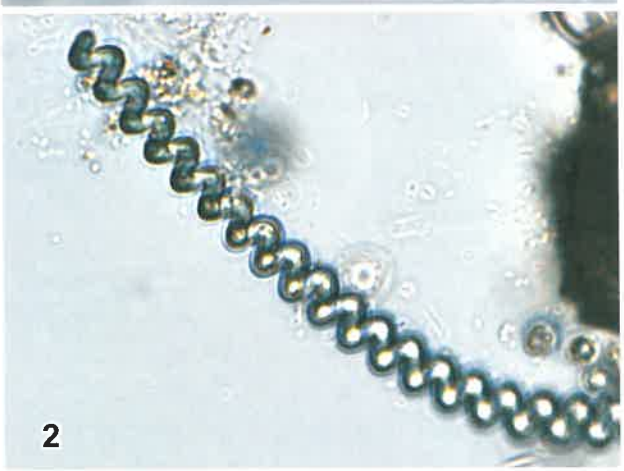
In chapter 2, Table 4, the toxigenic species

Planktothrix mougeotii (Bory et Gomont) Ananostidis et Komárek is listed. This species was recombined by Komárek et Anagnostidis (2005) and was given the new name:
Planktothrix isothrix (Skuja) Komárek et Anagnostidis. - Fig. 1.
Trichomes blue-green in masses, at first benthic, later free-floating, solitary often forming waterblooms. Trichomes long, slightly curved. Cells slightly shorter than wide, more or less constricted at cross walls, width (1.5) 2-5.5(9.5) μm. Apical cells cylindrical or widely rounded or flat, no calyptra.
Toxigenic cyanobacteria, common in eutrophic freshwaters, distributed world-wide.

As the genus *Spirulina* was not presented in chapter 2 , we add some information on the genus here (cf. *Arthrospira*).

Spirulina major Kützing ex Gomont 1892.
Fig 2





Trichomes solitary, pale to bright blue-green in colour, regularly coiled. Coils left-handed not joined to one another. Cell width is (2)2.4-3-4.5(5) μm, the distance between coils is (2-2.4)2.7-3.4-5 μm. Apical cells are rounded. Trichomes long with screw-like rotation and simultaneous rapid gliding.
Distributed in fresh and stagnant water. Cosmopolitan. (References, see Chapter 2).

8. Glossary

Acetylcholine – a reversible acetic acid ester of choline serving as a neurotransmitter of many interneural, neuromuscular and other cholinergic effector synapses.

Acidosis – excessively acid condition of the body fluids or tissues.

Adda – 3-Amino-9-methoxy-2,6,8-trimetyl-10-phenyldeca-4(E),6(E)-dienoic acid.

Akinete(s) – resting cells, (spores).

Alkaloid – nitrogenous organic bases found in plants, with medical or toxic properties, such as nicotine (general stimulant), caffeine (stimulant of central nervous system), morphine (analgesic) and cocaine (local anaesthetic). Alkaloids produced by cyanobacteria include anatoxin-a, homoanatoxin-a and saxitoxin.

Allelochemicals – are a class of biologically synthesized chemicals produced by water plants as a form of defence against algae.

Antagonistic – showing or feeling active opposition or hostility towards someone or something.

Antibiosis – an antagonistic association between two organisms (esp. micro organisms) in which one is adversely affected.

Apical cells - terminal cells of trichomes.

Ataxia – loss of full control of bodily movements.

Axenic – relating to a culture that is free from living organisms other then the species required.

Barrel-shaped – cylindrical, slightly convex sides, ends are not rounded (dolioform).

Benthic – organisms living on the bottom of stagnant or flowing water.

Bioactive – substance that have a biological effect on living matter.

Bioassay – bioassays are conducted to measure the effect of a substance on living organisms.

Breakpoint chlorination – superchlorination - was designed for the elimination of unwanted ammonia compounds of chlorines, the chloramines. As a side-benefit, it reduces algal colonies in the water.

Calyptra – a thickening cap on terminal cells of trichomes, often remnants of gelatinous sheaths.

Chlorosis – a disease of plants. Chlorosis is characterized by yellow condition of parts that are normally green. The disease is caused by circumstances inhibiting the formation of chlorophyll.

Chromatoplasm – in cyanobacteria, peripheral part of cell that contain pigments.

Clathrate – pierced with holes.

Cyanosis – is the bluish colouration of skin due to the presence of deoxygenated haemoglobin in blood vessels near the skin surface.

Cytotoxic – toxic to living cells.

Dermatotoxin – toxins for instance from the marine cyanobacterium *Lyngbya majuscula* that gives severe skin reactions, blistering when trapped beneath the swimming costumes (aplysiatoxin, debromoaplysia toxins).

Diazotrophs – microorganism, that fix atmospheric nitrogen.

Diffluent – flowing a part, dissolving.

Disciform – having a round or oval shape like a disc.

Endobiotic – living within another organisms.

Endotoxins – consist of polysaccharide chains, lipid A, and a connecting core unit. When present in nature, this structure contains traces of other, naturally occurring compounds and is referred to as endotoxin. The amounts of endotoxins are expressed as Endotoxin Units, EU ml-1.

Endogloeic – cells living within mucilage of other plants or animals, for example in the mucilage of *Microcystis* and *Woronichinia*.

Epispore – the thick outer coat of certain spores (e.g. akinetes).

Eukaryote – organisms with cells possessing a membrane-bounded nucleus. Eukaryotic cells are distinguished from prokaryotic cells by having somewhat denser (80S) ribosomes, and greater variety of membrane-bound organelles. The eukaryotes comprise algae, fungi, plants, slime moulds, animals and protozoans.

Eutrophication – usually rapid enrichment of bodies of water, by input of inorganic plant nutrients. It may occur naturally but can also be the result of human activities (e. g. discharge of sewage and animal waste).

Filament – trichome surrounded by sheath.

Gastroenteritis – inflammation in the gastrointestinal canal, typically resulting from toxins or infection, causing vomiting and diarrhoea

Hepatoenteritis – simultaneous inflammation in liver and intestines.

HPLC – High-performance liquid chromatography is a form of column chromatography used frequently in biochemistry and analytical chemistry (e.g. to identify microcystins).

Hypertrophic – extremely nutrient rich and polluted water body.

Heterocyte(s) – special cells of filamentous algae with thick several layered cell wall, active in nitrogen fixation.

Heteropolar – in cyanobacteria, when cells, trichomes thallus are morphological differentiated in a basal (usually attached) and an apical (free) part.

Hormogonium (hormogonia) – vegetative reproduction with small parts of trichomes. Fragmentation of trichomes forming distinct more or less mobile segments.
Hyaline – something that is translucent or transparent.
Hypovolaemia – a decrease in the volume of circulating blood.

Intercalary – arising between the base and the apex, e.g. with heterocytes intercalary

Isodiametric- in trichomes, the diameter of the cells are more or less constant along its length.

Isopolar – symmetric to the longitudinal axis of the filament.

Keritomy – net-like striated plasma in cells (visible in light microscope).

LD_{50} – the dose required at which 50 % of test animals die.

LPS – lipopolysaccharide – LPS is a constituent of the outer layer of the cell wall of Gram-negative bacteria and cyanobacteria. The LPS molecule contains a lipid region (lipid A), and a long covalently linked heteropolysaccharide. Lipid A is the primary toxic part of LPS.

Lenticular – shaped like a lentil, esp. by being biconvex

Meristematic zon – meristematic zones are connected with polarity of trichomes. They occur commonly in the genera with morphologically modified ends.

Mesotrophic – naturally eutrophicated waters with slightly increased nutrient concentrations.

Metameric – members of nostocales have a metameric morphology, when the heterocytes and akinetes are repeating the same pattern along the trichome. The positions of heterocytes and akinetes appear regularly at the same distance from each other.

Metaphyton – organisms free living among other algae, but not attached to them.

Microcystins – heptotoxins - are cyclic nonribosomal peptides produced by cyanobacteria. There exist over 80 microcystin variants. Microcystin-LR is a common toxin type that is very toxic.

Microcystin-LR equivalents – the protein phosphatase inhibition assay (PPIA) is an enzyme-based method to determine the concentration of microcystins. The assay is sensitive to peptide hepatotoxins and includes over 80 variants of microcystins as well as the nodularins. The PPIA is based on the standard curve that relates the phosphatase release and microcystin-LR concentration assessed calometrically. It is possible that other peptide hepatotoxins may be present and may inhibit protein phosphatases to varying extents. The results of the PPIA is, therefore, reported as microcystin-LR equivalents.

Mucilage – slimy fluid containing complex substances composed of various types of polysaccharides.

Mucilaginous – containing or composed of mucilage.

Nanocyte(s) – small reproductive cells, formed by multiple fission within the mother cell, and then separating.

Necridia, necrotic cell – in cyanobacteria, dead cells that function to aid separation of filaments for vegetative reproduction, hormogonia.

Obovate – ovate with the narrower end at the base.
Obovoid – egg-shaped with the narrow end attached to the stem.

Oligotrophic – bodies of water with low nutrient concentrations.
Paralytic – a person suffering from paralysis.

Parenchyma – a form of thallus in which true tissues are produced.

Phycocyanin – a chromoprotein that gives cyanobacteria their blue-green colour. Phycocyanin acts as an accessory photosynthetic pigment.

Pico-cyanobacterium, pico-cyanobacteria, colonial pico-cyanobacteria – cyanobacterium (cyanobacteria) with very small cell size, diameter1-4 μm (pico = small).

Planktic – free-floating in the plankton.

Plankton – microscopic organisms that are suspended, free-floating or swimming in the water column.

PSP toxins – Paralytic shellfish poisoning toxins.

Prokaryote – unicellular organisms whose small, simple cells lack a membrane-bounded nucleus, mitochondria, chloroplasts and other membrane-bounded organelles. Their DNA (in the form of a single circular molecule) is not housed within a nuclear envelope. The rest of the cytoplasm is enclosed by a plasma membrane. Smaller ribosomes than those of eukaryotes as well as granular inclusions are present in the cytoplasm. The prokaryotes include the bacteria (including mycoplasmas and actinomycetes) and the cyanobacteria. In modern classification, the prokaryotes are placed in a separate kingdom, Monera.

Proterozoic – a geological division (eon) of the Precambrian. The eon lasts from around 2500 million years to 590 million years ago. Proterozoic rocks contain few fossils, mainly cyanobacteria and soft-bodied animals. Cyanobacteria in fossils are distributed from about 630 Myr BP and onwards.

Pyrogenic – inducing fever.

Thallus – a colony of specific morphology, it is usually flat but among cyanoprokaryotes the colonies have diverse shape characteristic for different genera.

Thermocline – The zone between warm, fairly turbulent, surface water (epilimnion) and colder, relatively undisturbed, deeper water (hypolimnion), in which the temperature gradually decreases, in lake, reservoir or ocean. The metalimnion is the region of steep temperature drop between epilimnion and hypolimnion. The thermocline is the plane or surface of maximum rate of temperature drop with respect to depth.

Toxins – are poisonous substances produced by living cells or organisms.

Toxigenic – producing a toxin or toxic effect.

Trichome(s) – a row of cells, which are connected, cell division takes place division in one plane.

Ultraviolet irraditation – (uv) light has wavelengths in the band between those of the violet end of the visible light spectrum and roentgen rays (4-400 nm). Since nucleic acids absorb energy in the uv range of the electromagnetic spectrum, DNA absorption peaking at c. 260 nm can result in impairment of DNA replication.

Waterblooms – mass development of planktic algal species forming macroscopically visible floating colonies, give mostly green colour to the water, building foam.

Taxonomic Index

Other Species

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