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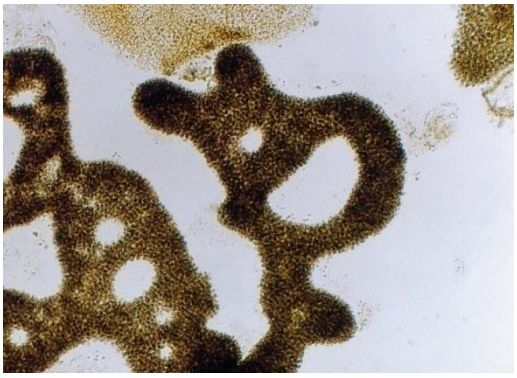
CHAPTER 1 INTRODUCTION (LEVEL 1)

CYANOBACTERIA

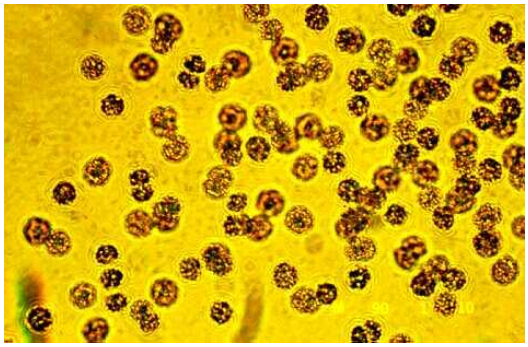
Cyanobacteria, also known as blue-green algae, blue-green bacteria or cyanophytes, are part of a primitive group of organisms which, according to fossil records, have existed for approximately 3.5 billion years [1, 2]. They are not true algae, they are gram-negative bacteria which contain chlorophyll and perform photosynthesis. Many cyanobacteria have a characteristic bluish-green colour because of phycocyanin pigment contained in the cells and hence the name blue-green algae, while some species may appear red due to the presence of the carotenoid and phycoerythrin pigments [3].

COLONY



Microcystis

SINGLE CELLS



Microcystis

STRAIGHT FILAMENTS

Phormidium

SPIRALING

Cylindrospermopsin

Coiled *Anabaena* showing heterocytes and akinetes

Coiled *Anabaena* showing heterocytes and akinetes

Figure 1-1 Different morphological cell forms of some cyanobacteria (photographs from AWQC photo collection, and 4, 5).

Cyanobacteria species display a remarkable diversity in cell morphology or form. The unicellular cyanobacteria have spherical, ovoid or cylindrical cells that can occur single-celled or may aggregate into irregular colonies. A slimy matrix secreted during the growth of the colony holds it together. Some cyanobacteria aggregate into regular colonies, or filaments, also called trichomes. Trichomes can be straight, or coiled (Figure 1-1).

The life cycle of cyanobacteria requires water, carbon dioxide, inorganic substances (such as phosphorus and nitrogen) and light. Although energy metabolism is primarily through photosynthesis where sunlight and carbon dioxide are used to produce energy-rich molecules and oxygen, some species can survive in complete darkness, while others have heterotrophic abilities [6]. Some cyanobacteria species also have specialised cells called heterocytes (formerly called heterocysts, but they aren't cysts at all) which enable them to fix atmospheric nitrogen. These cells are indicated in a filament of *Anabaena circinalis* in Figure 1-1. It is not surprising that cyanobacteria can live nearly anywhere on earth, from freshwater to salt and brackish water, from rainforests to the desert, in the air, in soil and other terrestrial habitats. It is also not surprising that cyanobacteria are adaptable organisms that can thrive under the harsh conditions in many regions affected by drought and climate change.

Although from an operational viewpoint high numbers of cyanobacteria can adversely impact a range of drinking water treatment processes such as coagulation and filtration, the main issue for the water supplier is the production by cyanobacteria of metabolites, in particular the algal toxins, or cyanotoxins.

Follow this link for a list of potentially toxic cyanobacteria their toxins, and where they have been found

FACTORS INFLUENCING OCCURRENCE

Cyanobacteria are a natural component of surface freshwater bodies. Their occurrence may vary radically with seasonal changes from only a few per unit volume in the water column to excessive numbers occurring as 'blooms' at the surface of a water body. Their distribution in the water column may vary from the surface of the water column, a few metres below the water surface or at the bottom of the water body.

UTILISATION OF THE AQUATIC ENVIRONMENT BY CYANOBACTERIA

Different cyanobacterial species can display quite different behaviour in their utilisation of the water body. Many cyanobacteria species (e.g. *Microcystis, Anabaena, Aphanizomenon* sp.) possess gas vacuoles that cause them to move up or down in the water column, depending on their stage in the daily photosynthetic cycle. This is illustrated in Figure 1-2 in a stylised cartoon drawing of the daily migration cycle of *Anabaena.* Buoyancy regulation is a mechanism that positions the cyanobacteria at the best depth for capturing light for optimum growth and may also allow them to scavenge nutrients from the water column [7]. This may be a significant advantage over other phytoplankton algae particularly in stratified lakes where turbulence is low and heavy cells tend to sink. This mechanism only works well when the water body is not too turbulent and is also deep. One consequence of this buoyancy regulation mechanism is that cyanobacterial colonies may all become buoyant at night and rise to the surface and form the characteristic surface scums often seen in the morning when a lake is calm.

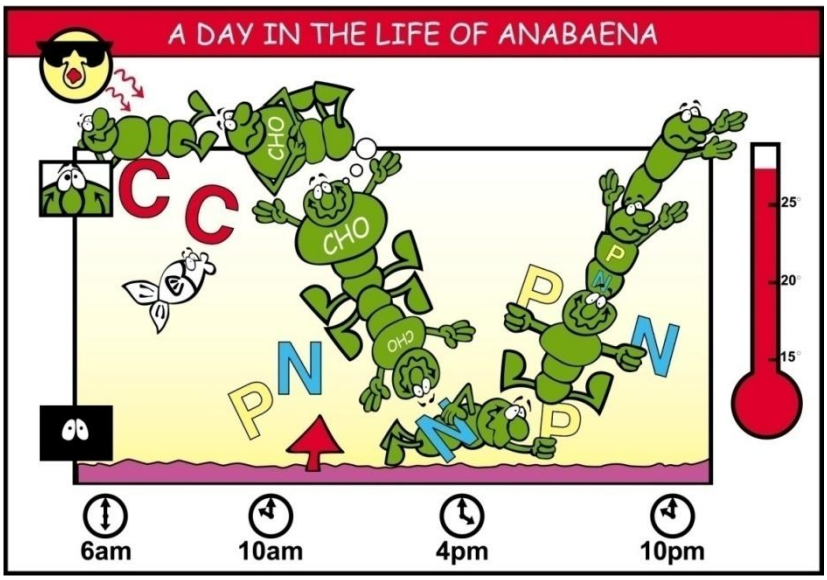


Figure 1-2 A stylised diagram of the daily cycle of buoyancy regulation and vertical migration in a lake by the cyanobacterium *Anabaena*

Other species tend to accumulate in the intermediate region of the water column (or metalimnion, between the warm upper layer and the cooler bottom layer, or hypolimnion). Examples are *Planktothrix* (*Oscillatoria*) *rubescens* and other red cyanobacteria. Under some conditions these cyanobacteria may also form surface scums. Examples of cyanobacteria that are often distributed uniformly through the water column are *Planktothrix (Oscillatoria) agardhii, Limnothrix (Oscillatoria) redekei* and *Cylindrospermopsis raciborskii.*

For more information on buoyancy regulation by cyanobacteria, click here

Non-planktonic, or benthic cyanobacteria can be found attached to sediments or rocks and other surfaces at depths that allow sufficient light penetration for photosynthesis. These cyanobacteria can form thick mats that may break off and float to the surface, particularly when oxygen produced by photosynthesis becomes concentrated within the mats. The *Phormidium* filament shown in Figure 1-1 is a species of benthic cyanobacteria.

THE CYANOBACTERIAL LIFE CYCLE

For one type of cyanobacteria, the filamentous, heterocystous cyanobacteria (Order *Nostocales*), the life cycle involves the planktonic population and benthic resting stages or akinetes. Akinetes are thick-walled reproductive structures that are found in sediments and are thought to provide a resting stage that may enable the survival of a species. They germinate when environmental conditions are appropriate, thereby providing a source of inoculum for subsequent populations, particularly from one season to the next [8]. Several akinetes are indicated in the *Anabaena* filaments shown in Figure 1-1. The life cycle of akinete-producing cyanobacteria can be summarised in a number of steps. First, the filaments of cyanobacteria grow by cell division. Akinete production and release follows, usually for the population to survive over winter. Finally, growth from the akinetes occurs, which is triggered by environmental factors, including light and temperature, with new cyanobacteria maturing and growing by cell division for the new season's population [8,9]. The cycle of akinete formation in the cyanobacterium *Anabaena* is illustrated in Figure 1-3.

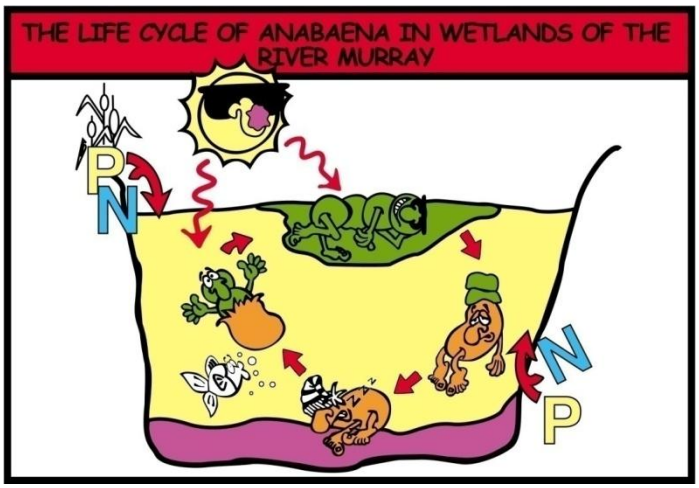


Figure 1-3 The typical life cycle of the cyanobacterium *Anabaena* showing akinete formation and germination

Other filamentous or single cell/colonial cyanobacteria are not known to form akinetes or other resting-stage cellular structures. It has been suggested that some of the normal or regular growth cells called vegetative cells may rest over winter in a state of senescence in the sediment. For example *Microcystis* can 'overwinter' as vegetative colonies on the lake sediments, where they may survive for several years, apparently without light or oxygen [10]. The new population may then appear in spring from the normal growth of these colonies by cell division.

FACTORS INFLUENCING GROWTH

Various cyanobacteria have the capacity to grow at a range of depths; this ability varies with species and is strongly influenced by nutrient and light availability (either the turbidity or the clarity of the water). Many cyanobacteria genera (e.g. *Planktothrix* and *Cylindrospermopsis*) are also adapted to grow in light limiting environments. This enables the cyanobacteria to utilise nutrient-rich environments at various depths. For example, bands of *Planktothrix* can occur at a depth of 12m and layers of *Cylindrospermopsis* filament at a depth of 7m. Some cyanobacteria, such as the filamentous *Anabaena* sp., prefer higher light intensities, and *Planktothrix* will form dense bands just below the water surface. The benthic cyanobacteria, (e.g. *Phormidium*, *Pseudanabaena* and *Oscillatoria*) thrive in shallow reservoirs with clear water as they are generally immobile in the water body. They can also colonise the shallow areas of larger reservoirs where they will be attached to rocks, sediment, or larger organisms such as macrophytes.

A complex interaction of environmental factors has been shown to contribute to cyanobacterial growth. These factors include light intensity, water temperature, pH, carbon dioxide concentration, nutrient availability (nitrogen, phosphorus, iron, and molybdenum), physical characteristics of the water body (shape and depth), water column stability, water flow rate (rivers) or horizontal movement due to inflows or wind (reservoirs and lakes) and aquatic ecosystem structure and function. Factors which favour the growth of cyanobacteria will be discussed below. If several of these factors occur simultaneously cyanobacterial growth will be optimised and potential bloom conditions may be present.

NUTRIENTS

Since cyanobacterial blooms often develop in water bodies enriched with nitrogen and phosphorus (eutrophic conditions), it has been assumed that they require high nutrient concentrations. This contrasts to observations that cyanobacterial blooms often occur when concentrations of dissolved phosphate are lowest. Experimental data have shown that the affinity for nitrogen or phosphorus of many cyanobacteria is higher than for many other photosynthetic microalgae. If dissolved phosphate (soluble reactive phosphate determined from filtered samples) is detected at concentrations of only a few micrograms per litre, cyanobacterial growth and biomass are not limited by phosphate availability [11]. Cyanobacteria effectively utilise phosphorus and out-compete green algae, especially in phosphorus-limiting environments, as they (1) have a greater affinity for phosphorus, (2) can store enough phosphorus to perform two to four cell divisions, which corresponds to a 4 - 32-fold increase in biomass [11] and (3) migrate to areas of higher phosphorus concentration in the water column. Cyanobacteria (e.g. *Microcystis* sp.) can store nitrogen in proteins (cyanophycin and phycocyanin), which can be utilised during nitrogen-limiting conditions. Other cyanobacteria (e.g. *Cylindrospermopsis*) can utilise atmospheric nitrogen and can thus proliferate and out-compete green algae in nitrogen-poor surface water where sufficient light is available. As a simple guide, the influence of nutrient levels on cyanobacterial growth can be measured in terms of total phosphorus levels in the water body. In general, a total phosphorus level of 10–25 μgL^{-1} presents a moderate risk in terms of the growth of cyanobacteria. For levels of less than 10 $\mu g\ L^{-1}$ there is a low risk of cyanobacteria growth, and a level greater than 25 $\mu g\ L^{-1}$ provides high growth potential. However, growth can be maintained at low phosphorus concentrations provided there is rapid recycling of the nutrient. This will be discussed further in Chapter 2.

In the past the ratio of total nitrogen to total phosphorous was thought to be a key parameter in the growth of cyanobacteria compared with other phytoplankton [12]. However, more recent studies have refuted this contention and it is no longer considered a controlling factor [13]. A more important issue is whether either nutrient could be considered limiting for cyanobacterial growth, or growth of other algae.

LIGHT

Cyanobacteria contain the photosynthetic pigment chlorophyll-a, but unlike other phytoplankton they also contain phycobiliproteins. These pigments are able to harvest light in the green, yellow and orange part of the spectrum (500-650 nm). This enables cyanobacteria to utilise light energy efficiently. High phytoplankton density leads to high turbidity and low light availability and under these conditions cyanobacteria can harvest light more effectively and therefore may be able to out-compete other phytoplankton. For example, in light limiting conditions, cyanobacterial growth rates are higher than those of green algae, which allows them to out-compete green algae in highly turbid waters.

Both turbidity and water colour can influence the amount of light received by cyanobacteria in a water body. Generally, the zone in which photosynthesis can occur is termed the euphotic zone. By definition, the euphotic zone extends from the surface to the depth at which 1 % of the surface light intensity is measured. The euphotic zone can be estimated by measuring the transmittance of the water with a 'Secchi' disk and multiplying the Secchi depth reading by a factor of approximately 2-3 (see Chapter 3 for more information about Secchi depth measurement). Those cyanobacteria that regulate their buoyancy via gas vesicles utilise optimum light conditions during the time they are in the euphotic zone. Light penetration into a water body is also important for growth of benthic cyanobacteria. The greater the light penetration, the deeper the benthic cyanobacteria can grow.

TEMPERATURE

Cyanobacteria have a wide range of temperature tolerance, but rapid growth rates are usually achieved when the water temperatures exceed 20°C. In temperate to tropical climates temperatures are favourable for cyanobacteria growth for a large part of the year. A distinct temperature gradient can develop between the warm upper water layer, which is rich in light and oxygen but deficient in nutrients (the epilimnion), and the cooler bottom layers which are light-poor, oxygen-poor but nutrient-rich (the hypolimnion). The area of temperature gradient in between is called the thermocline. This is called stratification and these conditions can be more conducive to the growth of cyanobacteria than other plankton. Thermal stratification of a water body is illustrated in Figure 1-4.

Although the main body of the lake or river may not be stratified, often warm, shallow, sheltered areas exist that can become stratified and provide ideal conditions for cyanobacteria growth, and thus increase the probability of cyanobacterial blooms. Source water abstraction points situated in these areas are more at risk of high cyanobacteria concentrations.

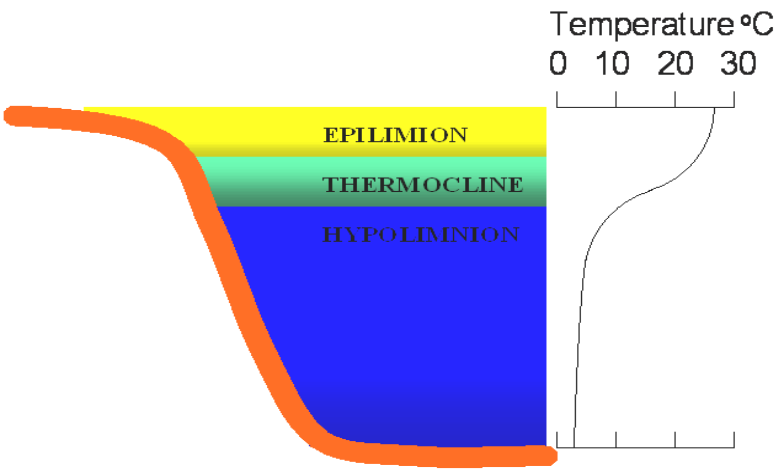


Figure 1-4 Cross section of a thermally stratified lake showing location of the epilimnion and hypolimnion and associated temperature changes.

For more information about stratification follow this link

CYANOTOXINS

Cyanobacteria produce a range of potent toxins with different modes of toxicity. Table 1-1 lists the major known toxins, the target organs of these toxins and the cyanobacteria that produce them. This list is evolving, for example new variants of microcystins are identified each year, and it is unlikely that all cyanotoxins have been discovered.

The majority of cyanotoxins are associated with well-known planktonic and bloom forming cyanobacteria that are free-floating in the water, such as *Microcystis*, *Anabaena* and *Cylindrospermopsis*, however some benthic or attached cyanobacteria, such as *Oscillatoria, Phormidium* and *Lyngbya* have also been shown to produce both neuro- and hepatotoxins (nerve toxins and liver toxins respectively) and should also be considered as a possible hazard with regard to toxicity [14, 15, 16].

Table 1-1 General features of the cyanotoxins

Toxin Group	Primary target organ in mammals	Cyanobacterial genera
Cyclic peptides		
Microcystins	Liver, possible carcinogen in this and other tissues	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis, Aphanizomenon ovalisporum*
Nodularin	Liver, possible carcinogen	*Nodularia, Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Alkaloids		
Anatoxin-a	Nerve synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon, Cylindrospermopsis*
Anatoxin-a(S)	Nerve synapse	*Anabaena*
Aplysiatoxins	Skin, possible tumour promoter	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsins	Liver and possibly kidney. Possible genotoxic and carcinogenic	*Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis, Anabaena, Lyngbya* (benthic)
Lyngbyatoxin-a	Skin, gastrointestinal tract, possible tumour promoter	*Lyngbya*
Saxitoxins	Nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharides (LPS)	Potential irritant; affects any exposed tissue	All

The cyanotoxins can broadly be grouped into cyclic peptides, alkaloids and lipopolysaccharides [6, 17]. Mechanisms of cyanobacteria toxicity are diverse and the mammalian health effects range from neurotoxicity (e.g. anatoxins and saxitoxins) or hepatotoxicity (e.g. microcystins, cylindrospermopsin and nodularin) to inflammatory or irritation effects (e.g. lipopolysaccharide endotoxins). These toxins have been responsible for numerous animal deaths [18]. Some cyanobacteria produce a metabolite, β-*N*-methylamino-L-alanine (BMAA), which may be involved in neurodegenerative disease [19].

For more detailed information on the cyanotoxins follow these links:

Peptide hepatotoxins (microcystins and nodularin)

Neurotoxins

Cylindrospermopsin

β-N-methylamino-L-alanine (BMAA)

Lipopolysaccharide endotoxins

While the unpalatable appearance of freshwater affected by heavy planktonic algal blooms has probably prevented significant human consumption with consequent fatalities, there is increasing evidence that low-level exposure may have chronic health effects in humans. Cyanobacteria have been implicated in episodes of human illnesses in Australia [20, 21], North America [22, 23, 24], the United Kingdom [25], Brazil [26] and Africa [27]. Many deaths of dialysis patients in Brazil from water contaminated with cyanotoxins were reported [28]. There is also epidemiological evidence from China of a link between cyanobacteria and cancer [29, 30].

Figure 1-5 shows the impact a toxic cyanobacterial bloom can have on wildlife dependent on a contaminated water source.

Figure 1-5 Toxic cyanobacterial blooms also affect wildlife reliant on a contaminated water source

For some examples of toxicity of benthic cyanobacteria follow this link

For examples of adverse human health effects follow this link

Toxic cyanobacteria have been recorded from every continent including Antarctica [31, 32]. Of the cyanobacterial blooms tested to date, 50-75% have been toxic [33]. However not all blooms of a particular species may be toxic. In fact toxicities of blooms of the same species can vary markedly both geographically and with time [34]. Toxicity depends on the relative proportions of toxic and non-toxic strains, and this proportion, and hence toxicity, can vary over time. It is for this reason that all cyanobacterial blooms should be considered toxic, unless proven otherwise by laboratory analyses. Monitoring must also be carried out on an ongoing basis due to the potential variation in toxicity. Monitoring of cyanobacteria is discussed in detail in Chapter 3. As mentioned previously, while initially toxicity appeared to be restricted to planktonic cyanobacteria, benthic forms which form mats in water bodies have also been shown to be toxic [35, 36]. This can cause problems for the water supplier as benthic cyanobacteria are usually submerged, and not readily visible compared with toxic planktonic blooms. This is also discussed further in Chapter 3.

The cyanotoxins are synthesised within the cyanobacteria cells and usually remain contained within the cells. However, cyanotoxins are released in substantial amounts during cell lysis (breaking of cells) and cell death [17, 3]. An exception appears to be cylindrospermopsin produced by *C. raciborskii,* where a substantial amount of the toxin is present in the surrounding water during a healthy bloom [37].

CYANOTOXIN DRINKING WATER GUIDELINES

Drinking water guidelines are designed to protect public health by suggesting safe levels for constituents that are known to be hazardous to health. The guideline level represents the concentration at which the water is safe to drink over a lifetime of consumption. The World Health Organization Guidelines for Drinking Water Quality [38] represent a scientific consensus on the health risks presented by microbes and chemicals in drinking water and are often used to derive guideline values for individual countries, states or regions. The guideline value is important for water supply authorities, as this value sets the concentration of a constituent that is tolerable in drinking water at the tap. For some countries the level is in the form of a recommendation from the health authorities. For other countries the level is a standard and compliance is monitored. For some water authorities the guidelines become part of the contractual obligations. They are required to comply with the guideline values as part of their standards of service.

Due to the current lack of strong toxicological data for a range of cyanotoxins, WHO has issued a guideline for only one cyanotoxin, microcystin–LR (1 μg/L),the most toxic variant of microcystins known thus far.

For a detailed summary of guidelines worldwide, and procedures for guideline derivation, go to:

International guidelines for cyanobacterial toxins and

Procedures for guideline derivation

CHAPTER 1 INTRODUCTION (LEVEL 2)

CYANOBACTERIA

POTENTIALLY TOXIC CYANOBACTERIA

Table 1-1(L2) Potentially toxic cyanobacteria, the toxins they can produce, and where they have been found to date.

Algal Species	Cyanotoxin	Location
Anabaena circinalis	Microcystins, Saxitoxins	France, Australia
Anabaena flos-aquae	Microcystins, Anatoxin-a	Canada, Norway
Anabaena lemmermanni, flos-aquae, circinalis	Microcystins, Anatoxin-a	Finland, Denmark
Anabaena spp.	Microcystins, Anatoxin-a	Egypt, Denmark, Finland, Germany, Ireland, Japan
Anabaena planktonica	Anatoxin-a	Italy
Anabaenopsis millerii	Microcystins	Greece
Aphanizomenon flos-aquae	Saxitoxin, Neosaxitoxin	USA
Aphanizomenon ovalisporum	Cylindrospermopsin	Israel, Australia
Aphanizomenon sp.	Anatoxin-a	Finland, Germany
Aphanocapsa cumulus	Microcystins	Brazil
Cylindrospermum	Anatoxin-a	Finland
Cylindrospermopsis raciborskii	Cylindrospermopsin, Saxitoxins	Australia, Brazil, Hungary
Haphalosiphon hibernicus (soil isolate)	Microcystins	USA
Microcystis aeruginosa	Microcystins	Worldwide
Microcystis flos-aquae	Microcystins	Australia
Microcystis botrys	Microcystin	Denmark
Microcystis viridis	Microcystins	Japan
Nodularia spumigena	Nodularins	Australia, Baltic Sea, New Zealand
Nostoc sp.	Microcystins	Finland, England
Oscillatoria limosa	Microcystins	Switzerland
Oscillatoria sp.	Anatoxin-a	Ireland, Scotland
Oscillatoria agardhii/rubescens group (=Planktothrix)	Microcystin	Denmark, China, Finland, Norway
Planktothrix formosa	Homoanatoxin-a	Norway
Planktothrix mougeotii	Microcystins	Denmark
Planktothrix sp.	Anatoxin-a	Finland
Plectonema (syn. *Lyngbya*) *wollei*	Saxitoxins	USA
Raphidiopsis curvata	Cylindrospermopsin, Deoxycylindrospermopsin	China
Umezakia natans	Cylindrospermopsin	Japan

Note: Most cyanobacteria outer cell wall components are implicated in gastro-intestinal disorders, skin and eye irritation and respiratory symptoms.

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FACTORS INFLUENCING OCCURRENCE

BUOYANCY REGULATION

Bloom forming cyanobacteria possess gas vesicles within cells that are collectively called gas vacuoles. These structures are rigid hollow cylindrical chambers made of protein which contain atmospheric gas [39] and provide cells with buoyancy. Some cyanobacteria can combine this positive buoyancy with the accumulation and loss of carbohydrate which acts as ballast to regulate their buoyancy and enables them to migrate up and down. The way this works is that colonies near the surface are exposed to high light and so have a high rate of photosynthesis and therefore build up carbohydrates within the cells. This makes them heavy and although they contain gas vacuoles the carbohydrate ballast makes them sink at a rate dependent upon their colony size and density of the cell. Large colonies sink faster than small ones. As the colonies sink down into a depth of lower light intensity they stop producing and start consuming carbohydrate by respiration [40]. The colonies then become buoyant again and float back up to the surface euphotic (higher light intensity) zone. Buoyancy regulation is a mechanism that positions the cyanobacteria at the best depth for capturing light for optimum growth and may also allow them to scavenge nutrients from the water column [41]. This may be a significant advantage over other phytoplankton algae particularly in stratified lakes where turbulence is low and heavy cells tend to sink.

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FACTORS INFLUENCING GROWTH

STRATIFICATION OF WATER BODIES

Stratification occurs when the surface layer of a water body (epilimnion) is warmed by sunlight. The resulting rise in temperature causes the water to be less dense and so it separates from the denser bottom layer (hypolimnion). The area between the two layers is known as the thermocline (Figure 1-4). As it is separated from the atmosphere, the hypolimnion is oxygen deficient, or anoxic. The upper, warmer, epilimnion can become wind-mixed and, because of its exposure, can freely exchange dissolved gases (such as O_2 and CO_2) with the atmosphere. The density change at the thermocline, caused by the temperature difference, acts as a physical barrier that prevents mixing of the upper and lower layers.

As there is little or no mixing between the surface layer and the hypolimnion, the latter becomes depleted of oxygen, or anoxic, due to microbial activity using up the available oxygen, which is not replenished by normal gaseous diffusion from the water column above. Under oxygenated conditions (i.e. well mixed water body) phosphorus rich sediments are sealed by an oxidised surface layer of an iron-phosphorus complex. However, under anoxic conditions the complex breaks down resulting in phosphorus, iron and manganese release from the sediments. In the case of phosphorous, this causes an increase in the internal nutrient loading to a water body. This, in turn, can result in an increase in cyanobacterial biomass. During stratified conditions, sediment-bound phosphorus can become a major nutrient source for cyanobacteria. The amount of phosphorus released from the sediments is governed by water exchange rates, sediment chemistry, temperature, mixing conditions, and sediment disturbance.

Usually, shallow (e.g. 2-3 m), wind-exposed lakes are non-stratified. Lakes of intermediate depth (e.g. 5-7 m) may develop transient thermal stratification for a few calm and sunny days, which is then disrupted by the next rain or wind event. In temperate climates deeper lakes can exhibit a stable stratification from spring to autumn. Thermal stratification of a water body influences the depth at which cyanobacteria are likely to be found, the light levels they receive, and the concentrations of nutrients in the water body.

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CYANOTOXINS

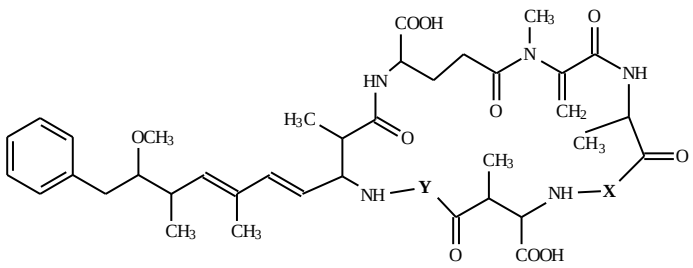
PEPTIDE HEPATOTOXINS (MICROCYSTINS AND NODULARIN)

The hepatotoxins are cyclic peptides, the most frequently encountered compounds of which are the microcystins. These are cyclic heptapeptides produced most commonly by *Microcystis aeruginosa* but also by other species of *Microcystis* and other genera such as *Planktothrix (Oscillatoria)*, *Anabaena*, *Nostoc, Anabaenopsis,* and *Hapalosiphon* [17]. A similar cyclic pentapeptide, nodularin, which is equally as toxic as the most toxic microcystins is commonly produced by *Nodularia spumigena* which is normally a brackish water cyanobacterium [42]. Other cyclic pentapeptide toxins have been characterised, e.g., motuporin isolated from a marine sponge [43] and [L-Har^2]-nodularin [44]. The structures of the peptide hepatotoxins are shown in Figure 1-1(L2).

Microcystins were initially considered to contain five invariant and two variant amino acids. One of the invariant amino acids is a unique β-amino acid called Adda. A two-letter suffix (XY) is ascribed to each individual toxin to denote the variant amino acids. X is commonly leucine (L), arginine (R) or tyrosine (Y), and Y, arginine, alanine (A) and methionine (M). Variants of all the "invariant" amino acids have now been reported, e.g., desmethyl amino acids and/or replacement of the 9-methoxy group of Adda by an acetyl moiety. Currently there are in excess of 70 variants of microcystin which have been characterised [17]. Of these 70 compounds, microcystin-LR is the microcystin most frequently found in cyanobacteria. Often more than one microcystin is produced by a particular strain of cyanobacterium [45]. The microcystin variants also differ in toxicity [46]. The literature indicates that hepatotoxic blooms of *M. aeruginosa* containing microcystins occur worldwide.

The cyclic pentapeptide nodularin contains amino acids similar or identical to those found in microcystins, namely arginine, glutamic acid, β-methylaspartic acid, N-methyl-dehydrobutyrine and also Adda [42]. Motuporin has arginine replaced by valine [43] and in [L-Har^2]-nodularin, arginine is replaced by homoarginine [44].

Return to level 1



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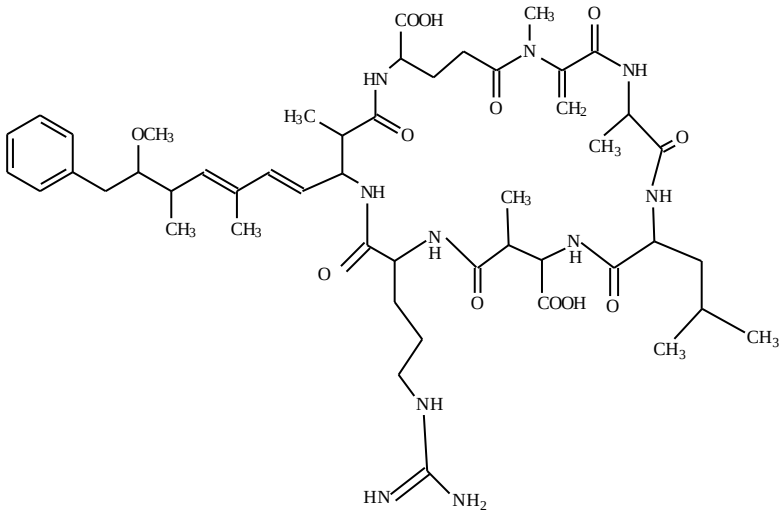

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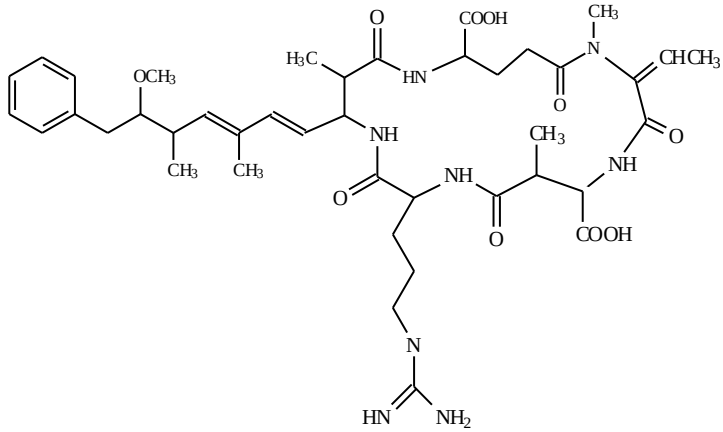


(3)

Figure 1-1(L2) Structures of peptide hepatotoxins; (1) - General structure of microcystins, (2) - microcystin-LR, (3) - nodularin

NEUROTOXINS

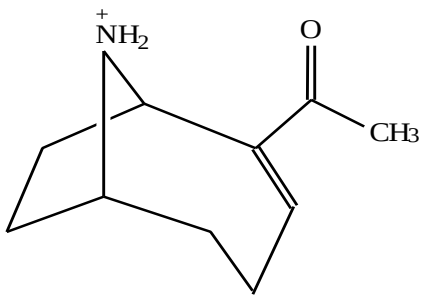
ANATOXINS

Toxins in this class identified to date are the neurotoxic alkaloids anatoxin-a, homoanatoxin-a and anatoxin-a(s). Anatoxins have been shown to be widespread in cyanobacteria in the northern hemisphere; only one report of anatoxin-a in cyanobacteria in the southern hemisphere has been confirmed [47].

SAXITOXINS (PARALYTIC SHELLFISH POISONS, PSPS)

The neurotoxic saxitoxins or paralytic shellfish poisons (PSPs) belong to one of a number of groups of toxins produced by dinoflagellates in the marine environment (Figure 1-2(L2)). Shellfish feeding on toxic dinoflagellates can themselves become toxic and hazardous if consumed, even causing human fatalities [48]. Poisoning incidents usually coincide with the sudden proliferation of these organisms to produce visible blooms, the so-called "red tides" [49].

In freshwater these toxins are produced by a fairly limited number of species of cyanobacteria. To date the only neurotoxic cyanobacterium encountered in Australia is *Anabaena circinalis,* which produces saxitoxins [50]. Elsewhere in the world, saxitoxins have been found to be responsible for neurotoxicity in the cyanobacterial species *Aphanizomenon flos-aquae* [51, 52], *Lyngbya wollei* [36] and *Cylindrospermopsis raciborskii* [53]. Saxitoxins in Danish lakes appear to be produced by *Anabaena lemmermannii* [54]. Toxin profiles are complex and variable, similar to those that have now been found in dinoflagellates and contaminated shellfish.



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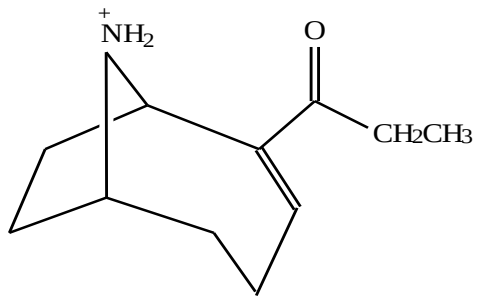

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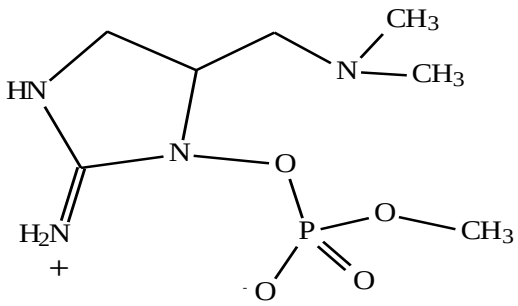
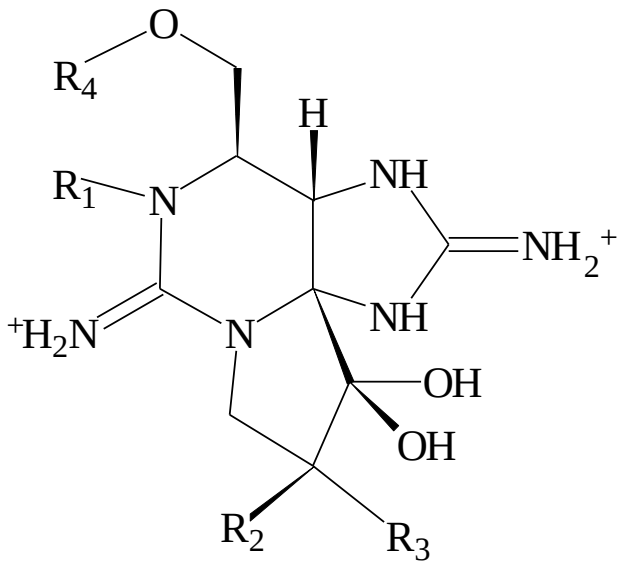


(3)

Figure 1-2(L2) Structures of the anatoxins; (1) - anatoxin-a, (2) - homoanatoxin-a, (3) - anatoxin-a(s)



	R1	R2	R3	Net Charge	Relative Toxicity
R4=$CONH_2$ (CARBAMATE TOXINS)					
STX	H	H	H	+2	1
neoSTX	OH	H	H	+2	0.924
GTX1	OH	H	OSO_3^-	+1	0.994
GTX2	H	H	OSO_3^-	+1	0.359
GTX3	H	OSO_3^-	H	+1	0.638
GTX4	OH	OSO_3^-	H	+1	0.726
R4 = $CONHSO_3^-$ (N-SULFOCARBAMOYL (SULFAMATE) TOXINS)					
GTX5 (B1)	H	H	H	+1	0.064
GTX6 (B2)	OH	H	H	+1	-
C1 (epiGTX8)	H	H	OSO_3^-	0	0.006
C2 (GTX8)	H	OSO_3^-	H	0	0.096
C3	OH	H	OSO_3^-	0	0.013
C4	OH	OSO_3^-	H	0	0.058
R4=H (DECARBAMOYL TOXINS)					
dcSTX	H	H	H	+2	0.513
dcneoSTX	OH	H	H	+2	-
dcGTX1	OH	H	OSO_3^-	+1	-
dcGTX2	H	H	OSO_3^-	+1	0.651
dcGTX3	H	OSO_3^-	H	+1	0.754
dcGTX4	OH	OSO_3^-	H	+1	-

Figure 1-3(L2) Structures of the saxitoxins (paralytic shellfish poisons (PSPs)). Toxicity data from [55]

The widespread occurrence of saxitoxins makes them an important class of cyanotoxins. In *A. circinalis* in Australia, toxin profiles appear to be relatively constant and dominated by the C toxins [56, 57]. There is also some limited evidence that this cyanobacterium can produce both neurotoxins and hepatotoxins [58], a phenomenon which has been reported elsewhere with *A. flos-aquae* [59, 60].

The saxitoxins are a relatively complex class of 18 compounds with widely differing toxicities which can be divided into three groups as shown in Figure 1-3(L2). They can also be divided into three groups based on the net charge of the molecule under acidic conditions [61]. This grouping comprises the saxitoxins (saxitoxin (STX), neosaxitoxin (neoSTX) and decarbamoyl derivatives) (charge +2;), the gonyautoxins (GTXs) including decarbamoyl derivatives (charge +1) and C toxins (charge 0). These properties form the basis of analytical methods involving high performance liquid chromatography (HPLC) (Chapter 3).

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CYLINDROSPERMOPSIN

In 1979 at Palm Island, Queensland, Australia there was a severe outbreak of hepatoenteritis in the population supplied with drinking water from a dam which had been treated with copper sulphate to kill a heavy bloom of algae [62]. Subsequent research on *Cylindrospermopsis raciborskii* from this source showed it to produce toxicological effects in animals consistent with the symptoms observed at Palm Island. On this basis it was subsequently suggested that the 1979 outbreak was caused by toxic *C. raciborskii* [63]. This species has also been responsible for cattle deaths in Queensland [64].

A hepatotoxic alkaloid toxin was isolated from *C. raciborskii* and named cylindrospermopsin (Figure 1-4(L2)) [65]. It has also subsequently been isolated from the cyanobacterium *Umezakia natans* in Japan [66] and *Aphanizomenon ovalisporum* in both Australia [67] and Israel [68]. Cylindrospermopsin can be classified as a hepatotoxic alkaloid but toxicological studies have shown that, while the principal organ affected is the liver, other organs such as the kidney are also affected [69]. A report that another toxic compound, 7-epicylindrospermopsin, was isolated from a strain of *Aph. ovalisporum* from Israel [68] suggests that the potential presence of other toxins, some possibly unknown at present, should also be considered when dealing with these cyanobacteria.

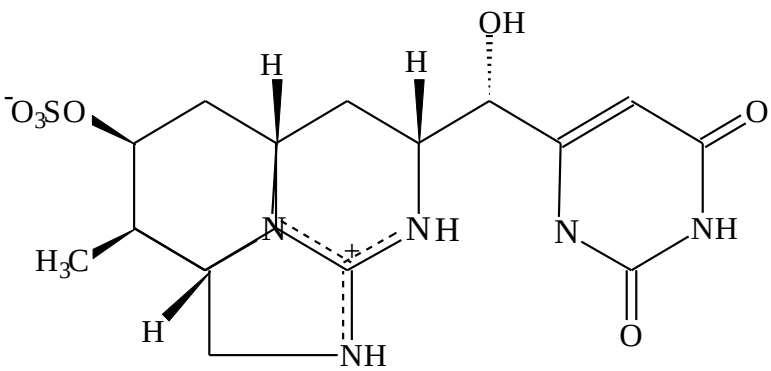

Figure 1-4(L2) Structure of cylindrospermopsin

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B-*N*-METHYLAMINO-L-ALANINE (BMAA)

The neurotoxic amino acid BMAA (β-methylamino-L-alanine) has been associated with a fatal human neurodegenerative disease, with similarities to Alzheimer's and Parkinson's diseases.

The disease (Amyotrophic Lateral Sclerosis/Parkinson's Dementia complex; ALS/PDC) was first described on Guam and BMAA was found to be produced by a symbiotic cyanobacterium living in specialized roots in cycads on the island [70]. BMAA has been reported in the brain tissue of patients who died of ALS/PDC [71] although a subsequent study found no BMAA in the brains of affected individuals [72]. BMAA is concentrated in various parts of the cycad plant [73] including the seeds which are used by the local people to produce flour. The flour is treated to remove toxins resulting in very little BMAA being ingested by this route. Consequently, flour was not considered to be a significant source of exposure to BMAA [74].

It has been hypothesised that, since flying foxes feed on cycad seeds and flying foxes are consumed by the people of Guam, this may be a route of exposure [75]. Subsequent analysis of flying foxes confirmed the accumulation of BMAA [76]. More recently, Cox et al. [70] described the biomagnification of BMAA from the cyanobacterium, through the cycads, in the flying foxes which feed on the cycad seeds and in humans who eat the flying foxes. Thus a plausible route of significant exposure has been identified. This hypothesis is supported by the decline in ALS/PDC in recent years mirroring the decline in flying fox numbers [77].

BMAA is found not only in its free form but also at higher levels bound in proteins, as are other normal protein amino acids, at all levels of the food chain (cyanobacteria, cycad plants including flour, flying foxes and brain tissue). This suggests that these proteins function as an endogenous neurotoxic reservoir slowly releasing free toxin [78].

Neurodegenerative diseases in other areas of the Pacific have also been associated with exposure to cycad material [79] which suggests that BMAA is involved in these disorders as well. A similar amino acid, β-N-oxalylamino-L-alanine (BOAA) is produced by the plant *Lathyrus sativus* and is responsible for a neurological disorder, neurolathyrism, when consumed [80].

Recent studies have suggested that BMAA is also produced widely by free-living cyanobacteria from freshwaters throughout the world. BMAA has also been found in brain tissue of not only people on Guam who had died of ALS/PDC but also Alzheimer's patients in Canada [71] although, as mentioned, a subsequent study could not reproduce these findings [72]. Other sources of BMAA, possibly free-living cyanobacteria, may contribute to these types of neurological disorders [70].

The detection of BMAA in a number of common cyanobacteria and the demonstrated capacity of BMAA to biomagnify raises some concern for the water industry. Research is needed to assess the level of risk of exposure from drinking waters. However, at this point in time, the association between BMAA and neuro-degenerative diseases must be considered tenuous.

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LIPOPOLYSACCHARIDE ENDOTOXINS

The lipopolysaccharide (LPS) endotoxins are perhaps the least understood of the toxins produced by cyanobacteria. These toxins are constituents of the outer wall of both cyanobacteria and heterotrophic gram-negative bacteria [81]. LPS endotoxins produced by cyanobacteria are less toxic than those produced by bacteria; however they may be responsible for illnesses such as gastroenteritis in human populations exposed to cyanobacteria [82]. Consequently the involvement of LPS endotoxins in episodes of human toxicity warrants further attention.

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EXAMPLES OF TOXICITY OF BENTHIC CYANOBACTERIA

Several dog deaths were linked to the presence of *Oscillatoria*-like species [83], *Phormidium favosum* mats containing anatoxin-a [84] and *Phormidium autumnale* containing both anatoxin-a and homoanatoxin-a [85]. Cattle have also died through ingestion of *Oscillatoria limosa* [86]. Baker et al. [87] investigated *Phormidium* aff. *formosum* and *Phormidium* aff. *amoenum* from two reservoirs and a recreational lake in South Australia and found them lethal to mice by intraperitoneal injection. Neuro and hepatotoxic affects have also been reported from *Calothrix parietina* and *Phormidium tenue* [88]. Izaguirre et al., [89] found several microcystin-producing *Phormidium, Oscillatoria* and *Lyngbya* species which were isolated from drinking water reservoirs and Seifert et al. [90] produced the first evidence of cylindrospermopsin and deoxy-cylindrospermopsin production by *Lyngbya wollei*.

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ADVERSE HUMAN HEALTH EPISODES DUE TO CYANOTOXINS

In view of the potential health risks of people drinking water that is contaminated by cyanotoxins, it is important to highlight the more serious suspected human poisonings that have been recorded:

Paulo Afonso gastroenteritis incident in the region of Bahia State in Brazil: In 1988 the people in the surrounding villages, who were supplied with conventional treated water from the newly built Itaparita Dam, experienced severe gastroenteritis (2000 cases were reported, of whom 88 people died). The investigation revealed that the source water from the Itaparita Dam contained very high concentrations (approximately 10^6 per millilitre) of *Anabaena* and *Microcystis* and people became sick after drinking boiled water from the dam [26].

Caruaru dialysis incident in Brazil: In 1996 an outbreak of severe hepatitis occurred at a Brazilian haemodialysis centre in Caruaru, Brazil. One hundred patients developed acute liver failure after receiving routine haemodialysis treatment; 52 of the affected patients died. The clinical symptoms included visual disturbances, nausea, vomiting, muscle weakness and painful hepatomegaly. Microcystins and cylindrospermopsin were found in the source water, in the water delivery tanker, and in the dialysis unit's holding tank as well as in the ion exchange resins and carbon filters from the dialysis centre's in-house treatment system. Microcystins were also detected in the blood sera and liver tissue of both live and deceased patients [28, 91].

Sewickley gastroenteritis incident in the United States of America: In 1975 approximately 62% of the people receiving piped water from the distribution network become ill, experiencing abdominal pains and diarrhoea. Due to a hole in the groundwater intake structure more than 40% of the source water supply came from the Ohio River. Cyanobacteria were found in the open finished-water reservoirs and it was concluded that the contamination of the distribution network was through these reservoirs [92].

Harare seasonal gastroenteritis incidents in Zimbabwe: Seasonal gastroenteritis in children is possibly due to the lysis of the cells of the annual *Microcystis* blooms that occur in the source water reservoir. The naturally-liberated cyanotoxin would probably not be effectively removed during the basic drinking-water purification process [93].

Armidale liver damage incident in Australia: The water in the Malpas Dam, which supplies water for the drinking water treatment plant for the town of Armidale was regularly treated for cyanobacteria with copper sulphate after complaints about taste and odour. In 1981, *Microcystis* scum formation around the abstraction point put additional stress on the drinking water treatment process, resulting in cyanobacteria cells passing through the treatment process

leading to re-growth in the open post-treatment drinking water tanks. Elevated enzyme activity in the sera of some town residents strongly suggests considerable liver damage. The presence of *Microcystis* and subsequent cyanotoxin release during the lysis of the cells may be responsible for the observed liver damage [21].

Palm Island poisoning incident, Queensland, Australia: In 1979, there was a major outbreak of hepato-enteritis amongst the children of the Aboriginal community after drinking water from the treatment works that received its source water from the Solomon Dam. Clinical symptoms included anorexia, vomiting, headache, painful liver enlargement, initial constipation followed by bloody diarrhoea and dehydration. It was concluded that the poisoning was due to the release of cyanotoxins during the lysis of the cyanobacteria cells after treating the surface water of the reservoir with copper sulphate. Subsequent evaluations confirmed that the poisoning was due to the presence of the cyanobacterium *Cylindrospermopsis raciborskii* in the dam [20].

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CYANOTOXIN DRINKING WATER GUIDELINES

INTERNATIONAL GUIDELINES FOR CYANOTOXINS

Guideline values for cyanotoxins are summarised in Table 1-2(L2).

Table 1-2(L2) Guideline values or standards for cyanotoxins in drinking water from various countries. (Information derived from websites and [94] unless otherwise stated).

Country	Guideline Value/Standard	Comments/Explanations
Argentina	Under revision	
Australia	1.3 µg L^{-1} total microcystins, guideline value	Australian Drinking Water Guidelines
Canada	1.5 µg L^{-1} cyanobacterial toxins as microcystin-LR MAC	Canada uses guidelines as the standard of water quality. The guidelines are expressed with the unit of Maximum acceptable concentrations (MAC). These are derived from tolerable daily intake (TDI) which in turn are derived from a calculated no-observed adverse effect level (NOAEL) from data from human or animal studies. To derive a MAC from a TDI adjustments are made for average body weight and drinking water consumption, as well as other considerations. In terms of health the guidelines ensure that the MACs are far below exposure levels at which adverse effects have been observed. For the case of cyanobacterial toxins the guideline is considered protective of human health against exposure to other microcystins (total microcystins) that may also be present
Czech Republic	1 µg L^{-1} microcystin-LR	Value as national legislation, follows WHO provisional guideline value.
China	1 µg L^{-1} microcystin-LR	WHO provisional guideline for microcystin-LR
France	1 µg L^{-1} microcystin-LR	Drinking water decree
Italy	1 µg L^{-1} microcystin-LR	WHO provisional guideline for microcystin-LR used as a reference by local authorities.
Japan	1 µg L^{-1} microcystin-LR	WHO provisional guideline for microcystin-LR
Korea	1 µg L^{-1} microcystin-LR	WHO provisional guideline for microcystin-LR.
New Zealand	MAV for cyanobacterial toxins: Anatoxin: 6.0 µg L^{-1} Anatoxin-a (S): 1.0 µg L^{-1} Cylindrospermopsin: 1.0µg L^{-1} Microcystins: 1.0 µg L^{-1} Nodularin: 1.0 µg L^{-1}	Maximum acceptable values (MAVs) for micro-organisms or organic determinands of health significance. MAVs are based on the WHO 'Guidelines for Drinking Water Quality'. They are the concentration of a determinand, which is not considered to cause any significant risk to the consumer over a lifetime of consumption of water. The method of derivation varies according to NZ conditions and the way in that the determinand presents a risk. However they are derived with the use of a TDI. The MAVs are standards in NZ. The Standards provide compliance criteria and

	Saxitoxins:1.0 µg L^{-1}	compliance is routinely monitored
Norway	1 µg L^{-1} microcystin-LR	Provisional WHO guideline for drinking water adopted
Oceania	None found	Clean drinking water supply to all people main current focus
Poland	1 µg L^{-1} microcystin-LR	National legislation for guideline value in drinking water
South Africa	0-0.8µg L^{-1} for microcystin-LR	Guideline levels for microcystins in potable water as a "Target Water Quality Range"
South America (Brazil)	1.0 µg L^{-1} for microcystins 3.0 µg L^{-1} for saxitoxins (equivalents) 15 µg L^{-1} for cylindrospermopsin	Guideline values for microcystins, saxitoxins and cylindrospermopsin, along with biomass monitoring programs. Guideline value for microcystins adopted as mandatory. Guideline values for equivalents of saxitoxins and for cylindrospermopsin included as recommendations. Use of algicides prohibited and toxicity testing/toxin analysis when cell counts exceed 10,000 cells/mL or 1mm^3 biovolume. .
Spain	1 µg L^{-1} microcystins	National legislation, maximum permissible amount in drinking water
Thailand	No guideline currently	Awareness for need for guidelines
United States of America	None currently known.	Maximum Contaminant Levels (MCLs) are the highest level of a contaminant that is allowed in drinking water. They are enforceable standards. Cyanobacteria and their toxins are listed as microbiological contaminants on the contaminant candidate list (CCL). This means that they are currently recognised as unregulated contaminants, but are known to occur in public water systems and may require regulation under the Safe Drinking Water Act. Contaminants on the CCL are a priority for the US Environmental Protection Agency with the aim to set MCLs
Uruguay	Under revision	
World Health Organisation	1.0 µg L^{-1} for microcystin-LR GV	Refer to World Health Organisation Guidelines for Drinking-Water Quality, 1996 [95]

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PROCEDURES FOR GUIDELINE DERIVATION

The guideline for microcystin–LR was derived using the following equation:

Guideline value ($\mu g\ L^{-1}$) = (TDI x Bw x PI)/DI

where:

TDI = An estimation of the amount of a substance in the drinking water expressed on a body mass basis ($\mu g\ kg^{-1}$), that can be ingested over a lifetime without significant health risks. The TDI ($\mu g\ kg^{-1} day^{-1}$) is calculated as (NOAEL or LOAEL) / Uncertainty factors. The NOAEL is the highest dose or concentration of a substance that causes no detectable adverse health effect. The LOAEL is the lowest observed dose or concentration of a substance at which there is a detectable adverse health effect. The source of uncertainty is from interspecies variation, intraspecies variation, adequacy of studies or databases and the nature and severity of the effect. The uncertainty values (factor of 10) thus ranges from 10 to 10000.

Bw = The average body weight of an adult (60 kg) or child (10 kg) or infant (5 kg).

PI = The portion of intake due to drinking water. This value is usually 10%. However, cyanotoxins intake is mainly via drinking water and is thus taken as 80 to 90%.

DI = The average drinking water consumption per day of an adult (2L) or child (1L) or infant (0.5L).

Therefore:

Guideline value (microcystin–LR as $\mu g\ L^{-1}$) = [(40/1000) x 60 x 0.8)]/2

= 0.96 $\mu g\ L^{-1}$

= 1 $\mu g\ L^{-1}$ microcystin-LR

where:

TDI = NOAEL is 40 $\mu g\ kg^{-1}\ day^{-1}$ and the uncertainty factor is 1000.

Bw = The average body weight of an adult is 60 kg.

PI = The portion of intake due to drinking water is 80%.

DI = The average drinking water consumption per day of an adult is 2L.

It is important to stress that the provisional guideline is only for microcystin–LR and thus excludes the toxicity of other microcystins that may be present [3, 6]. It is therefore advisable for drinking water suppliers not to base their guidelines on microcystin–LR alone. To overcome this problem, it has become common practice to use the 1.0 $\mu g\ L^{-1}$ microcystin-LR guideline value as a surrogate for all microcystin variants (total microcystins) to reduce the exposure risk. Therefore the frequently used guideline is 1.0 $\mu g\ L^{-1}$ microcystin equivalents (equivalent toxicity to microcystin-LR). The microcystin equivalents are calculated from the available microcystins variant toxicity data, assuming equivalent toxicity to microcystin–LR for those with no toxicity data available. Furthermore, the guideline value 1.0 $\mu g\ L^{-1}$ total microcystins is also based on the ELISA bioassay. This approach is frequently used by those water treatment facilities that do not have the capacity to monitor the full spectrum of microcystin variants, or by those that incorporate it as part of their Cyanobacteria Incident Management Framework.

Falconer [3] followed a similar approach to that of the WHO [96] in developing a proposed guideline for cylindrospermopsin:

Guideline value (cylindrospermopsin as $\mu g/L$) = [(30/1000) x 60 x 0.9)]/2

= 0.81 $\mu g/L$

= 1 $\mu g/L$

where:

TD = NOAEL is 30 $\mu g/kg/day$ and the uncertainty factors is 1000 (10 for intraspecies variation, 10 for interspecies variation, 10 for data adequacy).

Bw	=	The average body weight of an adult is 60 kg.
PI	=	The portion of intake due to drinking water is 90%.
DI	=	The average drinking water consumption per day of an adult is 2L.

It must be stressed that the guideline concentrations for both these toxins are not directly applicable to short term exposures as they aim to protect humans over a lifetime of consumption and are thus conservative [3]. This is very important for drinking water suppliers, as they may experience higher concentrations for short periods. Fitzgerald *et al*. [97] recommended that the safety factor of 10 could be omitted from the TDI calculation as the data are mainly based on subchronic exposure duration. The guideline for short-term exposure can thus be increased 10-fold. Subsequently, it was proposed that water utilities in Southern Australia use a guideline value of 10 $\mu g\ L^{-1}$ for microcystins as well as for nodularin as their alert levels. Falconer [3] argued that this value was too high and that a more conservative approach must be followed as people may be exposed to cyanotoxins several times a year. It is thus recommended that a concentration of 5 $\mu g\ L^{-1}$ be used for both the alert level and the drinking water guideline for alerting the health authorities regarding cyanotoxins.

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