Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management
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Chapter 3. CYANOBACTERIAL TOXINS

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The cyanotoxins are a diverse group of natural toxins, both from the chemical and the toxicological points of view. In spite of their aquatic origin, most of the cyanotoxins that have been identified to date appear to be more hazardous to terrestrial mammals than to aquatic biota. Cyanobacteria produce a variety of unusual metabolites, the natural function of which is unclear, although some, perhaps only coincidentally, elicit effects upon other biota. Research has primarily focused on compounds that impact upon humans and livestock, either as toxins or as pharmaceutically useful substances. Further ranges of non-toxic products are also being found in cyanobacteria and the biochemical and pharmacological properties of these are totally unknown. An overview of the currently identified cyanotoxins is given in section 3.1 and their toxicological properties are discussed in Chapter 4.

Studies on the occurrence, distribution and frequency of toxic cyanobacteria were conducted in a number of countries during the 1980s using mouse bioassay. Analytical methods suitable for quantitative toxin determination only became available in the late 1980s, but studies of specific cyanotoxins have been increasing since then. The results of both approaches indicate that neurotoxins are generally less common, except perhaps in some countries where they frequently cause lethal animal poisonings. In contrast, the cyclic peptide toxins (microcystins and nodularins) which primarily cause liver injury are more widespread and are very likely to occur if certain taxa of cyanobacteria are present. Section 3.2 presents an overview of the data currently available on the occurrence of cyanotoxins. It is noteworthy, however, that current knowledge is clearly biased by the inconsistent distribution of research effort around the world, with studies from Asia, Africa and South America beginning to appear in the 1990s. Because the ecological role of the toxins is unclear, it is not possible to use a functional approach to study the factors that enhance toxicity. Section 3.3 looks at the available data on relationships between environmental factors and toxin content and at the emerging understanding of genetic regulation of toxin production. Research into toxin production by cyanobacteria is increasing, and a better understanding of toxin function may provide a basis for predicting occurrence of toxicity in the future.

For assessing the health risk caused by cyanotoxins, an understanding of their persistence and degradation in aquatic environments is of crucial importance. Section 3.4 gives an overview of the current understanding of these processes. Because effects on aquatic biota may be relevant issues for water managers, and because public

concern could raise questions in this field for practitioners, section 3.5 briefly introduces the limited state of knowledge of cyanotoxin impacts on other aquatic organisms.

3.1 Classification

Mechanisms of cyanobacterial toxicity currently described and understood are very diverse and range from hepatotoxic, neurotoxic and dermatotoxic effects to general inhibition of protein synthesis. To assess the specific hazards of cyanobacterial toxins it is necessary to understand their chemical and physical properties, their occurrence in waters used by people, the regulation of their production, and their fate in the environment.

Cyanotoxins fall into three broad groups of chemical structure: cyclic peptides, alkaloids and lipopolysaccharides (LPS). An overview of the specific toxic substances within these broad groups that have been identified to date from different genera of cyanobacteria, together with their primary target organs in humans, is given in Table 3.1.

3.1.1 Hepatotoxic cyclic peptides - microcystins and nodularins

Globally the most frequently found cyanobacterial toxins in blooms from fresh and brackish waters are the cyclic peptide toxins of the microcystin and nodularin family. They pose a major challenge for the production of safe drinking water from surface waters containing cyanobacteria with these toxins. In mouse bioassays, which traditionally have been used to screen toxicity of field and laboratory samples, cyanobacterial hepatotoxins (liver toxins) cause death by liver haemorrhage within a few hours of the acute doses (see Chapter 4). Microcystins have been characterised from planktonic *Anabaena, Microcystis, Oscillatoria (Planktothrix), Nostoc,* and *Anabaenopsis* species, and from terrestrial *Hapalosiphon* genera. Nodularin has been characterised only from *Nodularia spumigena.*

The cyclic peptides are comparatively large natural products, molecular weight (MW) ≈ 800-1,100, although small compared with many other cell oligopeptides and polypeptides (proteins) (MW > 10,000). They contain either five (nodularins) or seven (microcystins) amino acids, with the two terminal amino acids of the linear peptide being condensed (joined) to form a cyclic compound. They are water soluble and, except perhaps for a few somewhat more hydrophobic microcystins, are unable to penetrate directly the lipid membranes of animal, plant and bacterial cells. Therefore, to elicit their toxic effect, uptake into cells occurs through membrane transporters which otherwise carry essential biochemicals or nutrients. As will be outlined in section 4.2, this restricts the target organ range in mammals largely to the liver. In aquatic environments, these toxins usually remain contained within the cyanobacterial cells and are only released in substantial amounts on cell lysis. Along with their high chemical stability and their water solubility, this containment has important implications for their environmental persistence and exposure to humans in surface water bodies (see section 3.4).

able 3.1 General features of the cyanotoxins

Toxin group[1]	Primary target organ in mammals	Cyanobacterial genera[2]
Cyclic peptides		
Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*
Nodularin	Liver	*Nodularia*
Alkaloids		
Anatoxin-a	Nerve synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Anatoxin-a(S)	Nerve synapse	*Anabaena*
Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsins	Liver[3]	*Cylindrospermopsis, Aphanizomenon, Umezakia*
Lyngbyatoxin-a	Skin, gastro-intestinal tract	*Lyngbya*
Saxitoxins	Nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharides (LPS)	Potential irritant; affects any exposed tissue	All

[1] Many structural variants may be known for each toxin group - see section 3.1 for details

[2] Not produced by all species of the particular genus

[3] Whole cells of toxic species elicit widespread tissue damage, including damage to kidney and lymphoid tissue

The first chemical structures of cyanobacterial cyclic peptide toxins were identified in the early 1980s and the number of fully characterised toxin variants has greatly increased during the 1990s. The first such compounds found in freshwater cyanobacteria were cyclic heptapeptides (that is they contain seven peptide-linked amino acids) with the general structure of:
cyclo-(D-alanine[1]-X[2]-D-MeAsp[3]-Z[4]-Adda[5]-D-glutamate[6]-Mdha[7])
in which X and Z are variable L amino acids, D-MeAsp[3] is *D-erythro*-β-methylaspartic acid, and Mdha is *N*-methyldehydroalanine (Figure 3.1 A). The amino acid Adda, (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, is the most unusual structure in this group of cyanobacterial cyclic peptide toxins.

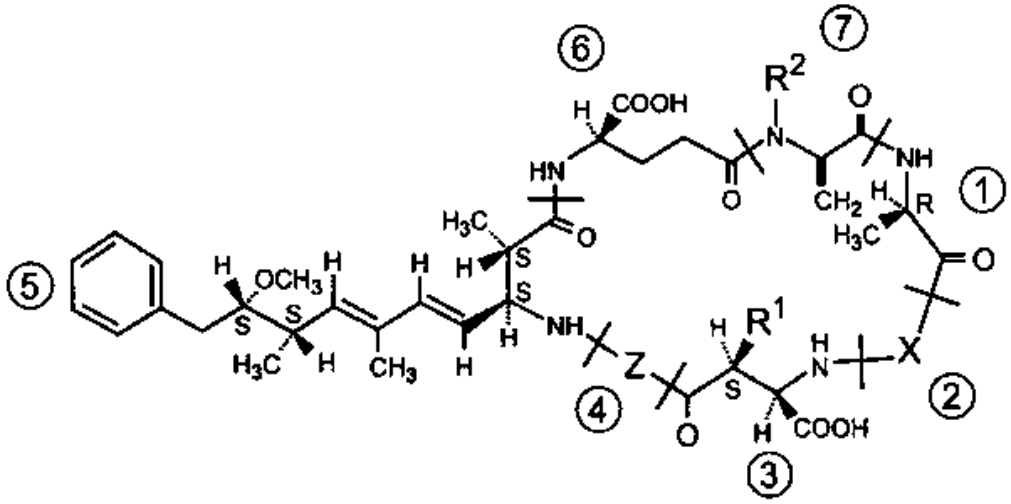
These compounds were first isolated from the cyanobacterium *Microcystis aeruginosa* and therefore the toxins were named microcystins (Carmichael *et al.,* 1988). Structural variations have been reported in all seven amino acids, but most frequently with substitution of L-amino acids at positions 2 and 4, and demethylation of amino acids at positions 3 and/or 7 (Figure 3.1A). About 60 structural variants of microcystins have been characterised so far from bloom samples and isolated strains of cyanobacteria (Table 3.2).

In one species of brackish water cyanobacterium, an identically acting and structurally very similar, cyclic pentapeptide occurs. It has been named as nodularin after its producer, *Nodularia spumigena.* The chemical structure of nodularin is cyclo-(D-MeAsp[1]-L-arginine[2]-Adda[3]-D-glutamate[4]-Mdhb[5]), in which Mdhb is 2-(methylamino)-2-dehydrobutyric acid (Figure 3.1B). A few naturally occurring variations of nodularins have been found: two demethylated variants, one with D-Asp[1] instead of D-MeAsp[1], the other with DMAdda[3] instead of Adda[3]; and the non-toxic nodularin which has the 6Z-stereoisomer of Adda[3] (Namikoshi *et al.,* 1994). The equivalent 6Z-Adda[3] stereoisomer of microcystins is also non-toxic. In the marine sponge, *Theonella swinhoei,* a nodularin analogue called motuporin has been found. It differs from nodularin only by one amino acid, having hydrophobic L-Val in place of the polar L-Arg in nodularin (de Silva *et al.,* 1992). The toxin might be cyanobacterial in origin because the sponge is known to harbour cyanobacterial symbionts.

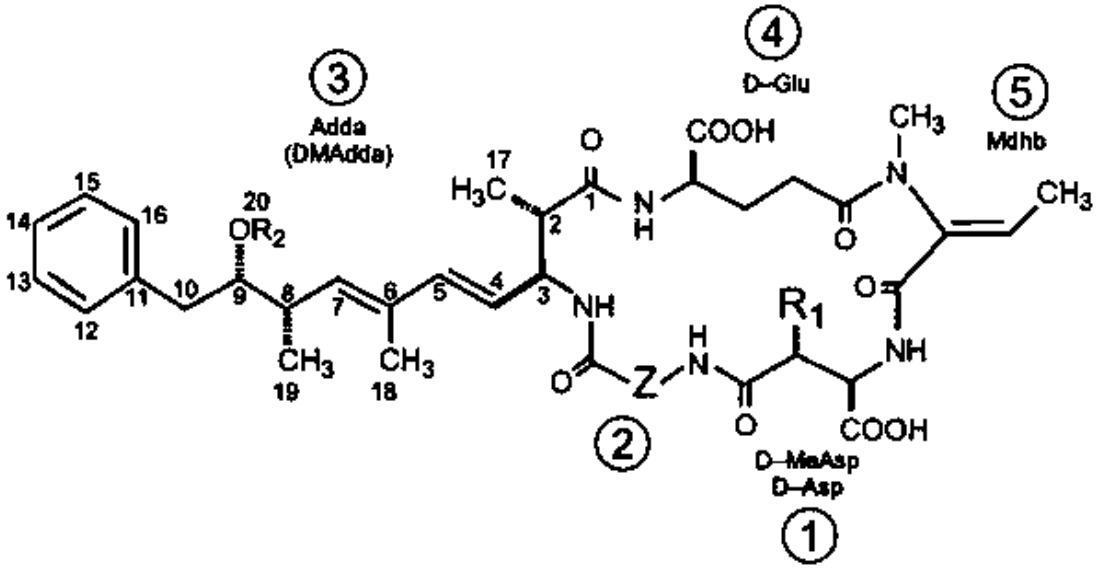
The mammalian toxicity of microcystins and nodularins is mediated through their strong binding to key cellular enzymes called protein phosphatases (see Chapter 4). In solution, microcystins and nodularins adopt a chemical "shape" that is similar, especially in the Adda-glutamate part of the cyanotoxin molecule (Rudolph-Böhner *et al.,* 1994; Annila *et al.,* 1996). Recent studies have shown that this region is crucial for interaction with the protein phosphatase protein molecule, and hence it is crucial for the toxicity of these cyanotoxins (Barford and Keller, 1994; Goldberg *et al.,* 1995). Microcystins show an additional characteristic of forming a covalent bond between the Mdha residue and the protein phosphatase molecule.

Figure 3.1 The structure of cyclic peptide toxins and cylindrospermopsin.

A. General structure of microcystins (MCYST), cyanobacterial heptapeptide hepatotoxins, showing the most frequently found variations. X and Z are variable L-amino acids (in MCYST-LR, X = L-Leusine (L) and Z = L-Arginine (R)); R^1 and R^2 are H (demethylmicrocystins) or CH_3; D-MeAsp is D-*erythro*-β-methylaspartic acid; Adda is (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid and Mdha is N-methyldehydroalanine (Dha = dehydroalanine) (see Table 3.2 for known microcystins); General structure of microcystins cyclo-(D-Ala[1]-X[2]-D-MeAsp[3]-Z[4]-Adda[5]-D-Glu[6]-Mdha[7])



B. Structures of nodularins (Z = L-arginine) and motuporin (Z = L-Valine). Mdhb is N-methyldehydrobutyrin; Nodularin R_1,R_2 = CH_3; D-Asp[1]Nodularin R_1 = H, R_2 = CH_3; DMAdda[3] Nodularin R_1 = CH_3, R_2 = H; Structure of nodularins cyclo-(D-MeAsp[1]-Z[2]-Adda[3]-D-Glu[4]-Mdhb[5])



C. Structure of cylindrospermopsin; Cylindrospermopsin MW 415; $C_{15}H_{21}N_5O_7S$

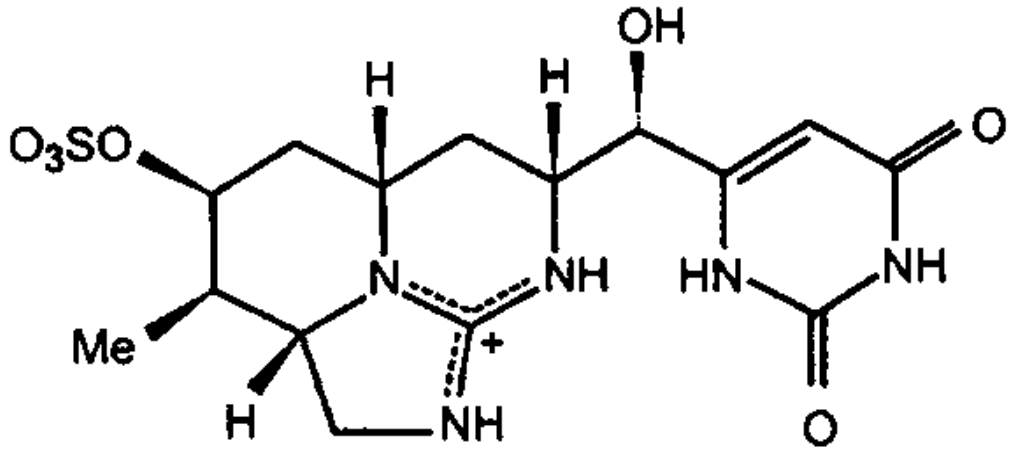


Table 3.2 The microcystins (MCYST) reported in the scientific literature

Microcystin	Molecular weight	Toxicity LD_{50}[2]	Organism[3]	Reference(s)
MCYST-LA	909	50	*M. aeruginosa*[s], *M. viridis*[s]	Botes *et al*., 1984; Kaya and Watanabe, 1990
MCYST-LAba	923	NR	*M. aeruginosa*[s]	Gathercole and Thiel, 1987
MCYST-LL	951	+	*M. aeruginosa*[b]	Craig *et al*., 1993
MCYST-AR	952	250	*Microcystis* spp.[b]	Namikoshi *et al.,* 1992a
MCYST-YA	959	NR	*M. aeruginosa*[s]	Botes *et al.,* 1985
[D-Asp[3],Dha[7]]MCYST-LR	966	+	*M. aeruginosa*[s], *Anabaena* sp.[s]	Harada et *al.,* 1991b; Sivonen *et al.,* 1992a
[D-Asp[3],Dha[7]]MCYST-EE(OMe)	969	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1998
MCYST-VF	971	NR	*M. aeruginosa*[s]	Bateman *et al.,* 1995
(D-Asp[3]]MCYST-LR	980	160-300	*A. flos-aquae*[s], *M. aeruginosa*[s], *M. viridis*[b], *O. agardhii*[s]	Krishnamyrthy *et al.,* 1989; Cremer and Henning, 1991; Harada *et al.,* 1990b; 1991a; Luukkainen *et al.,* 1993
[Dha[7]]MCYST-LR	980	250	*M. aeruginosa*[s], *Anabaena sp.*[s], *O. agardhii*[s]	Harada *et al.,* 1991b; Sivonen *et al*., 1992a; Luukkainen *et al.,* 1993
[DMAdda[5]]MCYST-LR	980	90-100	*Microcystis* spp.[b], *Nostoc* sp.[s]	Namikoshi *et al.,* 1992a; Sivonen *et al.,* 1992b
[Dha[7]]MCYST-EE(OMe)	983	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1998
[D-Asp[3],Dha[7]]MCYST-E(OMe)E(OMe)	983	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1998
MCYST-LF	985	+	*M. aeruginosa*[s]	Azevedo *et al.,* 1994

MCYST-LR	994	50	*M. aeruginosa*[s], *A. flos-aquae*[s] *M. viridis*[s]	Botes *et al.,* 1985; Rinehart *et al.,* 1988; Krishnamyrthy *et al.,* 1989; Watanabe *et al.,* 1988
[D-Asp[3],D-Glu(OCH_3)[6]]MCYST-LR	994	NR	*A. flos-aquae*[s]	Sivonen *et al.,* 1992d
[(6Z)-Adda[5]]MCYST-LR	994	>1,200	*M. viridis*[b]	Harada *et al.,* 1990a,b
[Dha[7]]MCYST-E(OMe)E(OMe)	997	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1998
[L-Ser[7]]MCYST-LR	998	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1992c
MCYST-LY	1,001	90	*M. aeruginosa*[s]	Stoner *et al.,* 1989
[L-Ser[7]]MCYST-EE(OMe)	1,001	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1998
[D-Asp[3],Ser[7]]MCYST-E(OMe)E(OMe)	1,001	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1998
MCYST-HilR	1,008	100	*Microcystis* spp.[b]	Namikoshi *et al.,* 1995
[D-Asp[3],ADMAdda[5]]MCYST-LR	1,008	160	*Nostoc* sp.[s]	Sivonen *et al.,* 1990a; Namikoshi *et al.,* 1990
[D-Glu(OCH_3)[6]]MCYST-LR	1,008	>1,000	*A. flos-aguae*[s], *Microcystis sp.*[s]	Sivonen *et al.,* 1992d; Bateman *et al.,* 1995; Rinehart *et al*., 1994
[D-Asp[3],Dha[7]]MCYST-RR	1,009	+	*O. agardhii*[b], *Anabaena* sp.[s], *M. aeruginosa*[s]	Krishnamyrthy *et al.,* 1989; Sivonen *et al.,* 1992a; Luukkainen *et al.,* 1994
[D-Asp[3], ADMAdda[5], Dhb[7]]MCYST-LR	1,009	+	*Nostoc sp.*[s]	Beattie *et al.,* 1998
[L-MeSer[7]]MCYST-LR	1,012	150	*Microcystis* spp.[b]	Namikoshi *et al., 1992a;* 1995
[Dha[7]]MCYST-FR	1,014	NR	*Microcystis sp.*[s]	Luukkainen *et al.,* 1994
[L-Ser[7]]MCYST-E(OMe)E(OMe)	1,015	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1998
[ADMAdda[5]]MCYST-LR	1,022	60	*Nostoc* sp.[s]	Sivonen *et al.,* 1990a; Namikoshi *et al.,* 1990
[D-Asp[3],ADMAdda[5]]MCYST-LHar	1,022	+	*Nostoc* sp.[s]	Sivonen *et al.,* 1992b
[D-Asp[3]]MCYST-RR	1,023	250	*O. agardhii*[s], *Anabaena* sp.[s], *M. aeruginosa*[s]	Meriluoto *et al.,* 1989; Sivonen *et al.,* 1992a; Luukkainen *et al.,* 1994
[Dha[7]]MCYST-RR	1,023	180	*M. aeruginosa*[s], *Anabaena* sp.[s], *O. agardhii*[s]	Kiviranta *et al.,* 1992; Sivonen *et al.,* 1992a; Luukkainen *et al.,* 1993
MCYST-LW	1,024	NR	*M. aeruginosa*[s]	Bateman *et al.,* 1995
MCYST-FR	1,028	250	*Microcystis* spp.[b]	Namikoshi *et al.,* 1992a
MCYST-M(O)R	1,028	700-800	*Microcystis* spp.[b]	Namikoshi *et al.,* 1992a
[Dha[7]]MCYST-HphR	1,028	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1992b
[D-Asp[3], Dha[7]]MCYST-HtyR	1,030	+	*Anabaena* sp.[s]	Namikoshi *et al.,* 1992b

[Dha^{7}]MCYST-YR	1,030	+	*M. aeruginosa*^{s}	Sivonen *et al.,* 1992c
[D-Asp^{3}]MCYST-YR	1,030	+	*Microcystis* spp.^{b}	Namikoshi *et al.,* 1992d
MCYST-YM(O)	1,035	56	*M. aeruginosa*^{b}	Botes *et al.,* 1985; Elleman *et al.,* 1978
[ADMAdda^{5}]MCYST-LHar	1,036	60	*Nostoc* sp.^{s}	Sivonen *et al.,* 1990a; Namikoshi *et al.,* 1990
MCYST-RR	1,037	600	*M. aeruginosa*^{s}, *M. viridis*^{s}, *Anabaena* sp.^{s}	Kusumi *et al.*, 1987; Painuly *et al.,* 1988; Watanabe *et al.,* 1988; Sivonen *et al., 1992a*
[(6Z)-Adda^{5}]MCYST-RR	1,037	>1,200	*M. viridis*^{b}	Harada *et al.,* 1990a,b
[D-Ser^{1}, ADMAdda^{5}]MCYST-LR	1,038	+	*Nostoc* sp.^{s}	Sivonen *et al.,* 1992b
[ADMAdda^{5},MeSer^{7}]MCYST-LR	1,040	+	*Nostoc* sp.^{s}	Sivonen *et al.,* 1992b
[L-Ser^{7}]MCYST-RR	1,041	+	*Anabaena* sp.^{s}, *M. aeruginosa*$^{s/b}$	Namikoshi *et al.,* 1992c; Luukkainen *et al.,* 1994
[D-Asp^{3},MeSer^{7}]MCYST-RR	1,041	+	*O. agardhii*^{s}	Luukkainen *et al.* 1993
MCYST-YR	1,044	70	*M. aeruginosa*^{s}, *M. viridis*^{s}	Botes *et al.,* 1985; Watanabe *et al.*, 1988
[D-Asp^{3}]MCYST-HtyR	1,044	160-300	*A. flos-aquae*^{s}	Harada *et al.*, 1991a
[Dha^{7}]MCYST-HtyR	1,044	+	*Anabaena* sp.^{s}	Namikoshi *et al.*, 1992b
MCYST-(H_4)YR	1,048	NR	*Microcystis* spp.^{b}	Namikoshi *et al.*, 1995
[D-Glu-$OC_2H_3(CH_3)OH^{6}$]MCYST-LR	1,052	>1,000	*Microcystis* spp.^{b}	Namikoshi *et al.*, 1992a
[D-Asp^{3},ADMAdda^{5}, Dhb^{7}]MCYST-RR	1,052	+	*Nostoc* sp.^{s}	Beattie *et al.*, 1998
MCYST-HtyR	1,058	80-100	*A. flos-aquae*^{s}	Harada *et al.*, 1991a
[L-Ser^{7}]MCYST-HtyR	1,062	+	*Anabaena* sp.^{s}	Namikoshi *et al.*, 1992b
MCYST-WR	1,067	150-200	*Microcystis* spp.^{b}	Namikoshi *et al.*, 1992a
[D-Asp^{3},ADMAdda^{5},Dhb^{7}]MCYST-HtyR	1,073	+	*Nostoc* sp.^{s}	Beattie *et al.*, 1998
[L-MeLan^{7}]MCYST-LR	1,115	1,000	*Microcystis* spp.^{b}	Namikoshi *et al.*, 1995

Aba	Aminoisobutyric acid
ADMAdda	*O*-Acetyl-*O*-demethylAdda
Dha	Dehydroalanine
Dhb	Dehydrobutyrine
DMAdda	*O*-DemethylAdda
E(OMe)	Glutamic acid methyl ester Δ
(H_4)Y	1,2,3,4,-tetrahydrotyrosine
Har	Homoarginine

Hil	Homoisoleucine
Hph	Homophenylalanine
Hty	Homotyrosine
MeLan	*N*-Methyllanthionine
M(O)	Methionine-*S*-oxide
MeSer	*N*-Methylserine
(6Z)-Adda	Stereoisomer of Adda at the Δ^6 double bond

[1] Several partial structures of microcystins have been reported in addition to those shown in this table (see Boland *et al.*, 1993; Craig *et al*., 1993; Jones *et al.,* 1995; Sivonen *et al.*, 1995)

[2] Toxicity determined i.p. mouse (µg kg^{-1}); the LD_{50} value is the dose of toxin that kills 50% of exposed animals; a '+' denotes a toxic result in a non-quantitative mouse bioassay or inhibition of protein phosphatase and 'NR' denotes 'Not reported'

[3] An '*s*' denotes toxins isolated from culture samples and a *'b'* denotes toxins isolated from bloom samples

Most of the structural variants of microcystin and nodularin are highly toxic within a comparatively narrow range (intra-peritoneal (i.p.) mouse toxicities largely in the range 50-300 µg kg^{-1} body weight (bw); see Table 3.2 and section 4.2). Only a few non-toxic variants have been identified. In general, any structural modifications to the Adda-glutamate region of the toxin molecule, such as a change in isomerisation of the Adda-diene (6(E) to 6(Z)) or acylation of the glutamate, renders microcystins and nodularins non-toxic (Harada *et al.*, 1990 a,b; Rinehart *et al*., 1994). Linear microcystins and nodularin are more than 100 times less toxic than the equivalent cyclic compounds. The linear microcystins are thought to be microcystin precursors and/or bacterial breakdown products (Choi *et al.*, 1993; Rinehart *et al.*, 1994; Bourne *et al.,* 1996).

Microcystins and nodularin have been characterised from axenic cyanobacterial strains (i.e. strains free of contaminating bacteria) and thus the cyanobacterial origin of these compounds is clear. At the present time, it is known that microcystins are produced by bloom forming species of *Microcystis, Anabaena, Oscillatoria (Planktothrix),* and *Nostoc* (see Table 3.2), by a species of *Anabaenopsis* and by a soil isolate of *Haphalosiphon hibernicus.* Nodularins have been found, with the exception of the marine sponge *Theonella* already mentioned, only in *Nodularia spumigena* (see section 3.2 for more details). Further species may yet be demonstrated to produce microcystin.

3.1.2 Neurotoxic alkaloids - anatoxins and saxitoxins

Mass occurrences of neurotoxic cyanobacteria have been reported from North America, Europe and Australia, where they have caused animal poisonings. In mouse bioassays death by respiratory arrest occurs rapidly (within 2-30 minutes) (see Chapters 4 and 13). Three families of cyanobacterial neurotoxins are known:

- anatoxin-a and homoanatoxin-a, which mimic the effect of acetyl choline,

- anatoxin-a(S), which is an anticholinesterase, and

- saxitoxins, also known as paralytic shellfish poisons (PSPs) in the marine literature, which block nerve cell sodium channels.

Anatoxin-a has been found in *Anabaena, Oscillatoria* and *Aphanizomenon,* homoanatoxin-a from *Oscillatoria,* anatoxin-a(S) from *Anabaena,* and saxitoxins from *Aphanizomenon, Anabaena, Lyngbya* and *Cylindrospermopsis.* Sixteen confirmed saxitoxins from cyanobacterial samples have been reported, some of which (e.g. the decarbamoyl-gonyautoxins) may be chemical breakdown products in some species (see section 3.4.2).

The alkaloid toxins are diverse, both in their chemical structures and in their mammalian toxicities. Alkaloids, in general, are a broad group of heterocyclic nitrogenous compounds (i.e. they contain ring structures with at least one carbon-nitrogen bond) usually of low to moderate molecular weight (< 1,000). They are produced, in particular, by plants and by some bacteria, and are invariably bioactive and commonly toxic. The non-sulphated alkaloid toxins of freshwater cyanobacteria (anatoxins and saxitoxin) are all neurotoxins. The sulphated PSPs, C-toxins and gonyautoxins (sulphated derivatives of saxitoxin) are also neurotoxins, but the sulphated alkaloid cylindrospermopsin blocks protein synthesis with a major impact on liver cells. Some marine cyanobacteria also contain alkaloids (lyngbyatoxins, aplysiatoxins) which are dermatoxins (skin irritants), but have also been associated with gastro-enteritis and more general symptoms such as fever (see Chapter 4).

Alkaloids have varying chemical stabilities, often undergoing spontaneous transformations to by-products which may have higher or lower potencies than the parent toxin. Some are also susceptible to direct photolytic degradation (see section 3.4).

Anatoxin-a

Anatoxin-a is a low molecular weight alkaloid (MW = 165), a secondary amine, 2-acetyl-9-azabicyclo(4-2-1)non-2-ene (Figure 3.2) (Devlin *et al.,* 1977). Anatoxin-a is produced by *Anabaena flos-aquae, Anabaena* spp. *(flos-aquae-lemmermannii* group), *Anabaena planktonica, Oscillatoria, Aphanizomenon* and *Cylindrospermum* (see section 3.2 for details). Homoanatoxin-a (MW = 179) is an anatoxin-a homologue isolated from an *Oscillatoria formosa (Phormidium formosum)* strain (Figure 3.2). It has a propionyl group at C-2 instead of the acetyl group in anatoxin-a (Skulberg *et al.,* 1992). The LD_{50} (lethal dose resulting in 50 per cent deaths) of anatoxin-a and homoanatoxin-a are 200 - 250 µg kg^{-1} bw (Devlin *et al.,* 1977; Carmichael *et al.,* 1990; Skulberg *et al.,* 1992).

Anatoxin-a(S)

Anatoxin-a(S) is a unique phosphate ester of a cyclic N-hydroxyguanine (MW = 252) (Figure 3.2) produced by *Anabaena flos-aquae* strain NRC 525-17 (Matsunaga *et al.,* 1989). It has more recently been identified in blooms and isolated strains of *Anabaena lemmermannii* (Henriksen *et al.,* 1997; Onodera *et al.,* 1997a). The LD_{50} of anatoxin-a(S) is 20 µg kg^{-1} bw (i.p. mouse) (Carmichael *et al.,* 1990). Structural variants of anatoxin-a(S) have not been detected.

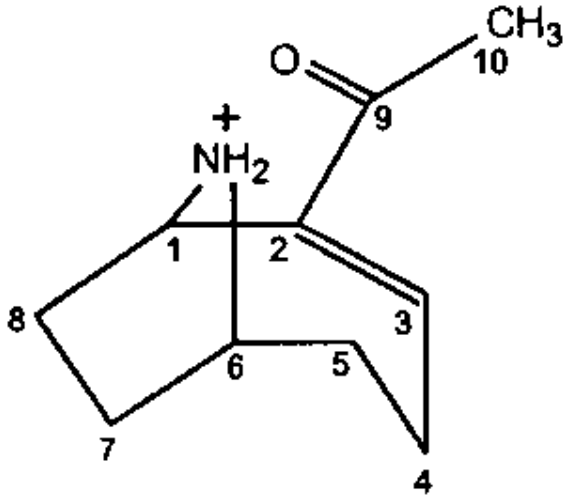
Saxitoxins

Saxitoxins are a group of carbamate alkaloid neurotoxins which are either non-sulphated (saxitoxins - STX), singly sulphated (gonyautoxins - GTX) or doubly sulphated (C-toxins)

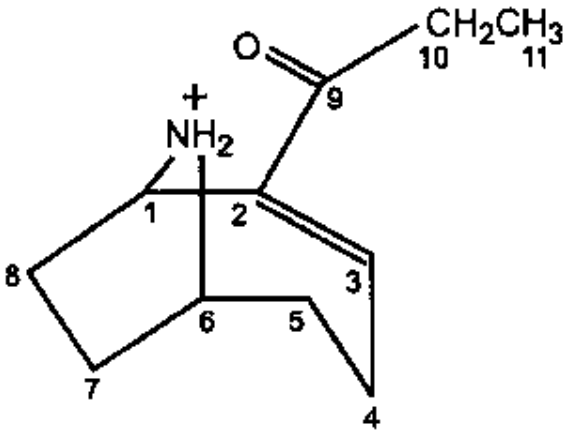
(Figure 3.2 and Table 3.3). In addition, decarbamoyl variants and several new toxins have been identified in some species.

Figure 3.2 The chemical structures of cyanobacterial neurotoxins, anatoxin-a, homoanatoxin-a, anatoxin-a(S), and the general structure of saxitoxins. Sixteen different saxitoxins have been reported from cyanobacteria (for details see Table 3.3). MW = molecular weight

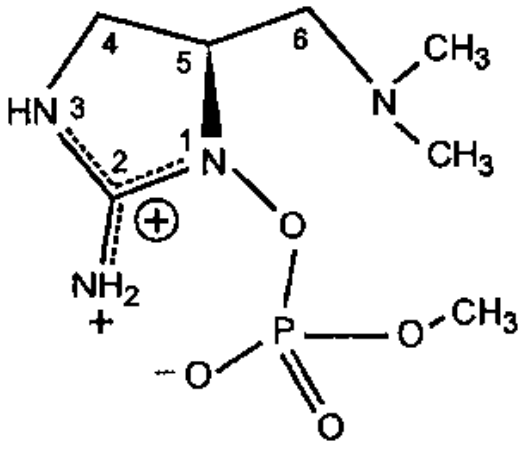
Anatoxin-a MW 165; $C_{10}H_{15}NO$



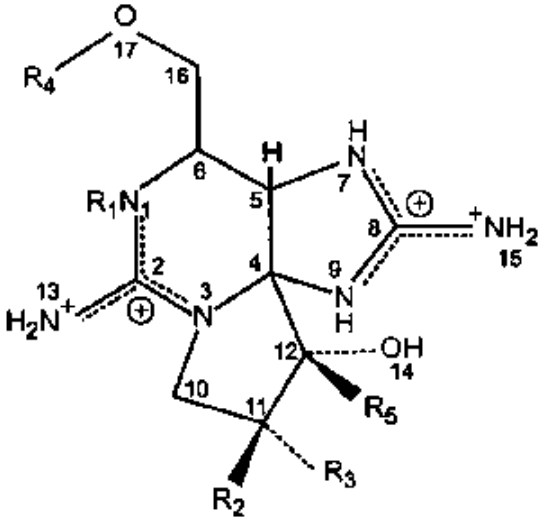
Homoanatoxin-a MW 179; $C_{11}H_{17}NO$



Anatoxin-a(S) MW 252; $C_7H_{17}N_4O_4P$



General structure of saxitoxins



Saxitoxins were originally isolated from shellfish where they are concentrated from marine dinoflagellates (so called "red tide" algae) and have caused deaths in humans (Anderson, 1994) (see section 4.1). Saxitoxins have been found in the cyanobacteria *Aphanizomenon flos-aquae, Anabaena circinalis, Lyngbya wollei* and *Cylindrospermopsis raciborskii.* The North American *Aphanizomenon flos-aquae* strains NH-1 and NH-5 contain mostly neosaxitoxin and less saxitoxin (plus a few unidentified neurotoxins). *Anabaena circinalis* strains (from Australia) contain mostly C1 and C2 toxins, with lesser amounts of gonyautoxins 2 and 3. The freshwater cyanobacterium *Lyngbya wollei* produced three known and six new saxitoxin analogues. *Cylindrospermopsis raciborskii* in Brazil was found to contain mostly neosaxitoxin and a smaller amount of saxitoxin.

Table 3.3 Saxitoxins reported from cyanobacterial strains and bloom samples (for the chemical structure see Figure 3.2)

Name of toxin	Variable chemical groups in toxins					Cyanobacteria			
	R_1	R_2	R_3	R_4	R_5	Aph[1]	Ana[2]	Lyn[3]	Cyl[4]
STX	H	H	H	$CONH_2$	OH	+	+		+
GTX2	H	H	OSO_3^-	$CONH_2$	OH		+		
GTX3	H	OSO_3^-	H	$CONH_2$	OH		+		
GTX5	H	H	H	$CONHSO_3^-$	OH		+		
C1	H	H	OSO_3^-	$CONHSO_3^-$	OH		+		
C2	H	OSO_3^-	H	$CONHSO_3^-$	OH		+		
NEO	OH	H	H	$CONH_2$	OH	+			+
GTX1	OH	H	OSO_3^-	$CONH_2$	OH		*		
GTX4	OH	OSO_3^-	H	$CONH_2$	OH		*		
GTX6	OH	H	H	$CONHSO_3^-$	OH		*		
dcSTX	H	H	H	H	OH		+	+	
dcGTX2	H	H	OSO_3^-	H	OH		+	+	
dcGTX3	H	OSO_3^-	H	H	OH		+	+	
LWTX1[3]	H	OSO_3^-	H	$COCH_3$	H			+	
LWTX2[3]	H	OSO_3^-	H	$COCH_3$	OH			+	
LWTX3[3]	H	H	OSO_3^-	$COCH_3$	OH			+	
LWTX4[3]	H	H	H	H	H			+	
LWTX5[3]	H	H	H	$COCH_3$	OH			+	
LWTX6[3]	H	H	H	$COCH_3$	H			+	

STX Saxitoxin

GTX Gonyautoxins

C C-toxins

dcSTX Decarbamoylsaxitoxin

LWTX Lyngbya-wollei-toxins

[1] Toxins found in *Aphanizomenon flos-aquae,* New Hampshire, USA (Ikawa *et al.,* 1982; Mahmood and Carmichael, 1986)

[2] Toxins reported in an *Anabaena circinalis* strain and bloom samples, Australia (Humpage *et al.*, 1994; Negri *et al.,* 1995; Negri *et al.,* 1997). dcGTX2 and dcGTX3 are probably break down products of C1 and C2 in this species (Jones and Negri, 1997). An asterisk in this column denotes toxins reported by Humpage *et al.,* 1994 for *Anabaena circinalis* based on retention time data, but not confirmed by mass spectrometry, and not found in subsequent studies

[3] Toxins detected in *Lyngbya wollei,* USA (Onodera *et al.*, 1997b)

[4] Toxins thus far found in *Cylindrospermopsis raciborskii,* Brazil (Lagos *et al.,* 1997)
Other neurotoxic cyanobacteria

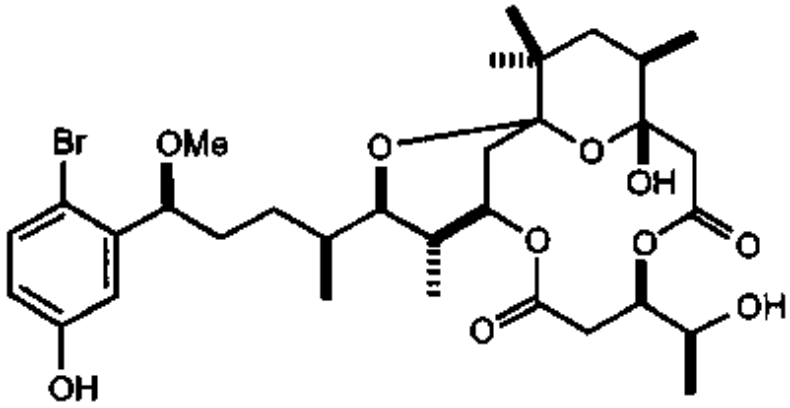
In marine *Trichodesmium* blooms from the Virgin Islands, a neurotoxic factor has been reported which was not anatoxin-a or anatoxin-a(S) but remains to be characterised (Hawser *et al.,* 1991).

3.1.3 Cytotoxic alkaloids

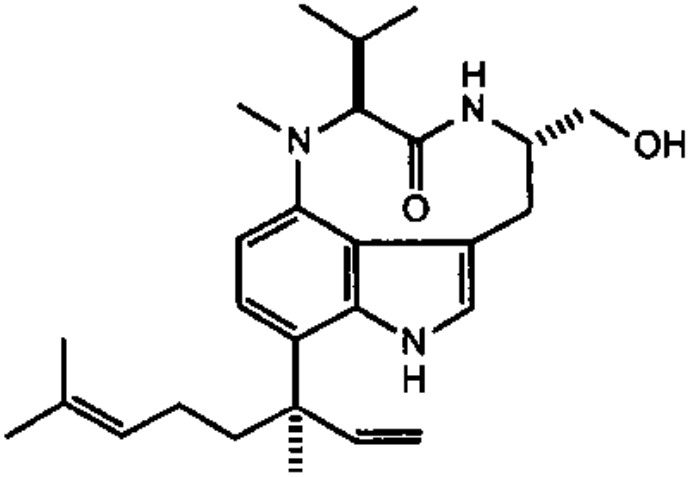
In tropical and subtropical waters of Australia, the alkaloid hepatotoxin cylindrospermopsin with a completely different mechanism of toxicity has caused health problems in drinking water supplies (see section 4.1). It is a cyclic guanidine alkaloid with a molecular weight of 415 (Figure 3.1C). It is produced by *Cylindrospermopsis raciborskii* (Hawkins *et al.,* 1985, 1997), *Umezakia natans* (Harada *et al.,* 1994) and *Aphanizomenon ovalisporum* (Banker *et al.,* 1997). In pure form, cylindrospermopsin mainly affects the liver, although crude extracts of *C. raciborskii* injected or given orally to mice also induce pathological symptoms in the kidneys, spleen, thymus and heart (see Chapter 4 for more details). Pure cylindrospermopsin has an LD_{50} in mice (i.p.) of 2.1 mg kg l^{-1} bw at 24 h and 0.2 mg kg l^{-1} bw at 5-6 days (Ohtani *et al.,* 1992). Recently, new structural variants of cylindrospermopsin have been isolated from an Australian strain of *C. raciborskii,* with one being identified as demethoxy-cylindrospermopsin (Chiswell *et al.,* 1999).

Figure 3.3 The chemical structures of debromoaplysiatoxin and lyngbiatoxin-a

Debromoaplysiatoxin



Lyngbyatoxin A



3.1.4 Dermatotoxic alkaloids - aplysiatoxins and lyngbyatoxin

Benthic marine cyanobacteria such as *Lyngbya, Oscillatoria* and *Schizothrix* may produce toxins causing severe dermatitis among swimmers in contact with the filaments (see section 4.2). The inflammatory activity of *Lyngbya* is caused by aplysiatoxins and debromoaplysiatoxin (Figure 3.3) which are potent tumour promoters and protein kinase C activators (Mynderse *et al.,* 1977; Fujiki *et al.,* 1990). Another strain of *Lyngby*a *majuscula* has caused dermatitis and severe oral and gastrointestinal inflammation. It was found to contain lyngbyatoxin-a (see Figure 3.3) (Cardellina *et al.,* 1979). Debromoaplysiatoxin along with other toxic compounds has also been isolated from other Oscillatoriaceae, such as *Schizothrix calcicola* and *Oscillatoria nigroviridis.*

3.1.5 Irritant toxins - lipopolysaccharides

Weise *et al.* (1970) were the first to isolate LPS from the cyanobacterium *Anacystis nidulans* and numerous reports of endotoxins in cyanobacteria have followed. Lipopolysaccarides are generally found in the outer membrane of the cell wall of Gram negative bacteria, including cyanobacteria, where they form complexes with proteins and phospholipids. They are pyrogenic and toxic (Weckesser and Drews, 1979). Lipopolysaccarides, as the name implies, are condensed products of a sugar, usually a hexose, and a lipid, normally a hydroxy C_{14}-C_{18} fatty acid. The many structural variants of LPS are generally phylogenetically conserved, i.e. particular orders, genera and occasionally species, have identical or similar fatty acid and sugar components contained in their cell wall LPS. It is generally the fatty acid component of the LPS molecule that elicits an irritant of allergenic response in humans and mammals.

Lipopolysaccharides are an integral component of the cell wall of all Gram negative bacteria, including cyanobacteria, and can elicit irritant and allergenic responses in human and animal tissues that come in contact with the compounds. There is considerable diversity of LPS composition among the cyanobacteria, but differences are largely related to phylogeny. Thus, different genera typically have distinct LPS compositions that are largely conserved within that genus (Kerr *et al.,* 1995). Cyanobacterial LPS are considerably less potent than LPS from pathogenic gram-

negative bacteria such as, for example, *Salmonella* (see Chapter 4). The chemical stability of cyanobacterial LPS in surface waters is unknown.

Structurally, LPS is a complex polymer composed of four regions. Region I, the O-antigen region, consists of repeating oligosaccharide units that may vary in structure, with numerous combinations of different sugar residues and associated glycosidic linkages. As suggested by its name, the O-antigen also exhibits several antigenic determinants that constitute the receptor sites for a number of lysogenic bacteriophages. Regions II and III are the outer core and backbone of a core polysaccharide. There is generally only minor variation in core structure between species. The backbone of the polysaccharide is connected to a glycolipid, lipid A (Region IV), via a short link normally composed of 3-deoxy-D-mannoocmiosonic acid (KDO). Lipid A is a disaccharide of glucosamines highly substituted with phosphate, fatty acids and KDO, although the proportion of KDO is low or absent in cyanobacteria compared with other bacterial LPS. The lipid A component is also acetylated with amide and ester-linked hydroxy fatty acids.

Recent studies of the fatty acid composition of Australian species of cyanobacteria (Kerr *et al.,* 1995) show a range of β-OH fatty acids ranging in size from C_{10} to C_{22}. Normal, saturated and branched chain acids have been detected. There was a stark predominance of straight chain 14:0 and 18:0 β-OH acids in *Microcystis* strains that was quite distinct from *Anabaena* and *Nodularia* strains where 16:0 β-OH predominated the LPS fatty acid fraction.

Although comparatively poorly studied, cell wall components, particularly LPS endotoxins from cyanobacteria may contribute to human health problems associated with exposure to mass occurrences of cyanobacteria. The few results available indicate that cyanobacterial LPS is less toxic than the LPS of other bacteria, such as *Salmonella* (Keleti and Sykora, 1982; Raziuddin *et al.,* 1983) (see also section 4.2). More studies are needed to evaluate the chemical structures and health risks of cyanobacterial LPS.

3.1.6 Other bioactive compounds

Cyanobacteria are known to produce several other bioactive compounds, some of which are of medical interest, as well as compounds toxic to other cyanobacteria, bacteria, algae and zooplankton (see section 3.5). Severe intoxication of fish embryos by crude extracts of *Planktothrix agardhii* has been reported by Oberemm *et al.* (1997). Skulberg *et al.* (1994) reported the presence of an unidentified "protracted toxic effect" in cyanobacterial samples that caused death within 4-24 hours in mice. Whether this effect was due to a specific cyanotoxin is unclear.

Cyanobacteria have been found to be a rich source of biomedically interesting compounds and therefore screening programmes for new bioactivities are underway. Cyanobacteria are known to produce antitumour, antiviral, antibiotic and antifungal compounds. Of the cyanobacterial extracts screened by a Hawaiian research group, 0.8 per cent showed solid tumour selective cytotoxicity (Moore *et al.,* 1996). Depsipeptides (peptides with an ester linkage) called cryptophycins isolated from a cyanobacterium, *Nostoc* sp. strain GSV 224, are promising candidates for an anticancer drug (Trimurtulu *et al.,* 1995). Recently, several new cyclic or linear peptides and depsipeptides from cyanobacteria have been characterised. Some are protease inhibitors, but the biological activity of the others remains to be characterised (Namikoshi and Rinehart, 1996). Many

of the cyanobacterial bioactive compounds possess structural similarities to natural products from marine invertebrates.

3.2 Occurrence of cyanotoxins

3.2.1 Mass occurrences of toxic cyanobacteria

The toxicity of cyanobacterial mass occurrences (blooms) was originally brought to the attention of scientists through reports of animal poisonings by farmers and veterinarians, with the first well documented case being reported from Australia in 1878 (Francis, 1878). In most, if not all, reported cases since that time, afflicted animals consumed water from water bodies where there was an obvious presence of a cyanobacterial scum on the water surface (see Ressom *et al.* (1994) and Yoo *et al.* (1995) for a list of reported animal poisonings). More recent measurements of cyanobacterial toxins using sensitive modem analytical methods have often revealed high frequencies of toxic blooms even when animal poisonings have not been reported (Table 3.4).

Throughout the world, it appears that liver-toxic (hepatotoxic, microcystin-containing) freshwater blooms of cyanobacteria are more commonly found than neurotoxic blooms. Liver-toxic blooms have been reported from all continents and almost every part of the world where samples have been collected for analysis. Nevertheless, mass occurrences of neurotoxic cyanobacteria are common in some countries and these have been reported from North America, Europe and Australia, where they have caused several animal poisonings. Blooms which have caused both liver and kidney damage due to the toxin cylindrospermopsin (and possibly related cyanotoxins) have been reported in Australia, Japan, Israel and Hungary.

In recent years, surveys have been carried out in a number of countries in South America, Africa, Australasia, Asia and Europe. The conclusion that can be drawn from these surveys is that toxic cyanobacteria are internationally ubiquitous, and that as further surveys are carried out more toxic cyanobacterial blooms and new toxic species will be discovered. This is particularly true of tropical and subtropical regions that are currently under-represented in the literature. It seems likely that every country in the world will have water bodies which support blooms of toxic cyanobacteria at some time or another. It is also important to note that mass occurrences of toxic cyanobacteria are not always associated with human activities causing pollution or "cultural eutrophication" (see Chapter 2). For example, massive blooms of toxic cyanobacteria have been reported in Australian reservoirs with pristine or near-pristine catchments (watersheds), and toxic benthic cyanobacteria have killed cattle drinking from oligotrophic, high-alpine waters in Switzerland.

3.2.2 Species composition and variation among toxic blooms

Cyanobacterial populations may be dominated by a single species or be composed of a variety of species, some of which may not be toxic. Even within a single-species bloom there may be a mixture of toxic and non-toxic strains. A strain is a specific genetic subgroup within a particular species, and each species may encompass tens or hundreds of strains, each with slightly different traits. Some strains are much more toxic than others, sometimes by more than three orders of magnitude. This can mean that one highly toxic strain, even when occurring in minor amounts amongst larger numbers of

non-toxic strains, may render a bloom sample toxic (Sivonen *et al.,* 1989a,b; Bolch *et al.,* 1997; Vezie *et al.,* 1998).

Table 3.4 Frequencies of mass occurrences of toxic cyanobacteria in freshwaters

Country	No of samples tested	% of toxic samples	Type of toxicity	Reference
Australia	231	42	Hepatotoxic	Baker and Humpage, 1994
			Neurotoxic	
Australia	31	84[1]	Neurotoxic	Negri *et al.*, 1997
Brazil	16	75	Hepatotoxic	Costa and Azevedo, 1994
Canada, Alberta	24	66	Hepatotoxic	Gorham, 1962
			Neurotoxic	
Canada, Alberta	39	95	Hepatotoxic	Kotak *et al.,* 1993
Canada, Alberta (3 lakes)	226	74[1]	Hepatotoxic	Kotak *et al.*, 1995
Canada, Saskatchewan	50	10	Hepatotoxic	Hammer, 1968
			Neurotoxic	
China	26	73	Hepatotoxic	Carmichael *et al.,* 1988b
Czech and Slovak Rep.	63	82	Hepatotoxic	Maršálek *et al.,* 1996
Denmark	296	82	Hepatotoxic	Henriksen *et al.,* 1996b
			SDF	
			Neurotoxic	
Former German	10	70	Hepatotoxic	Henning and Kohl, 1981
Dem. Rep.			SDF	
Germany	533	72[1]	Hepatotoxic	Fastner, 1998
Germany	393	22	Neurotoxic	Bumke-Vogt, 1998
Greece	18	?	Hepatotoxic	Lanaras *et al.,* 1989
Finland	215	44	Hepatotoxic	Sivonen, 1990a
			Neurotoxic	
France, Brittany	22	73[1]	Hepatotoxic	Vezie *et al.*, 1997
Hungary	50	66	Hepatotoxic	Törökné, 1991
Japan	23	39	Hepatotoxic	Watanabe and Oishi, 1980
Netherlands	10	90	Hepatotoxic	Leeuwangh *et al.,* 1983
Norway	64	92	Hepatotoxic	Skulberg *et al.*, 1994
			Neurotoxic	
			SDF	

Portugal	30	60	Hepatotoxic	Vasconcelos, 1994
Scandinavia	81	60	Hepatotoxic	Berg *et al.*, 1986
Sweden	331	47	Hepatotoxic	Willén and Mattsson, 1997
			Neurotoxic	
UK	50	48	Hepatotoxic	Codd and Bell, 1996
		28[1]		
USA, Minnesota	92	53	Unspecified	Olson, 1960
			Neurotoxic	
USA, Wisconsin	102	25	Hepatotoxic	Repavich *et al.,* 1990
			Neurotoxic	
Mean		59		

[1] HPLC was used to detect the toxin content of the samples

Some of the studies shown in the table have been conducted over several years while others lasted only one season. The relative share of cyanobacteria in the samples varied; low frequency of cyanobacteria led to low percentages of toxic samples in some studies. In most of the studies the method used to detect toxicity is mouse bioassay, normally with a 4-hour time limit (or longer when slow death factors (SDF) have been included). SDF may indicate low hepatotoxicity of samples or other unknown toxicity.

Toxic and non-toxic strains from the same cyanobacterial species cannot be separated by microscopic identification. The use of molecular genetic methods, in particular the use of molecular probes and primers that target specific toxin production genes, will lead to the development of more precise identification methods for toxic cyanobacteria in the future. To confirm that a particular cyanobacterial strain is a toxin-producer, it is important to isolate a pure culture of that strain, preferably free of other bacteria; then to detect and quantify toxin concentrations in the pure culture (either by bioassay or chemical analysis); and, where possible, to purify and characterise fully the toxins (for such examples see Tables 3.2 and 3.3). It is likely that the list of confirmed toxic species will increase in the future due to the isolation of new species and strains, and because of the use of improved isolation, culturing and analytical methods.

Microcystis sp., commonly *Microcystis aeruginosa,* are linked most frequently to hepatotoxic blooms world-wide (see Tables 3.2 and 3.5 for details and references for all toxic species). *Microcystis viridis* and *Microcystis botrys* strains also have been shown to produce microcystins. As noted in section 2.2, *Microcystis* is a non-nitrogen-fixing genus which is often dominant under nutrient-rich conditions (especially where there is a significant supply of ammonia), although it also forms blooms in less polluted waters. Microcystin-producing *Anabaena* sp. have been reported from Canada, Denmark, Finland, France and Norway. A recent study from Egypt revealed that 25 per cent of 75 *Anabaena* and *Nostoc* strains isolated from soil, rice fields and water bodies contained microcystins. *Planktothrix agardhii* and *Planktothrix rubescens* (previously called *Oscillatoria agardhii* and *O. rubescens)* are common microcystin producers in the Northern Hemisphere; toxic strains of these have been isolated from blooms in Denmark, Finland and Norway. In addition, these species were frequently shown to be dominant in microcystin containing blooms in China, in Germany and in Sweden. In Swiss alpine lakes, *Oscillatoria limosa,* which is benthic (i.e. it grows attached to sediments and rocks), is a microcystin producer. In spite of the widespread occurrence of

cyanobacterial blooms in Australia, *Planktothrix* blooms are rare there. This may be due to the higher temperature and tendency for elevated clay-derived turbidity in Australian water bodies.

Table 3.5 Toxic cyanobacteria species and their geographical distribution

Toxic species	Cyanotoxin	Location	Reference(s)
Anabaena flos-aquae	Microcystins	Canada	Krishnamurthy *et al.,* 1989; Harada *et al*., 1991
Anabaena ?	Microcystins	Denmark	Henriksen *et al.*, 1996b
Anabaena spp.	Microcystins	Egypt	Yanni and Carmichael, 1997
Anabaena spp. *(flos-aquae, lemmermannii, circinalis)*	Microcystins	Finland	Sivonen *et al.,* 1990b; 1992a
Anabaena circinalis	Microcystins	France	Vezie *et al*., 1998
Anabaena flos-aquae	Microcystins	Norway	Sivonen *et al*., 1992a
Microcystis aeruginosa	Microcystins	Worldwide	Several; see Rinehart *et al*., 1994 for a summary
M. viridis	Microcystins	Japan	Kusumi *et al.,* 1987; Watanabe *et al*., 1986
M. botrys	Microcystins	Denmark	Henriksen *et al.,* 1996b
Planktothrix agardhii	Microcystins	China	Ueno *et al*., 1996a
P. agardhii	Microcystins	Denmark	Henriksen *et al*., 1996b
P. mougeotii	Microcystins	Denmark	Henriksen *et al*., 1996b
P. agardhii	Microcystins	Finland	Sivonen, 1990b; Luukkainen *et al.,* 1993
P. agardhii	Microcystins	Norway	Krishnamurthy *et al.*, 1989; Meriluoto *et al*., 1989
Oscillatoria limosa	Microcystins	Switzerland	Mez *et al*., 1996
Nostoc sp.	Microcystins	Finland	Sivonen *et al*., 1990a, 1992b
Nostoc sp.	Microcystins	England	Beattie *et al*., 1998
Anabaenopsis millerii	Microcystins	Greece	Lanaras and Cook, 1994
Haphalosiphon hibernicus (soil isolate)	Microcystins	USA	Prinsep *et al*., 1992
Nodularia spumigena	Nodularins	Australia	Baker and Humpage, 1994; Jones *et al.,* 1994
N. spumigena	Nodularins	Baltic Sea	Sivonen *et al*., 1989b
N. spumigena	Nodularins	New Zealand	Carmichael *et al*., 1988a; Rinehart *et al*., 1988
Aphanizomenon ovalisporum	Cylindrospermopsin	Israel	Banker *et al*., 1997
Cylindrospermopsis raciborskii	Cylindrospermopsin	Australia	Hawkins *et al*., 1985; 1997
C. raciborskii	Cylindrospermopsin	Hungary	Törökné, 1997
Umezakia natans	Cylindrospermopsin	Japan	Harada *et al*., 1994

Anabaena flos-aquae	Anatoxin-a	Canada	Carmichael *et al*., 1975; Devlin *et al*., 1977
Anabaena spp.	Anatoxin-a	Finland	Sivonen *et al*., 1989a
Anabaena blooms	Anatoxin-a	Germany	Bumke-Vogt, 1998
Anabaena sp.	Anatoxin-a	Ireland	James *et al*., 1997
Anabaena sp.	Anatoxin-a (minor amounts)	Japan	Park *et al.,* 1993a
Anabaena planctonica bloom	Anatoxin-a	Italy	Bruno *et al.,* 1994
Aphanizomenon sp.	Anatoxin-a	Finland	Sivonen *et al.,* 1989a
Aphanizomenon blooms	Anatoxin-a	Germany	Bumke-Vogt, 1998
Cylindrospermum sp.	Anatoxin-a	Finland	Sivonen *et al*., 1989a
Microcystis sp.	Anatoxin-a (minor amounts)	Japan	Park *et al*., 1993a
Oscillatoria sp. benthic	Anatoxin-a	Scotland	Edwards *et al*., 1992
Oscillatoria sp. ?	Anatoxin-a	Ireland	James *et al.,* 1997
Planktothrix sp.	Anatoxin-a	Finland	Sivonen *et al.,* 1989a
Planktothrix formosa	Homoanatoxin-a	Norway	Skulberg *et al*., 1992
Anabaena flos-aquae	Anatoxin-a(S)	Canada	Matsunaga *et al.*, 1989; Mahmood and Carmichael, 1987
A. lemmermannii	Anatoxin-a(S)	Denmark	Henriksen *et al.,* 1997; Onodera *et al.,* 1997a
Anabaena circinalis	Saxitoxins	Australia	Humpage *et al*., 1994; Negri *et al*., 1995; 1997
Aphanizomenon flos-aquae	Saxitoxins	USA	Jackim and Gentile, 1968; Ikawa *et al*., 1982; Mahmood and Carmichael, 1986
Cylindrospermopsis raciborskii	Saxitoxins	Brazil	Lagos *et al.,* 1997
Lyngbya wollei	Saxitoxins	USA	Carmichael *et al*., 1997; Onodera *et al*., 1997b

The toxicity of the species listed in the table is in most cases verified by laboratory studies with isolated strains. A few bloom samples are also included from the new areas of occurrence where toxicity of the species is not verified by strain isolation but the toxins are determined in the bloom samples. The authors have suggested the listed species as the probable toxin producer (based on their dominance) but these reports should be treated as tentative until pure strains are studied.
Nostoc rivulare blooms in Texas, USA have caused poisoning of domestic and wild animals (Davidson, 1959) and, more recently, two unidentified *Nostoc* strains were shown to produce microcystins (Table 3.5).

The hepatotoxin, cylindrospermopsin, has been found in *Cylindro-spermopsis raciborskii* in Australia and Hungary, in *Umezakia natans* in Japan, and in *Aphanizomenon ovalisporum* in Israel (Table 3.5). In spite of their occurrence in Europe, it appears that cylindrospermopsin-producing genera most commonly form toxic blooms in subtropical,

tropical or arid zone water bodies. However, there have been reports of increasing occurrences of *Cylindrospermopsis raciborskii in* Europe and the USA (Padisák, 1997).

The neurotoxin, anatoxin-a, was first shown to be produced by *Anabaena flos-aquae* strains originating from Canada, and later by Finnish strains of unidentified *Anabaena* species, and in individual species of *Oscillatoria, Aphanizomenon,* and *Cylindrospermum,* by benthic *Oscillatoria* from Scotland, and by *Anabaena* and *Oscillatoria* in Ireland. It also was present in *Anabaena planctonica* blooms in Sardinia, Italy, in *Anabaena* and *Aphanizomenon* blooms in Germany, and in minor amounts in some Japanese bloom samples, as well as in *Anabaena* strains. Homoanatoxin-a has been characterised from an *Oscillatoria formosum (Phormidium formosum)* strain from Norway (see Table 3.5). To date, anatoxin-a(S) has been found only from *Anabaena* species: *A. flos-aquae* in the USA and Scotland, and *A. lemmermannii* in Denmark.

Aphanizomenon flos-aquae blooms and strains were found to contain saxitoxins in the USA and this species was for a long time the only known saxitoxin producer amongst the cyanobacteria. More recently, saxitoxins have been shown to be common in Australian rivers and reservoirs and to be produced by *Anabaena circinalis.* In North America, a benthic freshwater *Lyngbya wollei* was found to produce saxitoxins, as was a strain of *Cylindrospermopsis raciborskii* in Brazil (Table 3.5).

3.2.3 Cyanotoxin patterns in strains and species of cyanobacteria

Cyanobacteria may produce several toxins simultaneously. In general, more than one microcystin has been characterised from the strains listed in Table 3.2. Among neurotoxic strains, several PSPs are found in the same strain, although there are considerable variations between species (Table 3.3). Furthermore, simultaneous neurotoxin and hepatotoxin production has been noted; the best example studied being the *Anabaena flos-aquae* strain NRC 525-17 which produces anatoxin-a(S) (Matsunaga *et al.,* 1989) and several microcystins (Harada *et al.,* 1991a).

Microcystin

Although many strains produce several microcystins simultaneously, usually only one or two of them are dominant in any single strain. Qualitative variation in the microcystins present is most frequently found among strains of *Anabaena* but also in *Microcystis* (Sivonen *et al.,* 1995). Some taxa have a number of microcystins in common, such as planktonic *Anabaena, Microcystis* and *Planktothrix (Oscillatoria).* However, there is evidence of microcystin variants that are typical for certain cyanobacterial taxa.

Planktothrix and some strains of *Anabaena* produce only demethylmicrocystins (Table 3.2). *Planktothrix (Oscillatoria)* isolates from Finland (13 strains studied and toxins fully characterised) seem to produce one of two types of microcystin (D-Asp^3-RR or Dha^7-RR) (Sivonen *et al.,* 1995).

German field samples dominated by *Planktothrix* have also shown these microcystins, with dominance of one major demethylated microcystin in populations of *P. rubescens,* and two or three of these variants in populations of *P. agardhii* (Fastner *et al.,* 1998).

Microcystis strains from Japan appear to contain chiefly microcystin-LR, -RR and -YR, with some cultures showing all three variants, and some strains being dominated by one of them. These three microcystins are the only variants reported in several studies on *M. aeruginosa* and *M. viridis* (e.g. Watanabe, 1996). Many of the microcystins listed in Table 3.2 have been found only in minor amounts or, to date, have been found to be produced only by individual isolates.

In natural samples which usually contain many strains, or more than one toxin-producing species, different combinations of microcystins can be found. For example, in a *Microcystis* bloom from Homer Lake, USA, 19 different microcystins were characterised (Namikoshi *et al.,* 1992a, 1995) and in one Australian bloom of *Microcystis aeruginosa,* 23 microcystins were detected by high pressure liquid chromatography (HPLC), none of which were microcystin-LR (Jones *et al.,* 1995).

Microcystin-LR is often mentioned as the most frequently occurring microcystin, although such observations may be biased by the fact that a chemical standard for the analysis of microcystin-LR was the earliest to be commercially available (see Chapter 13). It has been reported to be the major toxin in bloom and strain samples from Portugal (Vasconcelos *et al.,* 1995. 1996), France (Vezie *et al.,* 1997), Canada (Kotak *et al.,* 1993) and frequently co-occurring with microcystin-RR and -YR in Japan (Watanabe *et al.,* 1988, 1989).

Geographical patterns are indicated by some survey results. Wide variation among the L-amino acids of microcystins has been reported for South Africa (Scott, 1991), frequent presence of demethylmicrocystins (RR and LR) has been seen in Finnish strains (Sivonen *et al.,* 1995) and microcystins in Danish samples show wide variation (Henriksen, 1996a). In part, these patterns probably reflect regional differences in dominance of cyanobacterial species or strains. Water bodies with regular dominance of specific taxa are likely to exhibit characteristic patterns of microcystin variants.

Nodularin

The cyanotoxin, nodularin, is found in waters where *Nodularia spumigena* is present; the most prominent areas being the Baltic Sea and brackish water estuaries and coastal lakes of Australia and New Zealand. However, the best known *Nodularia spumigena* bloom location, Lake Alexandrina, Australia, has salinities which are only slightly elevated above normal river water and at levels still suitable for drinking water. The presence of variants of nodularin in environmental samples is usually rather insignificant. In the Baltic Sea, the collection of samples for several years has shown nodularin to be the major compound present. The same is true for the almost 90 hepatotoxic *Nodularia* strains isolated from the same source (Sivonen *et al.,* 1989b; Lehtimäki *et al.,* 1997). Analyses of several strains isolated from blooms across Australia have revealed similar results, with nodularin variants being found rarely, and then only at low relative abundance (Jones *et al.,* 1994; Blackburn *et al.,* 1997).

Saxitoxins

There is much diversity of saxitoxin distribution in cyanobacteria from around the world (Table 3.3). In addition, the lack of analytical standards for many saxitoxins has probably restricted findings in some countries. Saxitoxin-producing *Anabaena circinalis* blooms

are widespread in Australian rivers and reservoirs, and the relative abundance of individual toxins is remarkably constant in toxin-producing strains, which is quite opposite to the microcystin-producing cyanobacteria. In all healthy *Anabaena circinalis* cultures examined in Australia, the relative composition of individual saxitoxins is very similar and dominated by C-toxins (Blackburn *et al.,* 1997). As blooms and cultures age, the proportion of decarbamoyl-gonyautoxins breakdown products increases at the expense of the C-toxins (see section 3.4). In North American *Aphanizomenon flos-aquae* and in Brazilian *Cylindrospermopsis raciborskii* samples, mostly neosaxitoxin and smaller amounts of saxitoxin have been detected. In the case of the *Aphanizomenon flos-aquae* from North America, only bloom samples and strains from New Hampshire, USA have been found to contain saxitoxins. Mat-forming *Lyngbya wollei* from Alabama, USA was found to produce decarbamoyl saxitoxin (dcSTX), decarbamoylgonyautoxin-2 and-3 (dcGTX2 and dcGTX3) and six new saxitoxins (see section 3.1).

3.2.4 Concentrations of cyanotoxins in surface waters

Information on the concentrations of cyanotoxins in surface waters has been appearing only recently in the international literature. In early studies (pre-1990s), the toxicity of bloom samples was determined by mouse bioassays, but this method is unsuitable for measuring the low concentrations of cyanotoxins that usually prevail in cyanobacterial populations when they do not accumulate in scums. The development of better analytical methods, in the first instance HPLC and more recently enzyme linked immunosorbent assay (ELISA), (and for microcystins and nodularins also the protein phosphatase assay) has made the quantification of total and individual toxins possible (see Box 3.1 and Chapter 13).

The quantitative determination of toxin concentrations is mostly performed from lyophilised (freeze-dried) cultures, bloom samples or seston (particulate material suspended in water, which contains not only cyanobacterial cells but usually other algae, some zooplankton, and possibly inorganic material such as soil and sediment particles) (see Chapter 13). Results are usually expressed as milligrams or micrograms of toxin per gram dry weight (dw). Whereas in cultures and bloom samples, the dry weight originates from cyanobacteria, it will encompass further particles (seston) in plankton samples taken outside of scum areas. The highest published concentrations of cyanotoxins from cyanobacterial bloom samples, measured by HPLC, are (see Table 3.6):

- microcystin - 7,300 µg g^{-1} dw from China and Portugal,
- nodularin - 18,000 µg g^{-1} dw from the Baltic Sea,
- cylindrospermopsin - 5,500 µg g^{-1} dw from Australia,
- anatoxin-a - 4,400 µg g^{-1} dw from Finland,
- saxitoxins - 3,400 µg g^{-1} dw from Australia,
- anatoxin-a(S) - 3,300 µg g^{-1} dw from the USA.

Box 3.1 Toxins and toxicity: what's in a name?

There is often a misunderstanding of the terms "toxicity" or "toxin content" when applied to cyanobacteria or a water sample that contains cyanotoxins. In particular, the terms are often taken to be synonymous, which they are not. Furthermore, the measurement units in which data are reported are not always carefully considered.

To some extent, the problem lies in the different ways in which toxin data can be expressed, and the way in which people think about the cyanobacterial or water sample, particularly in the context of their own professional background. For example, a water treatment plant operator may assume or expect a "toxicity" value to refer to the toxin concentration per litre of drinking water, a biologist may think of the same term to mean the amount of toxin per mass of cyanobacteria, whereas a toxicologist will normally take the term to reflect the amount of cyanobacteria needed to kill an animal.

Toxicity. In the strict sense, toxicity refers only to animal testing data, and is expressed as the amount of cyanobacteria lethal to an animal (usually normalised per kilogram of body weight). The commonly reported LD_{50} value, a measure of toxin potency, is the amount of cyanobacteria or pure toxin needed to kill 50 per cent of animals in an experimental trial, again normalised per kilogram of body weight. Thus, the lower the LD_{50} the more potent the cyanobacterial sample or pure toxin (see Table 3.2 for examples). Note, however, that cell growth assays may express results as EC_{50} (concentration reducing growth rate by 50 per cent).

Toxin concentration and toxin content. Toxin concentration can refer either to the amount of toxin per litre of water or the amount of toxin per mass of cyanobacterial bloom material. Therefore, particular attention must be paid to the units in which the data are reported. In the published literature on toxic cyanobacteria, the amount of toxin per mass of bloom material is often referred to as the toxin content. Strictly speaking, this is incorrect; the correct term should be the gravimetric, or per cyanobacterial mass, toxin concentration.

Toxin quota. This refers to the amount (mass or moles) of toxin per cyanobacteria cell.

Cell bound versus free toxin. In most circumstances almost all toxins are cell-bound (within the cells) and little or no toxin is found in solution except where a bloom is senescent or where an algicide has been applied.

Higher concentrations have been reported in unpublished studies and may be expected under some circumstances.

For the purposes of water treatment and public health management, toxin concentration per litre of water is often a more relevant unit, for example for relating ambient concentrations to guideline levels. Concentration per litre usually refers to toxins contained within the cells as well as dissolved in water, and taken from a defined volume of water. Very high concentrations of microcystins per litre of water (rather than per mass of cyanobacteria), have been reported up to 25,000 µg l^{-1} microcystin and up to 3,300 µg l^{-1} anatoxin-a(S) (Table 3.6). It should be noted, however, that these very high concentrations of toxins would be from scums or from very dense accumulations of cyanobacteria.

In one or two studies where many toxin concentration measurements have been made from more or less randomly collected plankton samples, the distribution of data revealed

a small number of samples with high toxin concentrations, some samples with moderate concentrations, and many samples with low or zero concentrations (Figure 3.4). However, such surveys tend to include a large proportion of samples where the particle content is not dominated by cyanobacteria. If cyanobacteria constitute only a small fraction of the total dry weight, toxin concentration per gram dry weight will necessarily be low. In contrast, if mass developments of toxic cyanobacterial species are investigated, cyanobacteria will constitute a large fraction of the total dry weight. In these cases, toxin content is often high. Recent German data show that the relative frequency of high, moderate and low toxin concentrations per gram dry weight is dependent on the particular species that is dominant (Figure 3.5).

For management, the option of estimating toxin content from the biomass of a dominant cyanobacterial species (i.e. the "toxin quota" per cell or biovolume) can be helpful. Survey data from Germany show that microcystin toxin quotas differ between taxa, but variation within most of the samples dominated by the same taxon is only moderate (two- to fivefold) (Figure 3.5). In contrast, an Australian investigation with other species of cyanobacteria showed substantial variation of toxin concentrations per dry weight, even in cases with dominance of the same cyanobacterial species (Negri *et al.,* 1997).

Table 3.6 Toxin concentrations reported in cyanobacterial bloom or water samples

Location	Period of study	No. of toxic samples (total no. of samples)	Toxins identified	Range of total concentrations (µg g^{-1} dw, unless otherwise indicated)	Analysis method	Reference
Microcystins						
Australia	1991	4	Microcystins, 24 unidentified	2,100-4,100[2]	HPLC	Jones *et al*., 1995
Canada, Alberta	1990	37(50)	Microcystin-LR	4-610	HPLC	Kotak *et al*., 1993
Canada, Alberta (3 lakes)	1990-93	168(226)	Microcystin-LR	1-1,550	HPLC	Kotak *et al*., 1995
China	1988	5(10)	Microcystin-RR, -LR	200-7,300	HPLC	Zhang *et al*., 1991
Czech and Slovak Rep.	1995-96	(63)	Microcystin-LR	4-6,835	HPLC	Maršálek *et al*., 1996
Denmark	1992-94		Microcystin-RR, -LR	3-2,800	HPLC	Christoffersen, 1996
Denmark	1993-95	198(296)	Microcystins	5-1,900	HPLC	Henriksen *et al*., 1996b
Finland	1994-95	17(20)	Microcystin-LR	> 10-800	HPLC	Lahti *et al*., 1997
France	1994	16(22)	Microcystins	70-3,970	HPLC	Vezie *et al*., 1997
France, L.	1994	19(30)	Microcystins	30-230	HPLC	Vezie *et al*.,

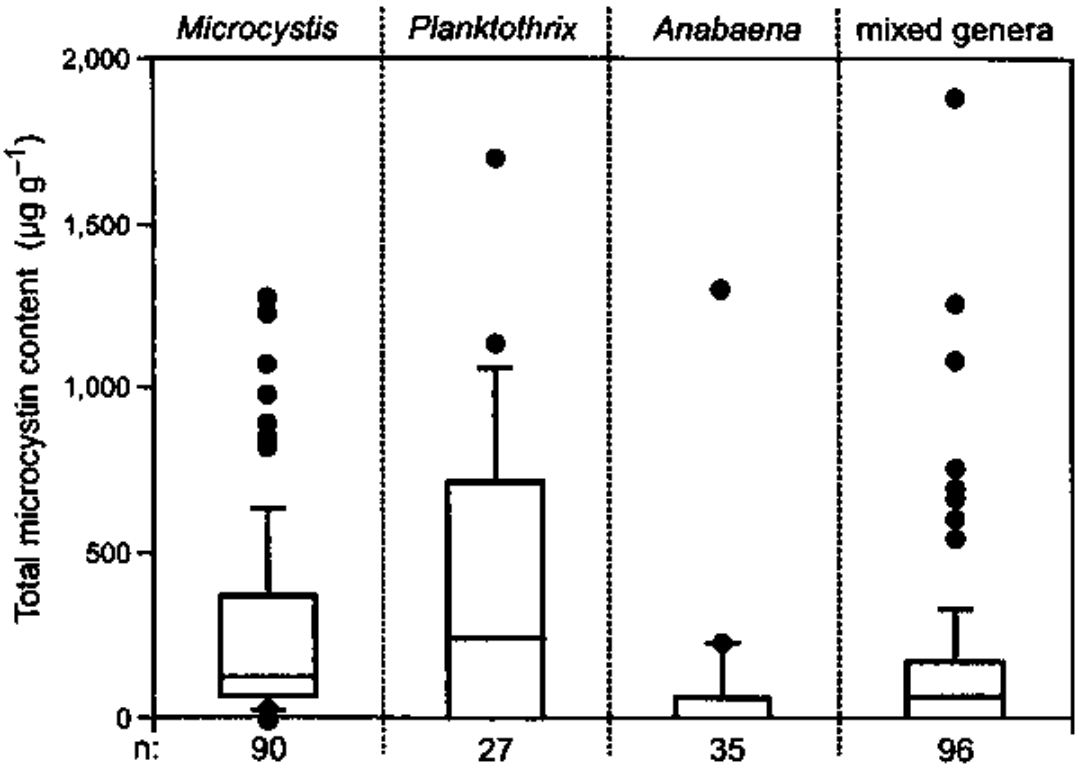
Grand-Lieu						1998
Germany	1992	8(15)	Microcystin-LR	36-360	HPLC	Fastner, 1994
Germany	1993	17(18)	Microcystins	0.15-36[1,2]	ELISA	Ueno *et al*., 1996b
Germany	1995-96	385(533)	Microcystins	1-5,000	HPLC	Fastner, 1998
Germany	1997	34	Microcystins, several	1-25,000 [1]	HPLC	Chorus *et al*., 1998
Japan	1990	12(14)	Microcystin-RR, -YR, -LR	160-950	HPLC	Watanabe *et al*., 1992
Japan	1988-92	11(19)	Microcystin-RR, -YR, -LR, [Dha[7]] -LR	70-1,610	HPLC	Park *et al*., 1993a
Japan, Lake Suwa	1980-91	13	Microcystin-RR, -YR, -LR	30-2,100	HPLC	Park *et al*., 1993b
Japan	1986-88	4(4)	Microcystin-RR, -YR, -LR	100-860	HPLC	Shirai *et al*., 1991
Japan	1992-95	18(22)	Microcystin-RR, -YR, -LR	0.04-480 [1]	HPLC	Tsuji *et al*., 1996
Japan	1993-95	46(57)	Microcystins	0.05-1,300 [1,2]	ELISA	Ueno *et al*., 1996b
Japan	1993-94	12(17)	Microcystins	0.06-94 [1,2]	ELISA	Nagata *et al*., 1997
Japan	1989-94	10(10)	Microcystins	300-15,600 [1,2]	ELISA	Nagata *et al*., 1997
				330-19,500 [1,2]	HPLC	
Portugal	1989-92	12(12)	Microcystin-LR plus six known and three unidentified microcystins	1,000-7,100	HPLC	Vasconcelos *et al*., 1996
Portugal	1994-95	28(29)	Microcystins	0.1-37 [1,2]	ELISA	Ueno *et al*., 1996b
South Africa	1985-86		Microcystin-FR, -LR, -YR, -LA, -YA, -LAba	5-420	HPLC	Wicks and Thiel, 1990
South Africa	1988-89	9(9)	Microcystin-YR, -LR, -FR, -YA, -LA, -LAba	40-630	HPLC	Scott, 1991
UK	1992	3(3)	Microcystins, 3	17-131 [1,2]	HPLC	Codd *et al*., 1995
USA, Wisconsin	1993	9	Microcystins	1,900-12,800 [2]	ELISA	McDermott *et al*., 1995
Nodularins						

Baltic Sea	1985-87	17(23)	Nodularin	< 100-2,400	HPLC	Sivonen *et al*., 1989b
Baltic Sea	1990-91	6(16)	Nodularin	300-18,000	HPLC	Kononen *et al*., 1993
Tasmania, Orielton Lagoon	1992-93	7(9)	Nodularin	2,000-3,500	HPLC	Jones *et al*., 1994
Anatoxins						
Denmark	1993-95	9(10)	Anatoxin-a(S)	4-3,300 [3]	ChE inhibition assay	Henriksen *et al*., 1997
Finland	1985-87	13(30)	Anatoxin-a	10-4,400	GC/MS	Sivonen *et al*., 1989a
Finland and Japan		3(3)	Anatoxin-a	0.4-2,600	LC/MS	Harada *et al*., 1993
Germany	1995-96	10(45)	Anatoxin-a	0.02-0.36 [1]	GC-ECD	Bumke-Vogt, 1998
Ireland	1995	2(2)	Anatoxin-a	10-100	HPLC (fluorimetric)	James *et al*., 1997
Japan	1988-92	9(14)	Anatoxin-a	0.3-16	TSP-LC/MS	Park *et al*., 1993a
Saxitoxins						
Australia	1990-92	11(11)	Saxitoxins	85-2,040	HPLC and FAB-MS	Humpage *et al*., 1994
Australia	1992-94	24(31)	Saxitoxins	50-3,400	HPLC	Negri *et al*., 1997
USA	1994	7(8)	Saxitoxins	5-60 [4]	HPLC/AOAC	Carmichael *et al*., 1997

dw	Dry weight
HPLC	High pressure liquid chromatography
ELISA	Enzyme linked immunosorbent assay
GC/MS	Gas chromatography/mass spectrometry
LC/MS	Liquid chromatography/mass spectrometry
GC-ECD	Gas chromatography-electron capture detection
TSP-LC/MS	Thermospray-liquid chromatography/mass spectrometry
FAB-MS	Fast atom bombardment-mass spectrometry
AOAC	Mouse bioassay done according to the Association of Official Analytical Chemists

[1] Given as µg l^{-1}
[2] Microcystin-LR used as standard
[3] Measured by enzyme inhibition
[4] Micrograms of STX equivalents

Figure 3.4 Cell-bound total microcystin content (measured by HPLC) of samples taken in Denmark between 1993 and 1995 and dominated by different cyanobacteria. Boxes show median values and the values within the 50 percentile range; bars indicate the 10th and 90th percentile; n = number of samples (Modified from Henriksen, 1996)



Further regional investigations are needed to clarify the toxins and species for which toxin quotas may be reasonably stable and, thus, predictable.

The further development of quantitative, pre-concentration methods coupled with the highly sensitive analytical methods noted above (see also Chapter 13) has also enabled the measurement of very low concentrations of extracellular, dissolved toxins in water. Data for dissolved toxin concentration, as reported in the scientific literature, are given in Table 3.7. Prevalence and degradation of dissolved toxins in water are discussed in section 3.4. Concentrations of microcystins dissolved in water vary from trace concentrations up to 1,800 µg l^{-1} or higher, following the collapse of a large, highly toxic bloom.

3.2.5 Seasonal variations in bloom toxin concentration

The timing and duration of the bloom season of cyanobacteria depends largely on the climatic conditions of the region. In temperate zones, mass occurrences of cyanobacteria are most prominent during the late summer and early autumn and may last 2-4 months. In regions with more Mediterranean or subtropical climates, the bloom season may start earlier and persist longer.

Figure 3.5 Cell-bound total microcystin content (measured by HPLC) of samples taken in Germany between 1995 and 1996 dominated by different cyanobacteria. A. On a dry weight basis; B. On a chlorophyll a basis; C. On a volume basis. *(Micro.* spp = *Microcystis* spp.; *Plankto. agardhii = Planktothrix agardhii; Plankto. rubescens = Planktothrix rubescens)* Boxes show median values and the values within the 50 percentile range; bars indicate the 10th and 90th percentile; n = number of samples (Modified from Fastner *et at.,* 1998)

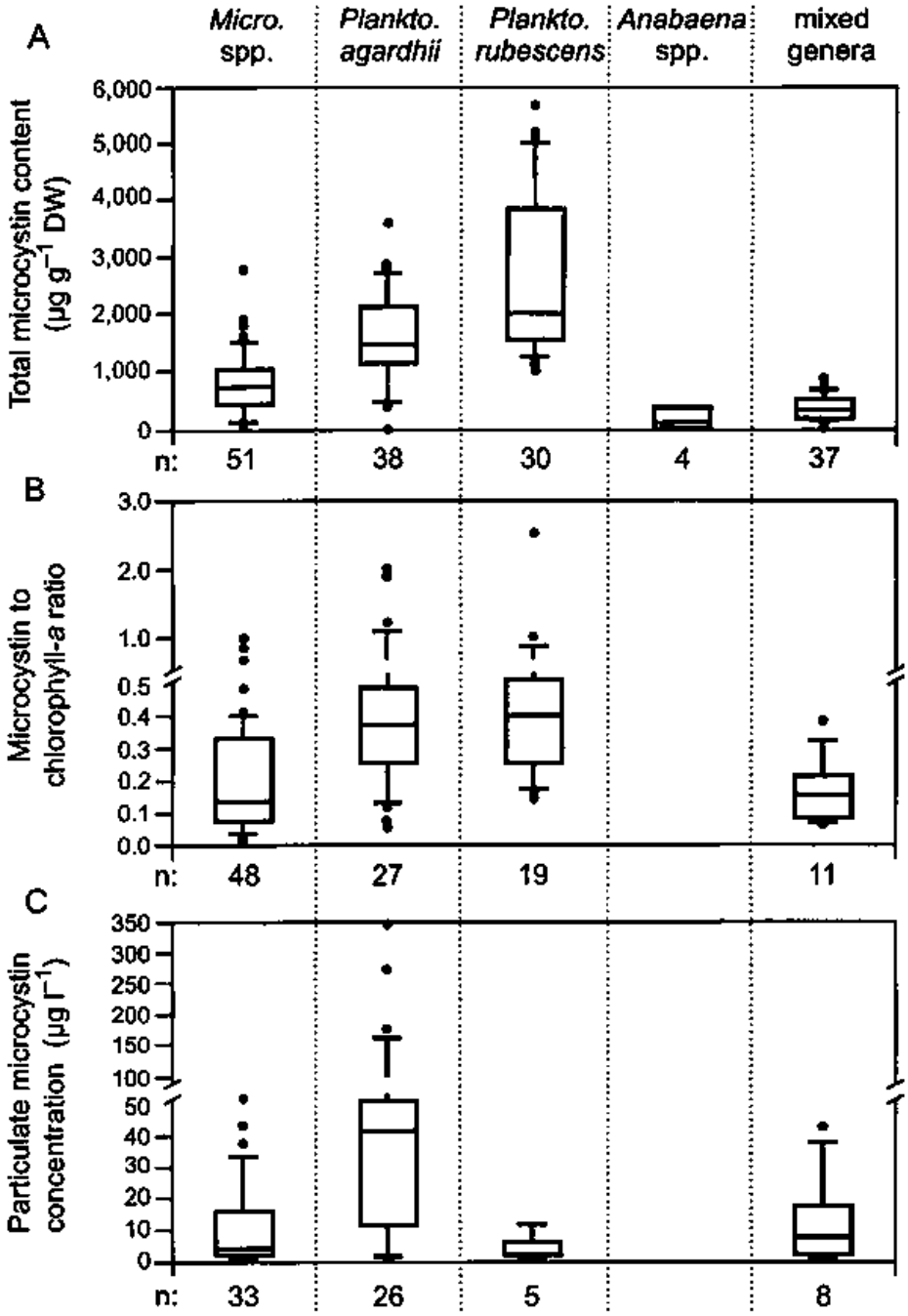


Table 3.7 Dissolved (extracellular) toxin concentrations measured in water samples

Location	Period of study	No. of toxic samples (total no. of samples)	Toxin identified	Concentration (µg l^{-1})	Analysis method	Reference
Australia		24	Microcystins	3-1.800[1]	HPLC	Jones and Orr, 1994
China	1993-94	130(835)	Microcystins	0.05-1.6	ELISA	Ueno *et al*., 1996a
Finland	1993-94	38(38)	Microcystin-LR	0.06-0,21	HPLC and ELISA	Lahti *et al*., 1997
Germany	1993-94	11(19)	Microcystins	0.07-0.76	ELISA	Ueno *et al*., 1996b
Japan	1992-95	9(22)	Microcystin-RR, -YR, -LR	0.02-3.8	HPLC	Tsuji *et al*., 1996
Japan	1993-95	26(38)	Microcystins	trace-5.6	ELISA	Ueno *et al*., 1996b
Japan	1993-94	4(13)	Microcystins	0.08-0.8	ELISA	Nagata *et al*., 1997
Thailand	1994	7(10)	Microcystins	0.08-0.35	ELISA	Ueno *et al*., 1996b
USA, Wisconsin	1993	27(27)	Microcystins	0.07-200	ELISA	McDermott *et al.,* 1995

HPLC High pressure liquid chromatography

ELISA Enzyme linked immunosorbent assay

[1] High range concentrations following treatment of a large bloom with algicide, which released intracellular microcystins (see section 3.4)

In France, four months is not uncommon, and in Japan, Portugal, Spain, South Africa and southern Australia blooms may occur for up to six months or longer. By contrast, in dry years, in tropical or subtropical areas of China, Brazil and Australia, cyanobacterial blooms may occur almost all year round, perhaps waning only briefly during reservoir overturn.

In shallow lakes, particularly in north-western Europe, populations of *Planktothrix agardhii (Oscillatoria agardhii)* may prevail perennially for many years. In deeper, thermally stratified lakes and reservoirs with moderate nutrient pollution, *Planktothrix rubescens (Oscillatoria rubescens)* may form blooms at the interface between the warmer upper and colder deeper layers of water during summer, but maintain high, evenly distributed density throughout the entire water body during winter. Both *Planktothrix* species may contain high amounts of microcystins (see Figure 3.5). Blooms of cyanobacteria, especially *Planktothrix agardhii,* have been found in winter under ice in Scandinavian and German lakes and can thus be an all year round problem.

Although toxic cyanobacteria occur in a large number of lakes, reservoirs and rivers in the world, quantitative reports on seasonal variation of cyanobacterial species composition and toxin concentration are rare. Only a few studies on seasonal, spatial and diel (day to night) variations in lakes have been published. Carmichael and Gorham (1981) showed a high degree of spatial variation of bloom toxicity that was due mostly to variations in the relative amounts of toxic *Microcystis aeruginosa* throughout the lake, rather than to substantial variations in cell toxin content. Other measurements of toxin concentrations in lakes have revealed similar trends; samples taken at the same time from different parts of the lake may show wide divergence in cyanotoxin content (Ekman-Ekebom *et al.,* 1992; Kotak *et al.,* 1995; Vezie *et al.,* 1998). A study in Alberta, Canada, showed considerable variation in toxin concentrations among the three lakes studied, both within and between years, even though the lakes were located within the same climatic region (Kotak *et al.,* 1995).

In any year or season, individual water bodies have their own populations of cyanobacteria and algae, the dominance of which is dependent not only on the weather, but on the specific geochemical conditions of the lake. If there are no major changes in these conditions, toxic blooms are likely to recur annually in those lakes that have a history of toxic blooms (Wicks and Thiel, 1990; Ekman-Ekebom *et al.,* 1992). Certain species, including the highly toxic *Planktothrix agardhii* and *P. Rubescens,* are known to produce maximum mass occurrences deep in the water column and which may be overlooked by surface monitoring of waters. Such situations may also cause problems for water treatment (see Chapters 2 and 9) (Lindholm and Meriluoto, 1991).

Studies over prolonged periods usually show that toxin concentration per gram dry weight may vary substantially over a time scale of weeks to months, but rarely from day to day as is sometimes reported. The maximum toxin concentration per gram dry weight is usually reported in summer or autumn, when cyanobacterial biomass dominates dry matter (Wicks and Thiel, 1990; Watanabe *et al.,* 1992; Park *et al.,* 1993b; Kotak *et al.,* 1995; Maršálek *et al.,* 1995; Vezie *et al.,* 1998). However, the time of toxin concentration maximum and biomass maximum are not necessarily coincident. Thus, there can be significant variation in the amount of toxin per mass of cyanobacteria over time, independently of changes in the size of the cyanobacterial population. The explanations for this are twofold. Firstly, there may be a waxing and waning of species or strains of quite different toxin quotas (i.e. toxin content per cell). Secondly, the toxin quotas may change up to five-fold in response to changes in environmental conditions (see section 3.3). A study by Kotak *et al.* (1995) found substantially higher concentrations of microcystin in blooms during the day than at night, whereas a study from Australia found no variation in microcystin content when samples were incubated during 24 hours at different depths in a reservoir (Jones and Falconer, 1994). Both findings need to be explored further.

High regional, seasonal, spatial, temporal and diel variations of toxin concentrations indicate that predicting or modelling the occurrence of toxin concentrations requires a comprehensive understanding of population (strain) development in different types of aquatic ecosystems, as well as of the variability of their toxin quotas. Data bases for such predictive models have yet to be compiled.

3.3 Production and regulation

Laboratory studies with pure strains of cyanobacteria have found that environmental factors can induce changes in toxicity or toxin concentration (on a per unit biomass basis), but usually by a factor of no more than three or four. On a per cell basis, the changes in toxin content are probably even smaller. These environmentally-induced changes are far less than the range of more than three orders of magnitude in toxin content measured between individual strains grown in culture under identical conditions. This lends support to the assumption that much, if not most, of the variation in toxicity of "monospecific" natural blooms is the waxing and waning of strains of the same species, but with varying toxin quotas. The factors that control the growth and toxin content of individual strains are as yet unknown, but clearly the genetic regulation of cyanotoxin production is an important area for further study and understanding (see section 3.3.3).

Both toxigenic (toxin producing) and non-toxigenic strains exist within many species of cyanobacteria. When grown in the laboratory, particular strains always produce much greater amounts of toxins than others. Indeed, the difference may be as much as three orders of magnitude or more (Bolch *et al.,* 1997). Several attempts to differentiate toxin producing (toxigenic) from non-toxic strains of the same species using microscopic methods have failed. The use of molecular biological methods to characterise toxic and non-toxic planktonic isolates has been initiated recently and will, in future, help to clarify the taxonomic status of these organisms, as well as the ecology of individual strain types (see section 3.3.3).

3.3.1 Regulation by chemical and physical factors

The production of toxin by a single cyanobacterial strain seems to be consistent and the spontaneous and permanent loss of toxin production has been seldom reported. The effects of several environmental factors on growth and toxin production by cyanobacteria have been studied in batch and continuous culture experiments. Culture age in batch cultures, and temperature, are the parameters most frequently examined, followed by light, nutrients, salinity, pH and micronutrient concentrations. Studies have been done with hepatotoxic *Microcystis, Oscillatoria (Planktothrix), Anabaena* and *Nodularia;* anatoxin-a producing *Anabaena, Aphanizomenon* and *Planktothrix;* and saxitoxin producing *Aphanizomenon* and *Anabaena circinalis* (Table 3.8). Microcystins and anatoxin-a are largely retained within cells when the conditions for the growth of the organism are favourable. The amount of microcystin in a culture increases during the logarithmic growth phase, being highest in the late logarithmic phase. The maximum anatoxin-a concentration is found during the logarithmic growth phase (Sivonen, 1996; Watanabe, 1996). While the variants of microcystins produced by a particular strain are rather constant, the ratios of individual microcystins may change with time, or under conditions of different temperatures and light. Environmental factors affect toxin content of cyanobacteria, but only within a range of less than an order of magnitude. The majority of studies indicate that cyanobacteria produce most toxins under conditions which are most favourable for their growth. For example, different cyanobacterial species have different light requirements: *Planktothrix* prefers low light intensities for growth, *Anabaena* moderate and *Aphanizomenon* high light intensities. All strains produce most toxin when grown under their optimum light conditions (Table 3.8). Two- to

threefold differences in toxin content (on a per unit biomass basis) have been reported in relation to light conditions.

Strains and species also differ slightly in their optimum growth temperatures. The toxin content in most studies was highest at temperatures between 18 °C and 25 °C, whereas low (10 °C) or very high temperatures (30 °C) decreased toxin content. Temperature gradients caused two- to threefold differences in toxin content.

In a study using mouse bioassay to detect effects of pH on toxin production, cells were found to be more toxic when grown at high and low pH (Van der Westhuizen and Eloff, 1983).

Table 3.8 Laboratory studies on cellular toxin concentrations in cyanobacteria

Parameter	Organism	Toxin(s)/ analysis method	Changes in toxin concentrations (dw)	Highest/lowest toxin production	Reference(s)
Temperature (°C)					
12.5-30	*Anabaena* spp. (2 strains), batch cultures	Microcystins HPLC	3.5-30 fold[1]	Highest at 25, lowest at 30; different toxins at different temperatures	Rapala *et al.,* 1997
10-28	*Anabaena* spp. (2 strains), continuous cultures	Microcystins HPLC	3-10 fold	Lowest at 10, highest at 25	Rapala and Sivonen, 1998
15-30	*Anabaena* spp. (2 strains), batch cultures	Anatoxin-a HPLC	3 fold	Lowest at 30, highest at 20	Rapala *et al.,* 1993
10-28	*Anabaena* spp. (2 strains), continuous cultures	Anatoxin-a HPLC	4-7 fold	Highest at 19-21, lowest at 10 and 28	Rapala and Sivonen, 1998
15-30	*Aphanizomenon* sp. (1 strain), batch cultures	Anatoxin-a HPLC	3 fold	Lowest at 30, highest at 20	Rapala *et al.,* 1993
10,25,34	*Microcystis aeruginosa* (1 strain), batch cultures	Microcystins mouse bioassay	5 fold	Highest toxicity at 25, lowest at 10	Codd and Poon, 1988
15-35	*Microcystis aeruginosa* (1 strain) batch cultures	Microcystins mouse bioassay HPLC	4 fold	Highest toxicity at 20; different toxins at different temperatures	van der Westhuizen and Eloff, 1985; van der Westhuizen *et al.,* 1986
18,25,35	*Microcystis aeruginosa* (1	Microcystins mouse	1.4 fold	Highest toxicity at 18, lowest at	Watanabe and Oishi,

	strain), batch cultures	bioassay		32	1985
10-30	*Nodularia spumigena* (2 strains), batch cultures	Nodularin HPLC	3-4 fold	Highest at 20, lowest at 10 or 30	Lehtimäki *et al.,* 1994
7-28	*Nodularia spumigena* (1 strain), batch cultures	Nodularin HPLC	3 fold	Highest at 19	Lehtimäki *et al.,* 1997
15-30	*Oscillatoria agardhii* (2 strains), batch cultures	Microcystins HPLC	7 fold	Strain dependent; lowest at 30	Sivonen, 1990b
Light (µmol m^{-2} s^{-1})					
2-100 continuous	*Anabaena* spp. (2 strains), batch cultures	Microcystins HPLC	3 fold	Highest at 25	Rapala *et al*., 1997
7, 19, 42 continuous	*Anabaena* spp. (2 strains), continuous cultures	Microcystins HPLC	2.5-15	Lowest at 10, highest at 25	Rapala and Sivonen, 1998
2-128 continuous	*Anabaena* spp. (2 strains), batch cultures	Anatoxin-a HPLC	3 fold	Highest at 26-44, lowest at 2	Rapala *et al.,* 1993
7, 19, 42 continuous	*Anabaena* spp. (2 strains), continuous cultures	Anatoxin-a HPLC	No effect	Highest at 19, lowest at 7	Rapala and Sivonen, 1998
2-128 continuous	*Aphanizomenon* sp. (1 strain), batch cultures	Anatoxin-a HPLC	4 fold	Highest at 128, lowest at 2	Rapala *et al.,* 1993
5-50 continuous	*Microcystis aeruginosa* (1 strain), batch cultures	Microcystins mouse bioassay	2.4 fold	Highest toxicity at 20	Codd and Poon, 1988
20-75 continuous	*Microcystis aeruginosa* (1 strain), continuous cultures	Microcystins HPLC	2.5 fold	Highest at 40	Utkilen and Gjølme, 1992
21-205 continuous	*Microcystis aeruginosa* (1 strain), batch cultures	Microcystins mouse bioassay	1.2 fold	Highest toxicity at 142, lowest at 21	van der Westhuizen and Eloff, 1985
7.5, 30, 75 continuous	*Microcystis aeruginosa* (1 strain), batch cultures	Microcystins mouse bioassay	3.8 fold	Highest toxicity at 30, lowest at 7.5	Watanabe and Oishi, 1985
25, 50, 80	*Nodularia*	Nodularin	No difference		Lehtimäki *et*

continuous	*spumigena* (2 strains), batch cultures	HPLC			*al*., 1994
2-155 continuous	*Nodularia spumigena* (1 strain), batch cultures	Nodularin HPLC	50 fold[1]	Higher at high irradiances, minimal at 2	Lehtimäki *et al*., 1997
12-95 continuous	*Oscillatoria agardhii* (2 strains), batch cultures	Microcystins HPLC	2.5 fold	Highest at 12-44	Sivonen, 1990b
Phosphorus (mg P l^{-1})					
0.05-5.5	*Anabaena* spp. (2 strains), batch cultures	Microcystins HPLC	5 fold	Highest at 5.5, lowest at 0.05	Rapala *et al*., 1997
0.05-5.5	*Anabaena* spp. (2 strains), batch cultures	Anatoxin-a HPLC	No difference	No statistically significant differences	Rapala *et al*., 1993
0.05-5.5	*Aphanizomenon* sp. (2 strains), batch cultures	Anatoxin-a HPLC	2 fold	Lowest at 0.05-0.1, highest at 0.5-5.5	Rapala *et al*., 1993
BG-11 and medium without P	*Microcystis aeruginosa* (1 strain), batch cultures	Microcystins Mouse bioassay	1.7 fold	Higher toxicity without P	Codd and Poon, 1988
0.0025, 0.025	*Microcystis aeruginosa* (1 strain), continuous cultures	Microcystins HPLC	2.3 fold	More toxin at 0.025	Utkilen and Gjølme, 1995
MA medium 1/1; dilutions 1/10, 1/20	*Microcystis aeruginosa* (1 strain), batch cultures	Microcystins Mouse bioassay	Less than 1	Highest toxicity with the original medium	Watanabe and Oishi, 1985
0.1-5.5	*Oscillatoria agardhii* (2 strains), batch cultures	Microcystins HPLC	1.8-2.5 fold	Lowest toxin at 0.1	Sivonen, 1990b
0.3, 0.6, 1.0	*Nodularia spumigena* (2 strains), batch cultures	Nodularin HPLC	Less than 1 fold	Lowest at 0.3	Lehtimäki et al., 1994
0-5.5	*Nodularia spumigena* (1 strain), batch cultures	Nodularin HPLC	4 fold	Lowest at 0-0.02, highest at 0.2-5.5	Lehtimäki *et al*., 1997
Nitrogen (mg N l^{-1})					
BG-11 medium, medium	*Microcystis aeruginosa* (1 strain), batch	Microcystins mouse bioassay	5 fold	Higher toxicity with the medium containing N	Codd and Poon, 1988

without N	cultures				
0.05-1	*Microcystis aeruginosa* (1 strain), continuous cultures	Microcystins HPLC	3 fold	Higher at high N	Utkilen and Gjølme, 1995
MA medium 1/1; dilutions 1/10, 1/20	*Microcystis aeruginosa* (1 strain), batch cultures	Microcystins mouse bioassay	2.5 fold	Highest toxicity with the original medium	Watanabe and Oishi, 1985
0.42-84	*Oscillatoria agardhii* (2 strains), batch cultures	Microcystins HPLC	5 fold	Higher at high N, lowest at low N	Sivonen, 1990b
Micronutrients					
Al, Cd, Cr, Cu, Fe, Mn, Ni, Sn, Zn; various concentrations	*Microcystis aeruginosa* (1 strain), batch cultures	Microcystins HPLC	1.7 fold	Less toxins at low Fe concentrations	Lukac and Aegerter, 1993
0.1-3.4 µg Fe l^{-1}	*Microcystis aeruginosa* (1 strain), continuous cultures	Microcystins HPLC	1.5 fold	More toxin at high Fe concentrations	Utkilen and Gjølme, 1995
0.03-1.2 µg Fe l^{-1}	*Microcystis aeruginosa* (1 strain), continuous cultures	Microcystins HPLC	0-3 fold	Less toxin at low Fe concentrations	Lyck *et al*., 1966
Salinity (‰)					
3, 5, 8, 11	*Nodularia spumigena (2* strains), batch cultures	Nodularin HPLC	No difference	No statistical difference	Lehtimäki *et al.,* 1994
0-30	*Nodularia spumigena* (1 strain), batch cultures	Nodularin HPLC	8 fold	Highest at 15, lowest at 0 and 30	Lehtimäki *et al.,* 1997
0-35	*Nodularia spumigena* (6 strains), batch cultures	Nodularin HPLC	5 fold	Highest at 12, lowest at 35	Blackburn *et al*., 1996
CO_2					
BG-11 medium, medium without CO_2	*Microcystis aeruginosa* (1 strain), batch culture	Microcystins mouse bioassay	6 fold	Higher toxicity with the medium containing CO_2	Codd and Poon, 1988
pH					
1-14	*Microcystis*	Microcystins	1.8 fold	Toxicity highest	van der

	aeruginosa (1 strain), batch cultures	mouse bioassay		at low and high pH	Westhuizen and Eloff, 1983

dw Dry weight

HPLC High performance liquid chromatography

[1] When the growth of the strains was poor the amount of toxins was also very low (less than 0.1 mg g^{-1} dw of cells); when these cases were compared to maximal toxin production more than ten-fold differences could be seen

In high concentrations of phosphorus, hepatotoxic strains produced more toxins, but for anatoxin-a production phosphorus had no effect (Table 3.8). The differences induced by low and high phosphorus concentrations vary between two- and fourfold. Similarly, in field studies, a positive correlation of total phosphorus with microcystin-LR concentration in cells of *Microcystis aeruginosa* (Kotak *et al.,* 1995) or in bloom material of *Microcystis* spp. (Lahti *et al.,* 1997b) has been found. Non-nitrogen fixing species, like *Microcystis* and *Oscillatoria,* produce more toxins under nitrogen-rich conditions. Nitrogen fixing species are not dependent on the nitrogen in the media for their toxin production (Rapala *et al*., 1993; Lehtimäki *et al*., 1997). In continuous cultures when the toxins were expressed in relation to cell protein rather than to dry weight, Utkilen and Gjølme (1995) found that nitrogen and phosphorus limited conditions had no effect on the toxin content of *Microcystis aeruginosa.*

Indications regarding the role of iron are contradictory (Lukac and Aegerther, 1993; Utkilen and Gjølme, 1995; Lyck *et al.,* 1996). While studying the effect of trace metals on growth and on toxin content of *Microcystis aeruginosa,* Lukac and Aegerter (1993) found that in batch cultures only zinc was required for both optimal growth and toxin production.

Orr and Jones (1998) have unified many of these rather disparate studies on the effect of growth conditions on cyanotoxin production. They showed that the rate of microcystin production by a cyanobacterial population in culture is directly proportional to its growth rate, no matter what environmental factor was limiting growth. Moreover, they showed that the amount of microcystin contained by a single cell of *Microcystis aeruginosa* (i.e. on a per cell or cell quota basis) is constant within a narrow range (two- to threefold).

3.3.2 Biosynthesis

To understand how cyanotoxins are produced, it is necessary to study the biochemical and genetic basis of toxin production. Knowledge of the biosynthetic pathways of cyanotoxins is in its early stage and no complete biochemical pathways are known. Biosynthesis of several cyanotoxins has been studied by feeding labelled precursors to a cyanobacterial culture and following their incorporation into the carbon skeleton of the toxins. Shimizu *et al.* (1984) used an *Aphanizomenon flos-aquae* strain to study biosynthesis of saxitoxin analogues. They proposed a new pathway for neosaxitoxin biosynthesis, the key steps of which are the condensation of an acetate unit, or its derivative, to the amino group bearing an α-carbon of arginine or an equivalent, and a subsequent loss of the carboxyl carbon and imidazole ring formation on the adjacent

carbonyl carbon. They established the origin of all the carbons in the toxin alkaloid ring system. The side-chain carbon was derived from methionine (Shimizu, 1986).

Anatoxin-a is related structurally to the tropane class of alkaloids found in higher plants. Based mainly on ^{14}C-labelled precursors and enzymatic studies, Gallon *et al.* (1990) and Gallon *et al.* (1994) suggested the biosynthesis of anatoxin-a to be analogous to that of tropanes. Anatoxin-a was proposed to be formed from ornithine/arginine via putrescine, which is oxidised to pyrroline, a precursor of anatoxin-a. Labelling experiments using ^{13}C NMR (nuclear magnetic resonance spectrometry) indicated that the carbon skeleton of anatoxin-a is derived from acetate and glutamate. The studies showed that C-1 of glutamic acid is retained during the transformation of anatoxin-a and not lost by decarboxylation, a finding incompatible with the tropane alkaloid theory (Hemscheidt *et al.,* 1995b). All of the carbons of anatoxin-a(S) are derived from amino acids. Three methyl carbons arise from L-methionine or other donors to the tetrahydrofolate C1 pool. L-arginine accounts for C-2, C-4, C-5 and C-6 carbons of the toxin (Moore *et al.,* 1992, 1993). The intermediate in the biosynthesis of anatoxin-a(S) from L arginine is (2*S*,4*S*)-4 hydroxyarginine (Hemscheidt *et al.,* 1995a). The structure of the cylindrospermopsin suggests a polyketide origin for the toxin (Moore *et al.,* 1993).

The origin of carbons in microcystin (Moore *et al.,* 1991) and in nodularin (Choi *et al.,* 1993; Rinehart *et al.,* 1994) have been studied by following the incorporation of labelled precursors into the toxins by NMR. Carbons C1-C8 of Adda in nodularin are acetate derived and the remaining carbons presumably originate from phenylalanine. Methyl groups in carbons 2, 4, 6, 8, and the O-methyl group in the Adda unit, originated from methionine. The D-Glu and L-Arg carbons C4-C5 were acetate derived, with C1-C2 being from glutamate. Methyldehydrobutyrine was possibly formed from threonine, its methyl group coming from methionine. The β-methylaspartic acid was found to originate from condensation of pyruvic acid (C3-C4) and acetyl-CoA (C1-C2) (Rinehart *et al.,* 1994). The studies on the carbon skeleton of nodularin, with some minor differences, agree with work on microcystin-LR by Moore *et al.* (1991). In their study, L-Leu and D-Ala units in microcystin had acetate incorporation. The dehydroamino acid in microcystin has been proposed to be formed from serine rather than from threonine (Rinehart *et al.,* 1994). Rinehart's group found linear nodularin, which was shown by culture experiments to be a precursor of cyclic nodularin. Three additional linear peptides were isolated from a bloom sample, one of them was possibly a precursor of cyclic microcystin-LR and the others possibly degradation products (Rinehart *et al.,* 1994).

3.3.3 Genetic regulation of cyanotoxin production

The genes and enzymes involved in cyanotoxin production are still mostly unknown. The first molecular biological studies on toxic cyanobacteria investigated the possible involvement of plasmids in toxin production. Four toxic strains of *Microcystis aeruginosa* contained plasmids, and no plasmid could be shown in one toxic and in several non-toxic strains (Schwabe *et al.,* 1988). More recently, a similar study in Australia found no evidence for plasmid involvement in microcystin synthesis (Bolch *et al.,* 1997). Gallon *et al.* (1994) studied an anatoxin-a producing *Anabaena* strain NCR 44-1, which spontaneously became non-toxic. They found that the size of a plasmid was reduced in that non-toxic clone, but this work has not been repeated or confirmed.

Multi-enzyme complexes and peptide synthetase genes are involved in hepatotoxin production. Several cyclic and linear peptides, often with D-amino acids, are known to be produced, non-ribosomally, by multi-domain peptide synthetases via the so-called thiotemplate mechanism in bacteria and lower eukaryotes. The best characterised are the synthesis of gramicidin S and tyrocidin by *Bacillus.* Peptide synthetase genes have been detected and sequenced (partly) in *Microcystis aeruginosa* (Meissner *et al.,* 1996) and in *Anabaena* (Rouhiainen *et al.,* 1994). Analogous polymerase chain reaction (PCR) products to the peptide synthetase genes have been shown by using DNA from *Microcystis* (Jacobs *et al.,* 1995; Arment and Carmichael, 1996) and *Nodularia* as a template. Dittman *et al.* (1997) showed, in knockout experiments, that peptide synthetase genes are responsible for microcystin production.

At least some strains which produce hepatotoxins also produce other small cyclic peptides (Namikoshi and Rinehart, 1996; Weckesser *et al.,* 1996) which are likely to be produced by nonribosomal peptide synthesis.

3.4 Fate in the environment

3.4.1 Partitioning between cells and water

It appears likely that cyanotoxins are produced and contained within the actively growing cyanobacterial cells (i.e. they are intracellular or particulate). Release to the surrounding water, to form dissolved toxin, appears to occur mostly, if not exclusively, during cell senescence, death and lysis, rather than by continuous excretion.

In laboratory studies, where both intracellular and dissolved toxins (microcystins/nodularin and saxitoxins) have been measured, it is generally the case that in healthy log phase cultures, less than 10-20 per cent of the total toxin pool is extracellular (Sivonen, 1990b; Lehtimäki *et al.,* 1997; Negri *et al.,* 1997; Rapala *et al.,* 1997). As cells enter stationary phase the increased rate of cell death may lead to an increase in the extracellular dissolved fraction. Even during log-phase cell growth in culture, a small percentage of cells in the population may be dying and lysing (and releasing intracellular toxins), even though there is an overall positive population growth. There are some indications that anatoxin-a may leak out of cells during growth especially in low light conditions. High concentrations of anatoxin-a, sometimes exceeding the intracellular pool of toxins, have been found in media in a batch culture study (Bumke-Vogt *et al.,* 1996).

In the field, healthy bloom populations produce little extracellular toxin. The range of measured concentrations for dissolved cyanotoxins, in all cases except those where a major bloom is obviously breaking down, is 0.1-10 µg l^{-1} (Lindholm and Meriluoto, 1991; Jones and Orr, 1994; Tsuji *et al.,* 1996; Ueno *et al.,* 1996b; Lahti *et al.,* 1997b) Cell-bound concentrations are several orders of magnitude higher (see Tables 3.6 and 3.7). In lakes or rivers, toxins liberated from cells are rapidly diluted by the large mass of water, especially if mixing of water by wind action or currents is vigorous (Jones and Orr, 1994). However, the concentration of dissolved toxins may be much higher in ageing or declining blooms. This is an important consideration for water treatment plant operators,

because it means that removal of healthy cyanobacterial cells intact from the raw water supply may obviate or substantially reduce the need for additional adsorptive (activated carbon) or oxidative (ozone or chlorine) toxin removal processes (see Chapter 9).

The release of toxins from cells is enhanced by chemical treatments for the eradication of cyanobacteria, especially the use of algicides (either copper-based or organic herbicides). Treatment of a bloom with copper sulphate, for example, may lead to complete lysis of the bloom population within three days and release of all the toxins into the surrounding water (Berg *et al.,* 1987; Kenefick *et al.,* 1992; Jones and Orr, 1994). The efficacy of copper sulphate treatment is, however, very much dependent on water chemistry, especially alkalinity, pH and dissolved organic content (see sections 8.5 and 9.2).

3.4.2 Chemical breakdown

The four main groups of cyanotoxins: microcystins, anatoxins, PSPs and cylindrospermopsins, exhibit quite different chemical stabilities and biological activities in water.

Microcystins

Microcystins, being cyclic peptides, are extremely stable and resistant to chemical hydrolysis or oxidation at near neutral pH. Microcystins and nodularin remain potent even after boiling. In natural waters and in the dark, microcystins may persist for months or years. At high temperatures (40 °C) and at elevated or low pH, slow hydrolysis has been observed, with the times to achieve greater than 90 per cent breakdown being approximately 10 weeks at pH 1 and greater than 12 weeks at pH 9 (Harada *et al.,* 1996). Rapid chemical hydrolysis occurs only under conditions that are unlikely to be attained outside the laboratory, e.g. 6M HCl at high temperature.

Microcystins can be oxidised by ozone and other strong oxidising agents, and degraded by intense ultra violet (UV) light. These processes have relevance for water treatment and are discussed in Chapter 9, although they are unlikely to contribute to degradation occurring in the natural environment.

In full sunlight, microcystins undergo slow photochemical breakdown and isomerisation, with the reaction rate being enhanced by the presence of water-soluble cell pigments, presumably phycobiliproteins (Tsuji *et al.,* 1993). In the presence of pigments the photochemical breakdown of microcystin in full sunlight can take as little as two weeks for greater than 90 per cent breakdown, or longer than six weeks, depending on the concentration of pigment (and presumably toxin, although this has not been tested). A more rapid breakdown under sunlight has been reported in the presence of humic substances (which can act as photosensitisers) in field concentrations ranging from 2-16 mg l^{-1} dissolved organic carbon (DOC). Approximately 40 per cent of the microcystins was degraded per day under summer conditions of insolation (Welker and Steinberg, 1998). In deeper or muddy waters, the rate of breakdown is likely to be considerably slower.

Anatoxins

Anatoxin-a is relatively stable in the dark, but in pure solution in the absence of pigments it undergoes rapid photochemical degradation in sunlight. Breakdown is further accelerated by alkaline conditions (Stevens and Krieger, 1991). The half-life for photochemical breakdown is 1-2 hours. Under normal day and night light conditions at pH 8 or pH 10, and at low initial concentrations (10 µg l^{-1}), the half-life for anatoxin-a breakdown was found to be approximately 14 days (Smith and Sutton, 1993). Anatoxin-a(S) decomposes rapidly in basic solutions but is relatively stable under neutral and acidic conditions (Matsunaga *et al.,* 1989).

Saxitoxins

In the dark at room temperature, saxitoxins undergo a series of slow chemical hydrolysis reactions. The C-toxins lose the N-sulphocarbamoyl group to form decarbamoyl gonyautoxins (dc-GTXs); while the dc-GTXs, GTXs and STXs slowly degrade to, as yet unidentified, non-toxic products. The half-lives for the breakdown reactions are in the order of 1-10 weeks, with more than three months often being required for greater than 90 per cent breakdown (Jones and Negri, 1997). Because dc-GTXs are much more toxic than C-toxins (by a factor of 10-100), a solution or water body containing a natural mixture of C-toxins and GTXs, for example from the lysis of an Australian bloom of *Anabaena circinalis,* will actually increase in toxicity over a period of up to three weeks, before toxicity begins to abate during the succeeding 2-3 months. Boiling an extract of *Anabaena* with predominant C-toxins may also substantially increase toxicity. Similar transformation reactions occur in living cells as they age in culture or in a natural bloom (Negri *et al.,* 1997). No detailed studies have been carried out on saxitoxin breakdown in sunlight, either with or without pigments.

Cylindrospermopsins

Cylindrospermopsin is relatively stable in the dark, with slow breakdown occurring at elevated temperature (50 °C) (Chiswell *et al.,* 1999). In sunlight and in the presence of cell pigments, breakdown occurs quite rapidly being more than 90 per cent complete within 2-3 days (Chiswell *et al.,* 1999). Pure cylindrospermopsin is relatively stable in sunlight.

3.4.3 Removal on natural sediments and soils

Microcystins appear to be retained only weakly on natural suspended solids in rivers and reservoirs; usually no more than 20 per cent of the total microcystin concentration is adsorbed. In a laboratory experiment, some of the dissolved anatoxin-a and microcystins were reported by Rapala *et al.* (1993) to be adsorbed on lake sediments. Percolation through clay soils may provide some cyanotoxin removal, but this will depend greatly on the type of clay, surface charge, cation concentration of the water, etc. Cyanobacterial cells and microcystins were retained in soil columns, but less efficiently in sediment columns, in laboratory experiments simulating the fate of cyanobacterial toxins in artificial recharge of groundwater and bank filtration (Lahti *el al.,* 1996). No data are available for other cyanobacterial toxins, but some removal may be expected, again depending on the chemical conditions of soil and water.

Sedimentation of living cells without lysis, for example through grazing by zooplankton and sinking of faecal pellets, may lead to accumulation and persistence of toxin material in sediments, although this process has received little scientific attention. As discussed in more detail below, microcystins retained in intact cells may persist for several months. Cells deposited in sediments may be subject to fairly rapid breakdown by sediment bacteria and protozoa, with the resultant release of toxins.

3.4.4 Biodegradation

Microcystins

In spite of their chemical stability and resistance to eucaryotic and many bacterial peptidases, microcystins are susceptible to breakdown by aquatic bacteria found naturally in rivers and reservoirs. These bacteria appear to be reasonably common and widespread. Degradative bacteria have been found in sewage effluent (Lam *et al.,* 1995), lake water (Jones *et al.,* 1994; Cousins *et al.,* 1996; Lahti *et al.,* 1997a), lake sediment (Rapala *et al.,* 1994; Lahti *et al.,* 1997a) and river water (Jones *et al.,* 1994). Nonetheless, one Finnish study showed a complete lack of degradation of microcystin over a three-month period by an inoculum taken in winter from the Vantaanjoki River (Kiviranta *et al.,* 1991). There is usually an initial lag phase with little loss of microcystin and this period can be as short as two days or more than three weeks, depending on the water body, climatic conditions, the concentration of dissolved microcystin and in some cases, although not all, the previous bloom history of a lake (Jones *et al.,* 1994; Rapala *et al.,* 1994; Lahti *et al.,* 1997b). Once the biodegradation process commences, removal of microcystin can be more than 90 per cent complete within 2-10 days. This may vary depending on the water body, initial microcystin concentration and water temperature (Jones *et al.,* 1994; Lahti *et al.,* 1997b).

Jones *et al.* (1994) isolated a species of aquatic *Sphingomonas* that initiated ring-opening of microcystin-LR to produce linear (acyclo-)microcystin-LR as a transient intermediate (Bourne *et al.,* 1996). This compound was nearly 200 times less toxic than the parent toxin. The products of complete bacterial degradation were non-toxic to mice at doses up to 500 µg kg^{-1} (compared with an LD_{50} for microcystin-LR of about 60 µg kg^{-1}). The same bacterium, however, did not degrade the closely related cyclic pentapeptide nodularin. In a strain of *Pseudomonas aeruginosa* from a Japanese lake, microcystin degradation appeared to proceed by attack on the Adda side chain of microcystin (Takenaka and Watanabe, 1997). Several bacteria were isolated from lake water and sediment in Finland capable of degradation of microcystins and some strains also degraded nodularin. One strain was identified as a *Sphingomonas* sp. and two of the strains belonged to the beta-subgroup of *Proteobacteria,* although the genera remains to be determined (Lahti *et al.,* 1997a).

Other cyanobacterial toxins

Little work has been undertaken on the biodegradation of anatoxins, saxitoxins or cylindrospermopsin. Anatoxin-a may be readily degraded by bacteria associated with cyanobacterial filaments. Laboratory studies using non-axenic strains of cyanobacteria found low concentrations of dissolved anatoxin-a in the culture medium (Kiviranta *et al.,* 1991; Rapala *et al.,* 1993) whereas high concentrations of anatoxin-a were found in the medium of a continuous culture using an axenic strain (free of contaminating bacteria) of

the same species (Rapala and Sivonen, 1998). A *Pseudomonas* sp. strain able to degrade anatoxin-a at a rate of 6-10 µg ml^{-1} per three days was isolated by Kiviranta *et al.* (1991). In the presence of lake sediment and natural bacteria, the half-life for breakdown of anatoxin-a in the laboratory was about five days (Smith and Sutton, 1993). In a recent study by Jones and Negri (1997) no bacterially-mediated degradation of saxitoxins from *Anabaena circinalis* was observed in a range of surface water samples.

3.4.5 Bioaccumulation

Microcystins bioaccumulate in common aquatic vertebrates and invertebrates, including fish (Carbis *et al.,* 1997; Beattie *et al.,* 1998), mussels (Eriksson *et al.,* 1989; Falconer *et al.,* 1992; Prepas *et al.,* 1997; Watanabe *et al.,* 1997) and zooplankton (Watanabe *et al.,* 1992). In mussels, the highest microcystin concentrations are found in the hepatopancreas, and in vertebrates they are found in the liver. Williams *et al.,* (1997) have shown covalent binding and accumulation of microcystin-LR in salmon liver and crab larvae. Whether the levels of microcystin accumulation are sufficient to pose a risk to humans is uncertain, and will depend on levels of consumption and the severity of toxic blooms in the area where fish or shellfish are caught or collected. Common advice given by water authorities is that the viscera of the fish should not be eaten, but caution should be taken in all cases where major toxic blooms occur.

Saxitoxins from marine "red tide" dinoflagellates are well known for their propensity to bioaccumulate in marine vertebrates and invertebrates, often with disastrous consequences for animals and humans that consume them. Similarly, saxitoxins from the freshwater cyanobacterium *Anabaena circinalis* may bioaccumulate in an Australian species of freshwater mussel to concentrations exceeding international guidelines (Shumway *et al.,* 1995) during as little as seven days exposure to a cell density of 100,000 cells per ml of a toxigenic strain (Negri and Jones, 1995). This cell density is commonly encountered in natural blooms of this species.

3.4.6 Persistence and stability in cells

Culture studies indicate that microcystins and nodularin degrade only very slowly (time scale of weeks), if at all, whilst contained within living cells (Sivonen, 1990b; Lehtimäki *et al.,* 1994, 1997; Rapala *et al.,* 1997; Orr and Jones, 1998). Similarly, scums of *Microcystis aeruginosa* that dry on the shores of lakes may contain high concentrations of microcystin for several months (Jones *et al.,* 1995). These toxins are released back into the water body when re-immersed. Thus there is the potential for significant localised concentrations of dissolved microcystin even in the absence of living cells or a recently collapsed bloom.

In a lake study carried out over two summer - autumn periods, Lahti *et al.* (1997b) found that dissolved microcystin was more persistent than particulate toxin, with 30 and 15 days respectively required for 90 per cent degradation to occur.

3.5 Impact on aquatic biota

Direct cyanobacterial poisoning of animals can occur by two routes: through consumption of cyanobacterial cells from the water, or indirectly through consumption of other animals that have themselves fed on cyanobacteria and accumulated cyanotoxins. As was outlined in section 3.4.5, cyanotoxins are known to bioaccumulate in common aquatic vertebrates and invertebrates, including fish, mussels and zooplankton. Consequently, there is considerable potential for toxic effects to be magnified in aquatic food chains. Such toxicity biomagnification is well known for anthropogenic pollutants such as heavy metals and pesticides. There is no reason to suspect that the situation would be any different with natural cyanotoxins.

It is difficult to ascribe the deaths of natural populations of aquatic animals, especially fish, unequivocally to cyanotoxin poisoning. One of the main reasons for this is because the collapse of a large cyanobacterial bloom can lead to very low concentrations of oxygen in the water column as a consequence of bacterial metabolism; consequent fish deaths may be due to the anoxia. The best evidence for the potential for toxic effects on aquatic organisms comes from controlled laboratory trials with exposure of animals to toxic cyanobacteria or cell-free solutions of cyanotoxins.

3.5.1 Effects on aquatic bacteria

The influence of cyanobacterial toxins on bacteria is not fully understood and the scientific literature gives a number of contradictory statements. According to some authors neither an extract of *Microcystis aeruginosa* nor pure microcystin-LR have a biocidal effect on *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* or *Pseudomonas hydrophila* (Foxall and Sasner, 1988). However, these limited tests should not be seen as general indicators of the potential impacts of cyanotoxins on aquatic bacteria. The majority of aquatic bacteria are yet to be cultured, and studies with common mammalian pathogens or "laboratory" bacteria should not be taken as all encompassing. It is quite possible that cyanotoxins impact on some species of aquatic bacteria and not others. Certainly, microcystins are not toxic to all bacteria because several species are known to degrade quite high concentrations of these toxins (see section 3.4.4). It is even possible that the slow release of cyanotoxins from the cell surface or from senescent cells may stimulate associations of particular bacterial types (see section 3.4.4) which may even act as symbionts.

Attempts have been made to use bacterial toxicity tests (based on inhibition of bacterial phosphorescence) to screen for the presence of cyanotoxins, especially microcystins. However it appears that the inhibition of bacterial phosphorescence is not related to the commonly known cyanotoxins. It has been suggested that the negative effect may be related to the presence of unidentified LPS endotoxins in the cell wall of the cyanobacterial cells (see sections 3.1 and 13.3).

3.5.2 Effects on zooplankton

Evidence of the potential effects of cyanotoxins on zooplankton from numerous studies, mostly in laboratory situations, is complex and inconsistent. The vast majority of published studies has been based on mouse bioassay data describing cyanobacterial toxicity, with only a few more recent studies having used analytical methods such as HPLC (see section 13.4) to quantify individual toxins. Overall, it appears that cyanobacteria may exhibit a deleterious effect on zooplankton, but the effect is highly variable between genera and species, and even between clones of individual zooplankton species. One of the main questions yet to be resolved is whether the observed inhibitory effects are due to the putative poor nutritional value of cyanobacteria, to the known cyanotoxins, or to other unidentified compounds. There is evidence in the literature to support all three effects as being significant, at least with particular species under experimental growth conditions. A major difference in study design is whether organisms are exposed to cyanotoxins dissolved in water, or fed with toxic cyanobacteria. The latter is likely to lead to a substantially higher dose. Furthermore, Jungmann and Benndorf (1994) reported that exposure of *Daphnia* to dissolved microcystins showed effects only at concentrations several orders of magnitude above those found in field samples. They did, however, observe toxicity to *Daphnia* by unidentified metabolites other than microcystins from *Microcystis.*

There is dramatic variation among zooplankton species in their response to toxic (and even non-toxic) cyanobacteria. For example, DeMott *et al.* (1991) showed that the four species of zooplankton differed in their sensitivity to hepatotoxins by almost two orders of magnitude, but toxicity was observed only at very high concentrations that are scarcely encountered in natural water bodies (48 h LC_{50} ranging from 450 to 21,400 µg of microcystin per litre). Snell (1980) found that there was a genotype-dependent response of the rotifer *Asplanchna girodi* to toxic *Anabaena flos-aquae* and *Lyngbya* sp. Hietala *et al.* (1997) observed a variation in susceptibility of more than three orders of magnitude in the acute toxicity of *Microcystis aeruginosa* to 10 clones of *Daphnia pulex.* Both DeMott *et al.* (1991) and Laurén-Määttä *et al.* (1997) suggested that clone and species differences between zooplankton susceptibilities to toxic cyanobacteria may lead to selection pressures in favour of resistant strains or species in water bodies where toxic cyanobacteria occur frequently.

Benndorf and Henning (1989) found that the toxicity of a field population of *Microcystis* was increased by the feeding activity of *Daphnia galeata* over a period of a few months. A possible explanation for this phenomenon is offered by DeMott *et al.* (1991) who demonstrated that a number of zooplankton species will avoid grazing on toxic cyanobacteria, but continue to graze on non-toxic species. Similar results have also been shown for grazing by the phytoplanktivorous fish *Tilapia* and silver carp. Thus, grazing pressure from zooplankton and some fish may lead to the selective enrichment of toxic cyanobacterial strains over time.

It is likely that under natural conditions in water bodies, certain species and strains of zooplankton may be affected by cyanotoxins, whereas others will be unaffected. As such, cyanotoxins may influence the zooplankton community structure, especially during times when cyanobacteria are dominant within the phytoplankton.

3.5.3 Effects on fish

If fish are dosed with cyanotoxins by i.p. injections or by force-feeding, they develop similar symptoms of intoxication as laboratory mammals. The question relevant for field exposure is whether cyanotoxins enter healthy fish. For example, Tencalla *et al.* (1994) showed that gastrointestinal uptake by gavage (force-feeding) caused massive hepatic necrosis followed by fish deaths, whereas immersion of adults and juveniles in contaminated water did not cause toxic effects. Other reported evidence suggests that immersion in toxic cyanobacteria or cyanotoxins may be harmful to fish. Differences in sensitivity may be pronounced between species: goldfish were found to be nearly 30 times less susceptible to i.p. microcystin than mice (Sugaya *et al.,* 1990). Release of toxic compounds from mass developments of cyanobacteria was considered to be the cause of fish kills by Penaloza *et al.* (1990). Histopathological investigations offish deaths during cyanobacterial blooms in the UK, indicated that the cause of death was mostly due to damage of the gills, digestive tract and liver (Rodger *et al.,* 1994). The gill damage was probably caused by the high pH induced by cyanobacterial photosynthesis activity prior to the bloom collapse, together with the higher level of ammonia arising from the decomposition of the cyanobacteria. However, gill damage may have enhanced microcystin uptake and thus led to liver necrosis. Damage to gills by dissolved microcystin-LR has been shown experimentally in *Tilapia* and trout (Garcia, 1989; Gaete *et al.,* 1994; Bury *et al.,* 1996).

Other pathological symptoms ascribed to toxic cyanobacterial blooms include damage to the liver, heart, kidney, gills, skin and spleen (Garcia, 1989; Råbergh *et al.,* 1991). Garcia (1989) and Rodger *et al.* (1994) carried out experiments on trout, while Råbergh *et al.* (1991) experimented on carp. The latter study highlighted degenerative changes in kidney tubules and glomeruli. The effect of microcystins on European carp, *Cyprinus carpio,* under natural field conditions in Australia has been described by Carbis *et al.* (1997) as atrophy of hepatocytes, gills with pinpoint necrosis, epithelial ballooning, folded lamellar tips, exfoliation of the lamellar epithelium, elevated asparate aminotransferase activity and serum bilirubin concentrations. Laboratory studies indicate that dissolved microcystins may affect fish embryos (Oberemm *et al.,* 1997) and behaviour of fish (Baganz *et al.,* 1998).

The most definitive effect of microcystin on fish concerns Atlantic Salmon reared in net pens in coastal waters of British Columbia and Washington State, USA. As yet unidentified microcystin-producing organisms produce a progressive degeneration of the liver in salmon smolts placed into open-water net pens (Anderson *et al.,* 1993). The disease, referred to as Net Pen Liver Disease (NPLD), has resulted in significant economic losses for the mariculture industry.

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