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Quantitative algal toxin analysis by LC-MS/MS: a critical component of bloom risk assessment

ALS offers NATA-accredited quantitative analysis of key algal toxins by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for a range of surface water matrices, including dams, reservoirs, catchments, lakes and rivers. These analyses support drinking water management, bloom response, environmental investigations and risk assessment, with reporting limits well below current guideline and health alert values where available.

Toxic algal blooms

Phytoplankton comprise a diverse group of microscopic, photosynthetic organisms found in aquatic systems, including eukaryotic algae and cyanobacteria (blue-green algae). They are a natural and essential component of surface waters and typically occur at low concentrations. However, when environmental conditions favour rapid population growth, phytoplankton populations can increase sharply, resulting in the formation of blooms. These conditions commonly include elevated nutrient availability, warm temperatures and limited water mixing, such as those found in slow-flowing rivers and thermally stratified lakes (Huisman *et al.*, 2018).

While most phytoplankton are non-toxic, some species can produce algal toxins—secondary metabolites whose occurrence and dynamics are influenced by species composition and environmental conditions. These toxins may occur within cells (intracellular) or in the surrounding water (extracellular), either during active growth or following cell damage and breakdown. Blooms dominated by toxin-producing species may compromise water supplies and have been associated with livestock, wildlife and domestic animal poisonings, as well as adverse human health outcomes through drinking-water consumption or recreational contact.

Two toxin groups are of primary concern:

- **Hepatotoxins**, including microcystins, cylindrospermopsin and nodularin, which primarily affect the liver
- **Neurotoxins**, including anatoxins and saxitoxins, which interfere with nervous system function.

Impact on human health and the environment

Toxic algal blooms pose recognised risks to human health, livestock, wildlife and aquatic ecosystems. Several algal toxins, including cylindrospermopsin and microcystins, pose additional challenges for environmental management because they exhibit high chemical stability, allowing them to persist in the environment and resist degradation by conventional measures such as boiling (Chiswell *et al.* 1999). As a result, these toxins may remain biologically available and accumulate within food webs (Papadimitriou *et al.*, 2012), increasing the potential for adverse ecological and human health outcomes. Human exposure may occur through ingestion of contaminated drinking water, recreational contact (including swimming and water sports), or consumption of contaminated aquatic organisms (Chorus & Welker 2021). Consumption of shellfish contaminated with saxitoxins is a well-established cause of paralytic shellfish poisoning globally (Etheridge 2010).

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Algal toxins of concern

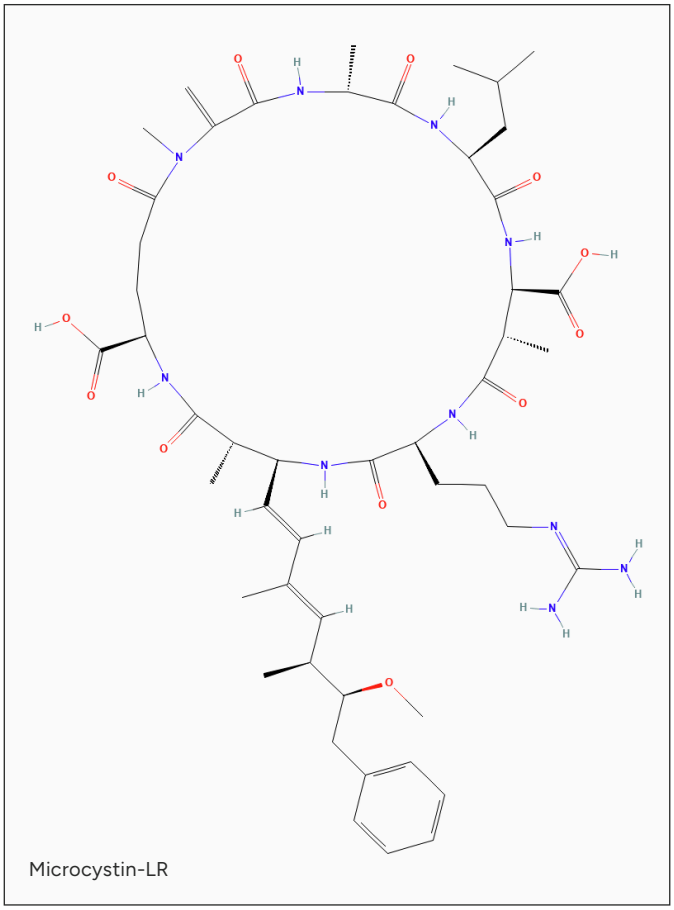
The following algal toxins are commonly targeted in monitoring programs due to their potential occurrence in blooms and relevance to water management:

Cylindrospermopsin

Cylindrospermopsin (CYN) and its analogue deoxycylindrospermopsin (deoxyCYN) are cytotoxins produced by several freshwater cyanobacteria, including *Raphidiopsis raciborskii, Umezakia ovalisporum, Aphanizomenon flos-aquae* and *Raphidiopsis curvata.* Once considered confined to tropical regions, CYN-producing species are now also reported across more temperate climates.

Microcystin

Microcystins are a large family of cyclic peptide hepatotoxins produced by various cyanobacteria, predominantly *Microcystis aeruginosa* in Australia. They are among the most frequently monitored cyanotoxins in Australian drinking water sources. Microcystins are chemically stable and can persist under a wide range of environmental conditions; microcystin-LR is the most extensively characterised and among the most toxic analogues.



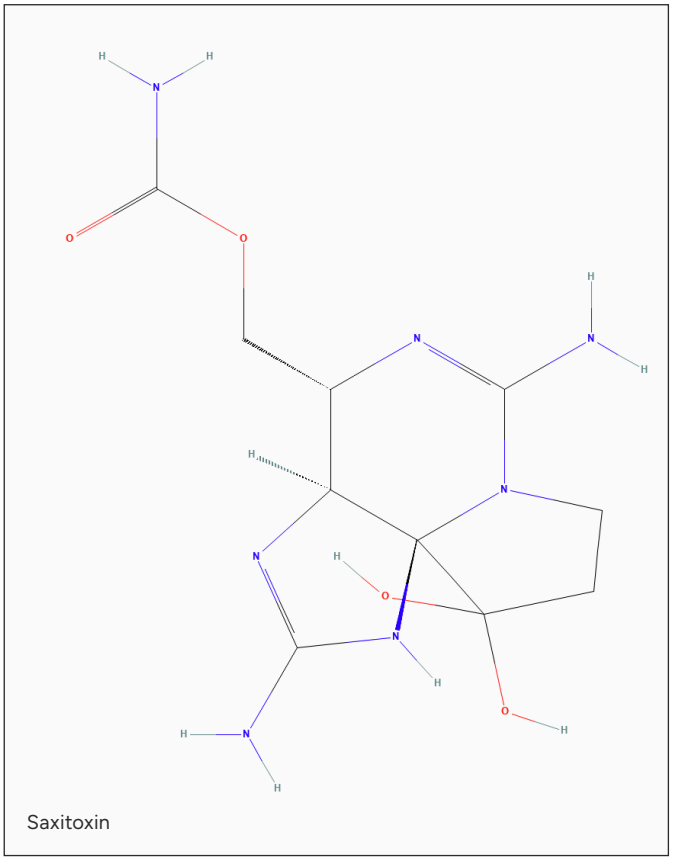
Microcystin-LR

Nodularin

Nodularin is a cyclic pentapeptide hepatotoxin produced primarily by *Nodularia spumigena*, but it has also been reported in other genera, including *Nostoc* and *Iningainema*. Structurally and toxicologically similar to microcystins, nodularin is commonly associated with brackish and estuarine systems but may also occur in inland saline waters.

Saxitoxin

Saxitoxins are neurotoxins belonging to the paralytic shellfish toxin (PST) family, which includes saxitoxin, neosaxitoxin, Ctoxins and gonyautoxins. They can be produced by both cyanobacteria and marine dinoflagellates. In Australia, saxitoxin production has been linked primarily to *Dolichospermum circinale*, with blooms typically dominated by C1-C4 saxitoxin congeners.



Saxitoxin

Anatoxins

Anatoxins are fast-acting neurotoxins with several analogues, including anatoxin-a and anatoxin-a(s), produced by multiple cyanobacterial genera such as *Anabaena flos-aquae*. They are associated with acute neurotoxicity following ingestion.

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Regulatory context

Management of phytoplankton and algal toxins in drinking water is guided by the Australian Drinking Water Guidelines (ADWG), which adopt a preventive, risk-based framework rather than relying solely on fixed numeric compliance limits (NHMRC 2011, updated 2022-2025). The ADWG emphasise early detection, a graduated response and verification monitoring, integrating source-water surveillance, treatment optimisation and targeted analytical testing to manage potential human-health risks associated with algal blooms. The framework is implemented nationally through State and Territory drinking water legislation, with individual jurisdictions applying the ADWG through their own regulatory and operational arrangements.

Under the current Guidelines, a health-based guideline value of 1.3 µg/L (expressed as microcystin-LR toxicity equivalents) is specified for total microcystins in drinking water. For other algal toxins, including cylindrospermopsin, nodularin, saxitoxins and anatoxins, definitive guideline values are not specified due to limitations in available toxicological data. Instead, the ADWG apply an Alert Levels Framework, using cell counts or biovolume as early indicators, with direct toxin analysis required to confirm risk and inform management actions (NHMRC 2011, updated 2022-2025).

The guidelines explicitly note that cell enumeration alone is insufficient to assess human-health risk, and that quantitative toxin measurements are essential to distinguish the presence of potentially toxigenic species from the actual occurrence of extracellular or bioavailable toxins. This accommodates the highly variable, site-specific nature of bloom dynamics and toxin production.

Within this regulatory context, quantitative LC-MS/MS analysis of algal toxins plays a critical role in confirming toxin presence and concentrations relevant to risk assessment, supporting operational decision-making and demonstrating due diligence.

Analytical approach

Quantitative algal toxin analysis typically follows the identification of potentially toxigenic taxa through routine monitoring, including phytoplankton identification and enumeration by microscopy, and detection of algal toxin genes by qPCR, both of which are services offered by ALS.

Quantitative algal toxin analysis is performed by LC-MS/MS with electrospray ionisation, delivering reporting limits substantially below guideline and indicative health alert values. This provides sensitive detection and robust quantification to support water management, bloom response and risk-based decision-making.

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Method specifications

ALS provides NATA-accredited quantitative algal toxin analysis for a suite of toxins, including cylindrospermopsin and its analogue deoxycylindrospermopsin, anatoxin, microcystin analogues, saxitoxin analogues and nodularin (table 1).

TABLE 1. Details of algal toxin analysis method

Compound	Method code	Analysis name	CAS number	Limit of reporting (µg/L)
Cylindrospermopsin (CYN) **Total**‡, Deoxycylindrospermopsin (deoxyCYN) **Total**‡	WP248 EP248	W-ALG-CYNL	143545-90-8 344941-42-0	0.05
Cylindrospermopsin (CYN) **Intra- and extra-cellular**†*, Deoxycylindrospermopsin (deoxyCYN) **Intra- and extra-cellular**†*	WP248 EP248	W-ALG-CYNL-EXT-INT	143545-90-8 344941-42-0	0.05
Anatoxin-a	WP248 EP248	W-ALG-ANA	64285-06-9	0.1
Microcystins, MC-LR, MC-YR, MC-RR, **Total**‡	WP249 EP248	W-MCYSTINL	101043-37-2 101064-48-6 111755-37-4	0.2
Microcystins, MC-LR, MC-YR, MC-RR, **Intra- and extra-cellular**†*	WP249 EP248	W-MCYSTINL-EXT-INT	101043-37-2 101064-48-6 111755-37-4	0.2
Nodularin	WP249 EP248	W-ALG-NODL	118399-22-7	0.2
Saxitoxin	WP263 EP263	W-ALG-SAX	35523-89-8	0.5
Saxitoxin analogues	WP263 EP263	W-ALG-SAX	Not applicable	0.3-1.9 0.5

** Extracellular toxins are measured in the filtrate. † Intracellular toxins are released following cell lysis. ‡ Total includes both intra- and extra-cellular toxins.*

Sampling and holding time requirements

Samples must be collected using the appropriate containers and stored under the specified conditions (table 2).

TABLE 2: Sampling requirements for algal toxin analysis

Parameter	Requirement
Bottle type	100 mL amber glass
Preservative	Sodium thiosulfate (chlorinated supplies only)
Storage	Cool to approximately 4°C immediately after sampling
Maximum holding time	30 days

References

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