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Quantitative Algal Toxins Analysis

INTRODUCTION

Following several extensive research and development project, ALS WRG (Melbourne) now offers **NATA accredited analysis of Algal (Cyanobacterial) Toxins by LCMSMS** from various water sources including dams/reservoirs, catchments, freshwater lakes and rivers with this latest update including Saxitoxins.

WHAT ARE ALGAL TOXINS?

Algal toxins is a generic term applied to a group of toxins produced by Aquatic Cyanobacteria or blue-green algae. Of particular importance and concern are those species known to be associated with the production of toxins. These toxins can be either contained intracellularly or expressed extracellularly and therefore present in the surrounding water [1].

The growth of cyanobacteria and blooms are favoured by nutrient enrichment, combined with warm temperatures and calm stable water conditions such as those occurring in slow-moving rivers and thermally stratified lakes[1]. As well as contaminating water supplies, blue-green algae have been implicated in the poisoning of animals including stock, amphibians, fish, pelicans, waterfowl, and zooplankton and harmful to human health.

There are several toxin types produced of which two in particular are of concern:

- Hepatotoxins – predominately **Microcystins, Cylindrospermopsin and Nodularin** which may cause liver damage.
- Neurotoxins – predominately **Anatoxins & Saxitoxins**, which may cause damage to the nervous system.

METHOD INFORMATION

ALS METHOD CODE
EP248WRG CYN, do-CYN and Anatoxin-a
EP249 Microcystins and Nodularin
EP263 Saxitoxins (Pending NATA)
LIMITS OF REPORTING (LOR)
EP248WRG 0.05-0.1µg/L
EP249 0.3µg/L
EP263 0.5-3.0 µg/L
METHOD REFERENCE
Various

ALGAL TOXINS OF CONCERN

Cylindrospermopsin (CYN)
CYN and the analogue deoxycylindrospermopsin (do-CYN) are general cytotoxins produced by a variety of freshwater cyanobacteria however it is understood that *Cylindrospermopsis raciborskii* is the principal producer of CYN. Historically CYN has only been detected in tropical areas however the distribution of CYN producers is now expanding into more temperate climates.

Microcystins
Are a large group of cyclic peptides produced by a range of cyanobacteria, and in Australia predominately produced by *Microcystis aeruginosa*. Microcystins are the most significant water quality issue in relation to cyanobacterial blooms in south-eastern Australia [1]. These toxins are extremely stable in water and can survive in both cold and warm water. Of these, Microcystin-LR is the best characterised and also one of the most toxic variants studied.

Nodularin
Is a cyclic pentapeptide hepatotoxin which is only produced by the cyanobacterium *Nodularia spumigena*. It is structurally similar to microcystins and exerts a comparable toxicity to Microcystin-LR. *N. spumigena* occurs primarily in brackish water and may form blooms in estuarine, brackish and freshwater inland lakes.

Saxitoxins
A member of the Neurotoxin group which includes saxitoxin, neosaxitoxin, C-toxins and gonyautoxins. Saxitoxins can be produced by cyanobacteria and also various dinoflagellates under the name paralytic shellfish poisons (PSPs). In Australia, only *Anabaena circinalis (aka Dolichospermum circinalis)* has been found to produce Saxitoxins. The prevalent toxins produced by blooms of *A. cicinalis* are the C-toxins.

Anatoxins
Are members of the neurotoxin group which includes anatoxin-a. Anatoxin-a is a cyanotoxin with acute neurotoxicity and has been isolated from *Anabaena flos aquae.*

REGULATORY GUIDELINES

The Australian Drinking Water Guidelines (ADWG) 2011includes a health based guideline for the total concentration of Microcystins in drinking water that should not exceed 1.3µg/L expressed as microcystin-LR Toxicity Equivalents.

Due to a lack of adequate data, the ADWG 2011 has set no guidelines values for concentrations of Cylindrospermopsin, Nodularin or Saxitoxins. Although no guideline values have been set, in all cases, cell numbers detected through traditional microscopy techniques should only be used as preliminary signals and as triggers for toxin testing to enable assessment of potential health risks[1].

ANALYSIS OF ALGAL TOXINS

A number of methods are required to cover the quantitative analysis of the algal toxins of importance found in Australian waters. The various algal toxins are analysed via ESI LC-MS/MS or LC-DAD generating limits of reporting significantly lower than current ADWG guidelines or indicative health alert values.

The **Limits of Reporting** (LOR) are listed in the table below:

Compounds	CAS Number	LOR (µg/L)
Cylindrospermopsin (CYN)	143545-90-8	0.05
Deoxycylindrospermopsin (do-CYN)	344941-42-0	0.05
Anatoxin-a	64285-06-9	0.1
Microcystins (as Microcystin-LR Toxicity Equivalents	n/a	0.3
Nodularin	118399-22-7	0.3
Saxitoxins (8 toxins plus Saxitoxin Toxicity Equivalents (STE)	Various	0.5 – 2.0 (STE = 3.0)

IMPACT ON HUMAN HEALTH AND ENVIRONMENT

Toxic blue-green algal blooms are recognised as posing serious health risks to humans and a range of terrestrial and aquatic animals and plants. Human health is at risk through the ingestion of contaminated water via drinking or other aquatic recreational activity or through the consumption of toxin laden foods (e.g. fish, shellfish).

The toxicity of Cylindrospermopsin and Microcystins are of concern due to their extreme chemical stability, are slow to breakdown through natural processes and remain potent even after boiling. Because these toxins can accumulate in the food chain, they pose a potential threat to aquatic organisms living in regions prone to cyanobacterial blooms. The consumption of shellfish containing relatively high concentrations of Saxitoxins/PSPs is known to cause human toxicity.

SAMPLING AND HOLDING TIME REQUIREMENTS

	Algal Toxins (Includes: CYN, do-CYN, Anatoxin-a, Microcystins, Nodularin & Saxitoxins)
Bottle Type	500mL Amber Glass
Preservative	Sodium Thiosulfate ($Na_2S_2O_3$) – chlorinated supplies only
Storage	Cool to 4°C from sampling
Holding Time	30 days

REFERENCES

[1] *National Health Medical Research Council, Australian Drinking Water Guidelines 6 2011 – Microorganisms: Cyanobacteria and their toxins.*

[2] *Marine Drugs 2010, Cylindrospermopsin: A Decade of Progress on Bioaccumulation Research. Susan Kinnear.*

[3] *National Wildlife Health Centre, Field Manual of Wildlife Diseases, Algal Toxins Chapter 36. Lynn H Creekmore.*

[4] *Health Canada, Environmental and Workplace Health, Blue-Green Algae (Cyanobacteria) and their Toxins.*