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LABORATORY SERVICES

Cyanobacteria and Algal Toxin Analysis

Authored by David Bumgarner and Wade DeLong - Microbac Laboratories, Inc., Ohio Valley Division

Cyanobacteria, also known as blue-green algae, include unicellular and colonial species, and are so common that they can be found in almost every conceivable marine and freshwater environment. These organisms provide vital ecological functions, including photosynthesis, and are a global source of nitrogen and carbon. However, under certain conditions, algal blooms result in large blue-green masses that clog fresh water lakes worldwide and can become a major nuisance in the late summer. Because cyanobacteria also produce water soluble toxins that are lethal to animals including humans, they are considered a major global problem threatening the ecosystem. Cyanobacteria toxins have been linked to both human and animal deaths all over the world and this has spurred a significant amount of research on the harmful algal blooms (HAB), the cyanobacteria toxins, and analytical methods to identify and quantify the levels of these toxins (1). The number of toxins produced by the cyanoHABs is quite large, but only a few have been identified as critical health concerns. The toxin groups of primary concern include the microcystins, nodularin, cylindrospermopsin, and anatoxin.

The microcystins are a group of compounds that are known to be hepatotoxins (liver toxins). They are classified as cyclic heptapeptides, consisting of a seven-member ring made up of five non-protein amino acids and two protein amino acids. It is the protein amino acid content that distinguishes the various microcystins. Nodularins are similar to the microcystins in structure and health effects, but are composed of only five amino acids in the peptide ring. Cylindrospermopsin is another cyanotoxin that is a highly water-soluble polycyclic uracil derivative, and is also toxic to the liver and kidneys. Anatoxin, also known as Very Fast Death Factor, is a potent alkaloid derived from a species of cyanobacteria known as Anabaena.

Algal Toxin Analysis

Two analytical approaches are practiced at state and commercial laboratories to identify the presence and levels of the algal toxins in freshwater. Screening procedures have been developed to measure approximate levels of the microcystin toxins. One option includes the use of enzyme-linked immunosorbent assays (ELISA), which is used to measure microcystins as a class. An indirect approach has been proposed that involves the analysis of the water soluble pigment, phycocyanin, which is characteristically produced by the cyanobacteria. The later technique utilizes fluoroescence spectrometry, and has been investigated as an early warning system detecting cyanotoxins in drinking water supplies (2). The definitive method, however, for the analysis of the individual toxins is liquid chromatography/ tandem mass spectrometry (LC/MS/MS). While the screening

options may be faster and less expensive, they are unable to speciate individual toxins, and may fail to detect toxins (false negatives) (2). The World Health Organization (WHO) has established an advisory limit of 1 ug/L for Microcystin-LR in drinking water (1). At this time, no advisory limits have been established for nodularin, cylindrospermopsin or anatoxin, although each has received increasing attention as emerging contaminants of concern (3).

Analytical Approach – LC/MS/MS

The **Microbac Laboratories, Inc., Ohio Valley Division** (OVD) has developed an LC/MS/MS method for the analysis of the cyanotoxins. Samples are collected in 40 mL amber glass vials and sample preparation consists of three freeze/thaw cycles to release the water soluble toxins. Samples are injected directly into the LC/MS/MS, where the analytes are separated in a gradient phase through a C18 reversed phase column. The analysis utilizes electro-spray ionization (ESI) in positive mode and multiple reaction monitoring (MRM) for detection of characteristic mass fragments. Analyte identification is achieved by monitoring two mass fragments, and quantitation is performed using linear regression calibration curves of the primary mass fragment.

Proposed Analyte List

The following toxins can be determined to a reporting limit of 0.5 ug/L.

Microcystin-LR
Microcystin-YR
Microcystin-RR
Microcystin-LA
Nodularin
Cylindrospermopsin
Anatoxin a

In addition to the specialty testing for cyanobacteria described above, **Microbac Laboratories, Inc.** has testing capabilities for the many known contributors to algal bloom (phosphorus, nitrogen, etc). For more information or analytical support with initial assessment, quantification, speciation and/or treatment efficacy, please contact us at microbac_info@microbac.com.

References:

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(2) Schmidt, W.; Petzoldt, H.; Imhof, L.; Moldaenke, C. Use of cyanopigment determination as an indicator of cyanotoxins in drinking water. Water Sci. Technol. 2009; 59(8):1531-1540.

(3) Boyer, G.; Dyble, J.; Harmful Algal Blooms, A newly emerging pathogen in water.
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For more information, please contact: microbac_info@microbac.com