

References

- (1) Health Canada (2017). Guidelines for Canadian Drinking Water Quality: Guideline Technical Document — Cyanobacterial Toxins. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario. (Catalogue No H144-38/2017EPDF).
- (2) Analysis of individual and total microcystins in surface water by on-line preconcentration and desalting coupled to liquid chromatography tandem mass spectrometry; G. Munoz, S. Vo Duy, A. Roy-Lachapelle, B. Husk, S. Sauvé, *Journal of Chromatography A*, (2017) 1516:9-20.
- (3) Règlement sur la qualité de l'eau potable, Loi sur la qualité de l'environnement (chapitre Q-2, a. 31, 45, 45.2, 46, 87, 115.27, 115.34 et 124.1), chapitre Q-2, r. 40.
- (4) Health Canada (2012). Guidelines for Canadian Recreational Water Quality, Third Edition. Water, Air and Climate Change Bureau, Healthy Environments and Consumer Safety Branch, Ottawa, Ontario. (Catalogue No H129-15/2012E).
- (5) Groupe scientifique sur l'eau (2017). Cyanobactéries et cyanotoxines dans l'eau potable et l'eau récréative. Dans *Fiches synthèses sur l'eau potable et la santé humaine*. Repéré sur le site de l'Institut national de santé publique du Québec : <https://www.inspq.qc.ca/eau-potable/cyanobacteries>.
- (6) Guide d'identification des fleurs d'eau de cyanobactéries (3e édition) (http://www.environnement.gouv.qc.ca/eau/eco_aqua/cyanobacteries/guide.htm)
- (7) U.S. EPA (United States Environmental Protection Agency). 2015. Drinking Water Health Advisory for Two Cyanobacterial Toxin. EPA 820F15103, Washington, DC; June 2015.
- (8) U.S. EPA (United States Environmental Protection Agency). 2017. Recommendations for Cyanobacteria and Cyanotoxins Monitoring in Recreational Waters. EPA 820-R-17-001, Washington, DC; June 2017.
- (9) U.S. EPA (United States Environmental Protection Agency). 2015. Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water. EPA 815-R-15-010, Washington, DC; June 2015.
- (10) Les significations environnementales dans ce document sont adaptées du site du Ministère de l'Environnement et de la Lutte contre les changements climatiques (MELCC), section eau (<http://www.environnement.gouv.qc.ca/eau/flrivlac/guides-protocoles.htm>).
- (11) Les significations environnementales dans ce document proviennent du site du Centre d'expertise en analyse environnementale du Québec (CEAEQ) (http://www.ceaeq.gouv.qc.ca/methodes/methode_para.htm).
- (12) Ces trois mesures ont été adaptées du protocole de mesure proposé par Water Rangers (<https://waterrangers.ca/fr/>). Cette organisation à but non lucratif propose aux citoyens des kits de mesure de la qualité d'eau.
- (13) Algues bleu vert - Pour connaître la manière de se protéger en présence d'une fleur d'eau d'algues bleu vert ou pour s'informer à ce sujet (<http://www.environnement.gouv.qc.ca/eau/algues-bv/precautions.htm>).
- (14) Prévenir les effets sur la santé liés aux algues bleu vert (<https://www.quebec.ca/sante/conseils-et-prevention/sante-et-environnement/algues-bleu-vert/#precautions-a-prendre-en-presence-d-algues-bleu-vert>).
- (15) Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques (MDDELCC) et Conseil régional de l'environnement des Laurentides (CRE Laurentides), 2017. Protocole d'échantillonnage de la qualité de l'eau, 4e édition, Québec, Direction de l'information sur les milieux aquatiques, ISBN 978-2-550-78284-1 (PDF), 9 p. <http://www.environnement.gouv.qc.ca/eau/rsvl/protocole-echantill-qualite.pdf>
- (16) L'analyse génomique de type 16S <https://www.youtube.com/watch?v=fCd6B5HRaZ8>
- (17) Detection of Cyanotoxins in Algae Dietary Supplements; A. Roy-Lachapelle, M. Sollic, M. F. Bouchard and S. Sauvé, *Toxins* (2017), *Toxins* 2017, 9(3), 76.

A special thanks to Ms. Stéphanie McFayden (Health Canada), Ms. Sylvie Blais (MELCC), Ms. Kodja and the office of communications of the Faculty of Arts and Sciences for their contribution to the analysis report. If you have any questions regarding the report, please contact Ms. Dana F. Simon (df.simon@umontreal.ca).

Appendix 1 : Analytical methods

The samples are analyzed by high resolution mass spectrometry using certified standards for the 12 microcystin (MC) variants and the six other cyanotoxins. The MC_{tot} value is the measurement of about 200 microcystin variants (including 12 variants). The MC_{tot} is analyzed by the MMPB² method.

Appendix 2 : Cyanotoxins, abbreviations and types of toxic effects

Table 1. Cyanotoxins, abbreviation and types of toxic effects

Microcystins (MC)		
Mode of toxicity known to date: Known hepatotoxicity ¹ (liver damage)		
Microcystins-RR	Microcystins -LA	[D-Asp3]-RR (dmMC-RR)
Microcystins -YR	Microcystins -LY	HtyR (MC- HtyR)
Microcystins -LR	Microcystins -LW	[D-Asp3]-LR (dmMC- LR)
Microcystins -WR	Microcystins -LF	HilR (MC- HilR)
Other cyanotoxins	Toxicity mode known to date	
Anatoxin-a (ANA-A)	Neurotoxic (damage to the nervous system)	
Homoanatoxin-a (HANA-a)	Neurotoxic	
Anabaenopeptin-A (AP-A)	Cytotoxic (cell damage)	
Anabaenopeptin-B (AP-B)	Cytotoxic	
Cylindrospermopsin (CYN)	Hepatotoxic et cytotoxic	

Appendix 3: Other recommendations and health risks

Health Canada recommends that when levels of MC_{tot} in treated water are detected above 400 ng/L, drinking water authorities should inform the public in the affected area so that another appropriate source of water (bottled water) is used in infant formula.¹ *The Australian National Health and Medical Research Council* has established a maximum recommendation of 1300 ng/L for MC_{tot}.¹ In the United States, the Environmental Protection Agency (EPA) has not established regulations or guidelines for cyanotoxins. However, in 2015 the EPA issued drinking water health advisories recommending a maximum exposure of 300 ng/L for microcystins (MC_{tot}) and 700 ng/L for cylindrospermopsin (CYN) for children under six years old.⁷ Peak concentrations of 1600 ng/L microcystins (MC_{tot}) and 300 ng/L cylindrospermopsin (CYN) are recommended for adults.⁹ Several states have developed their own customized action plan for monitoring recreational waters and assisting water managers.⁸ It should be noted that recommendations and opinions are not legal regulations. For recreational waters, these values are not used to manage algae bloom episodes. To this end, the MSSS recommends that people recognize a water bloom using MELCC tools (identification guide)⁶ and, if necessary, follow the recommended precautions recommended by the MSSS.⁵

Health risk: In large quantities, cyanobacteria or cyanotoxins pose a risk to human health. You can expose yourself to risk by:

- The consumption of insufficiently treated drinking water;

-
- Accidental ingestion of water during activities such as swimming in a waterbody affected by a bloom.^{5,13,14}
 - The consumption of food (fish, molluscs, vegetables, etc.) or dietary supplements contaminated with algae.
 - Direct or indirect contact of your skin or face with water during recreational activities (swimming, windsurfing, boating, fishing, etc.).^{13,14}
 - Inhalation of water droplets from the air (this is a less known exposure route).^{13,14}

To protect yourself during episodes of cyanobacteria blooms in lakes and streams, the Department of Health and Services recommends that you take precautions, which differ based on the type of recreational activities and other uses of water.^{1,15}

Appendix 4: Toxicity factors

Toxicity depends on several factors:

- variations in the density of cyanobacteria;
- the succession of cyanobacterial species and strains present and whether or not they produce cyanotoxins;
- variations in environmental conditions that do or do not favor the production of cyanotoxins by cyanobacteria.

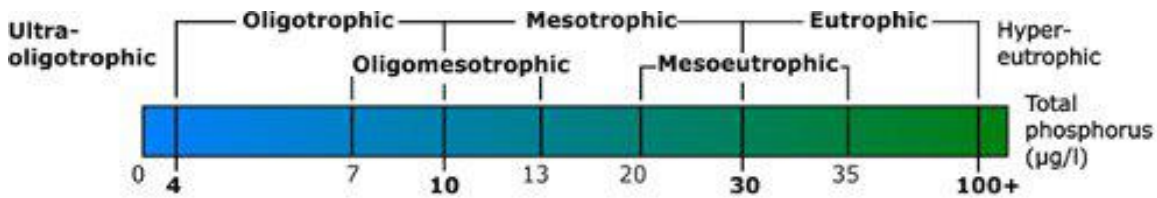
Appendix 5: Eutrophication^{10,11}

Eutrophication (lake aging) is the process of gradually enriching a lake with nutrients (such as nitrogen and phosphorus), changing its state from oligotrophic (which means sparse) to eutrophic (which means well-fed). With eutrophication, we can see an increase in algae, cyanobacteria and aquatic plants, a decrease in the water clarity, and a disappearance of sport fish species.

Eutrophication can be accelerated by human activities on the shoreline and in the watershed. Depending on who lives in the watershed, sources of phosphorus inputs mainly come from domestic or municipal wastewater discharges or certain fertilized industries (eg: food and paper), poorly maintained or obsolete septic systems, golf courses, leaching and runoff from farmland or logging, etc. Premature aging is one of the major problems affecting resort lakes and lakes in agricultural and urban areas.

In theory, the concentration of total phosphorus at the sample location could correlate to the oligotrophic level. However, it cannot be concluded that the lake has this trophic level; this would require a more complete water quality monitoring and an assessment should be based on several points mentioned below. The trophic level classification diagram below is an indication of the state of the state point at the sampling point.

Diagram: The trophic level of lakes¹⁰



Assessing the state of eutrophication would require more comprehensive monitoring of water quality.¹⁵ Such an assessment should, at a minimum, be based on the following:

- a minimum of three sampling dates per year during the ice-free period;
- a testing location opposite the deepest point of the lake;
- a sample not limited to the surface, but integrating, for example, the first meter of the water column;
- the same sample divided into subsamples for each of the parameters (rather than a separate sample for each parameter);
- the cumulative data of two parameters chlorophyll *a* and water clarity in addition to phosphorus.

Appendix 6: pH, alkalinity and hardness

- **pH:** Since the pH scale is logarithmic, this indicates a factor of 10 between each unit. For example, a pH of 5 is 10 times more acidic than a pH of 6 and 100 times more than a pH of 7. pH influences the toxicity of several elements by governing a large number of chemical reactions. Rivers and lakes range from 5 (acidic) to 9 (basic). In natural waters with little human activity, the pH depends on the nature of the soil and rock. In addition, a high density of cyanobacteria, algae or aquatic plants can vary the pH of the water within a day. While breathing, plants and algae release carbon dioxide (CO₂) into the water and make it more acidic. In contrast, when they photosynthesize during the day, the CO₂ they capture make it less acidic.
- **Alkalinity:** The sensitivity of an aquatic environment to acidification varies with alkalinity. Alkalinity is measured as an equivalent concentration of calcium carbonate (CaCO₃) and is affected by surrounding soil, bedrock, vegetation and industrial waste. High alkalinity is not necessarily a sign of poor water quality. An acidification sensitivity level is considered high (alkalinity of <10 mg/L CaCO₃), average (10-20 mg/L CaCO₃) and low (> 20 mg/L CaCO₃).
- **Hardness :** Hardness is related to alkalinity and they often change together. The hardness is based on the calcium and magnesium content; these minerals are often dissolved when the water comes in contact with rocks like limestone. The reverse process can produce scale buildup inside the pipes. Water is considered to be soft (0-20 mg/L), moderately soft (21-60 mg/L), moderately hard (61-120 mg/L), hard (121-180 mg/L) and very hard (> 180 mg/L).



Appendix 7: Genomic analysis of type 16S¹⁹

16S genomic analysis is used to confirm whether or not cyanobacteria are present in the received sample. Since microorganisms are very difficult to identify even with a microscope, scientists have developed a quick and accurate way to detect them in environmental samples using their DNA. Once the DNA is extracted, we use the polymerase chain reaction (PCR) technique that creates multiple copies of a DNA fragment so we can identify them as a fingerprint. The last step is to read the information present in the bacteria's DNA within the lake water sample. For this, we use a machine called DNA sequencer (Miseq Illumina sequencer). We insert the multiple copies of the "bacterial fingerprint" and the machine sequences the data that, once processed through the bioinformatics technology, inform us about the quantity and identity of the species present in the sample.