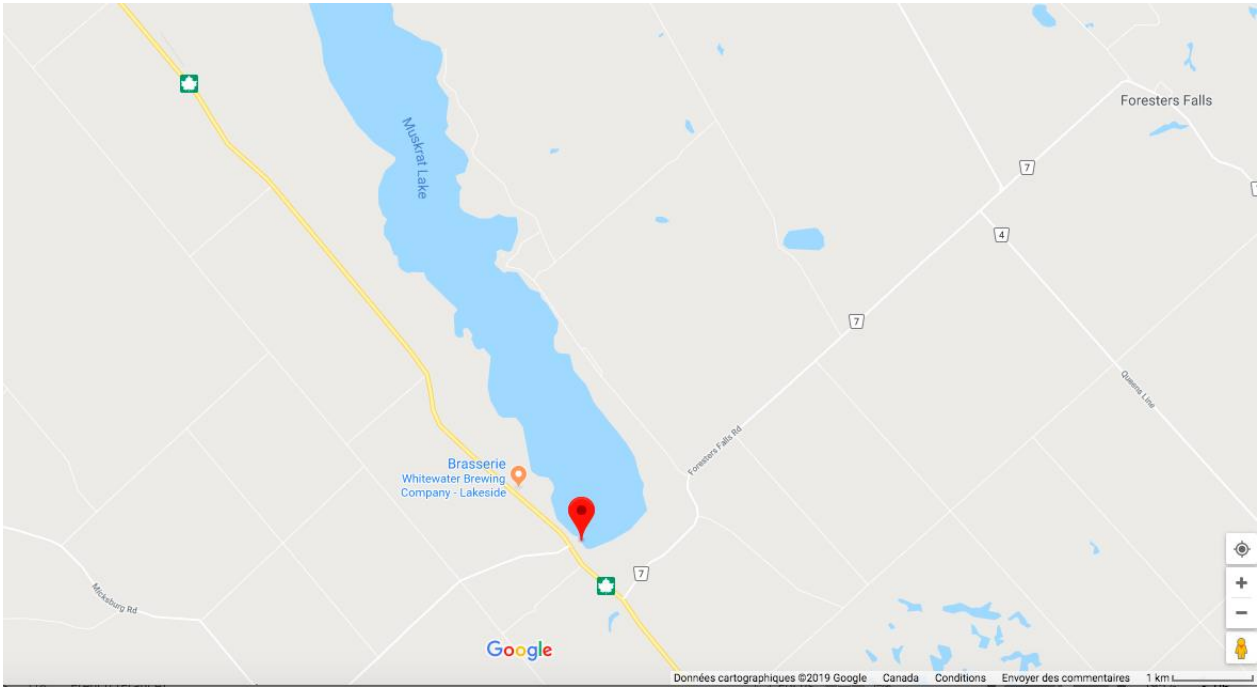
ANALYSIS REPORT

Adopt a Lake Campaign, University of Montreal (UdeM)



500 m

Lake sampled: Muskrat Lake Boat launch
Sample date: 19 September 2018
Sampler: Julie Sylvestre
The analysis was conducted under the supervision of the Environmental Chemistry Laboratory of UdeM.

Context and purpose: The results presented below are intended to provide a picture of the water quality and the presence of cyanotoxins in Muskrat Lake (located on the map above) on September 30, 2018. Please note that the sample show the water's state for the sampled location at the time of sampling only. **At this time and place, Muskrat Lake had toxins and a level of nitrogen and phosphorus that indicates a meso-eutrophic state.**

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1) Cyanotoxin analysis results

Several species or strains of cyanobacteria with toxic potential are likely to produce more than one type of cyanotoxin. [1] The cyanotoxins measured in your sample are summarized below.

- Lake Muskrat's result for cyanotoxin analysis (in nanograms per litre, ng/L)

Variants of microcystins (MC) and total microcystins (MC_{tot})							
MC-RR	MC-YR	MC-LR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF
<MDL	<MDL	307±28	<MDL	<MDL	<MDL	<MDL	<MDL
dmMC-RR	MC-HtyR	dmMC-LR	MC-HilR	MC_{tot}*			
<MDL	<MDL	<MDL	<MDL	N/A			
Other cyanotoxins							
CYN	ANA-a	HANA-a	AP-A	AP-B			
<MDL	<MDL	<MDL	88±21	83±30			

The method detection limits (MDLs) range from 0.02 ng/L to 0.12 ng/L. <MDL: Not detected. N/A: no sample.

* MC_{tot}[2] is the measurement of 200 microcystin variants. The figures after (±) indicate the result's variation (Appendix 1).

Please see Appendix 2 to interpret the above abbreviations.

ANALYSIS: Muskrat Lake contains very few microcystins, below the recommended standards for limits presented below. Among other cyanotoxins, AP-B and AP-B were also detected. There is no recommendation for Anabaenopeptins (AP-A and AP-B).

Tap water (after water treatment) has a recommended maximum acceptable level for MC-LR[1] of 1,000 ng/L of, a standard set by the World Health Organization (WHO) in 2003. Health Canada recommends a maximum concentration MC_{tot}[1] of 1500 ng/L. Some jurisdictions enforce these standards: Quebec's standard for tap water is 1500 ng/L MC-LR (equivalent toxicity) as detailed under the *Regulation respecting the quality of drinking water* in Québec (RQEP). [3] For recreational water (swimming), Health Canada recommends a concentration of <20,000 ng/L. [4] See Appendix 3 for other recommendations and health risks.

Understanding environmental significance of Cyanobacteria and Cyanotoxins: In aquatic ecosystems when temperature, nutrient and light conditions are favorable, cyanobacterial blooms (blue-green algae) can reach densities visible to the naked eye, which also known as an algae bloom. For a Quebec body of water to be considered affected by a cyanobacterial bloom, the density of cyanobacteria must be at least 20,000 cells per milliliter. [4] In Quebec, the Ministry of Health and Social Services (MSSS) recommends that people recognize an algae bloom using MELCC[6] tools. Please note: blooms may or may not be toxic. Their level of toxicity may also vary during short periods of time and from different places in the same waterbody. Toxicity depends on several factors (Appendix 4). Blooming is usually seasonal (spring to fall) and transient (average is less than 30 days). [1] Some may occupy a very small area while others may extend over the entire waterbody.

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2) Nutrient analysis results for Muskrat Lake

- **Phosphorus** (micrograms of phosphorus (P) per liter, µg/L)

Location name	Total phosphorous (µg P/L)	Dissolved phosphorous (µg P/L)	Soluble Orthophosphates (µg P/L)
Muskrat Lake	27.0±4.0	10.4±3.2	5.5±1.3

The detection threshold for total phosphorus, dissolved and soluble orthophosphate is 2 µg P/L. The units µg P/L represent the number of micrograms of phosphorus per liter in the sample. "N/A" means that no sample was taken. The digits after (±) indicate the result's variation.

ANALYSIS: The total phosphorus concentration measured in this sample does not seem to indicate eutrophication (see explanations below). For a diagram showing the eutrophication levels and more information, refer to Appendix 5. As a reminder, it is impossible to diagnose the state of a lake with a single sample; the sample only shows us the state of the lake for the sampled location and when the sampling took place.

Understanding the environmental significance of phosphorus: [10,11] Phosphorus (P) is dissolved or associated with particles. In both surface water and wastewater, P is mostly in the form of particulate phosphorus. Orthophosphates (PO_4^{3-}) are a type of dissolved phosphorus that are used by cyanobacteria, algae and aquatic plants and is a nutrient essential for their growth. However, high concentrations and other favourable conditions (low current, adequate transparency, heat, etc.), leads to excessive growth. Excessive growth of cyanobacteria is often attributed to premature aging (eutrophication) of the lake (Appendix 5).

- **Nitrogen** (in milligrams of nitrogen (N) per liter, mg/L)

Location name	Total nitrogen (mg N/L)	Dissolved nitrogen (mg N/L)	Ammoniacal nitrogen (mg N/L)	Nitrites and nitrates (mg N/L)
Muskrat Lake	0.61±0.07	0.48±0.01	0.049±0.009	0.034 ±0.000

The limit of quantification for total and dissolved nitrogen is 0.01 mg N/L, for ammoniacal nitrogen is 0.005 mg N/L and for nitrite and nitrate is 0.001 mg N/L. mg N/L is the number of milligrams of nitrogen per liter of sample. "N/A": no sample was taken. The digits after (±) indicate the result's variation.

ANALYSIS: The nitrogen levels at this sample location and sample time do not seem to indicate over-fertilization (see explanations below and Appendix 5).

Understanding the environmental significance of nitrogen: [10,11] Total nitrogen is the sum of different forms of nitrogen. Dissolved nitrogen, ammoniacal nitrogen and nitrites and nitrates are found in more or less significant quantities in aquatic ecosystems. There is no criterion for total nitrogen toxicity. However, a concentration greater than 1.0 mg/L of total nitrogen in surface water is indicative of a problem of over-fertilization. In natural waters, ammoniacal nitrogen and nitrites and nitrates mainly originate through

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leaching from agricultural land as well as municipal and industrial wastewater. Moreover, just like phosphorus, nitrogen is a nutrient essential for plant and algae growth. High concentration may contribute to excessive growth of cyanobacteria (Appendix 5).

3) Lake Muskrat's results for pH, hardness and alkalinity [12]

Location name	pH	Alkalinity (mg/L)	Total hardness (mg/L)
Lac Muskrat	8.4	120	250

The pH, alkalinity and hardness data represent a sampling location's specific state at the time of sampling.

Understanding the environmental significance of pH: [10]
pH is measured on a scale of 0 to 14. A pH of 7 indicates neutral water; values below 7 indicate acidic conditions, and values above 7 are characteristic of basic conditions. Rivers and lakes range from 5 (acidic) to 9 (basic). A high density of cyanobacteria, algae or aquatic plants can change the water's pH in a day (Appendix 6).

Understanding the environmental significance of alkalinity and hardness in water:[10]
The sensitivity of an aquatic environment to acidification (ability to change pH quickly) varies with its alkalinity. Sensitivity to acidification can be high (alkalinity of <10 mg/L $CaCO_3$), average (10-20 mg/L $CaCO_3$) and low (> 20 mg/L $CaCO_3$). 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), very hard (> 180 mg/L) (Appendix 6).

ANALYSIS: Muskrat Lake has a basic pH, a low acid sensitivity level and a hard water. For further explanation, refer to Appendix 6.

4) Genomic Analysis results Lake Muskrat Lake

When it is possible to sample foam, 16S genomic analysis is used to confirm whether cyanobacteria were present in the sample or not (Appendix 7).

ANALYSIS: Lake Muskrat is mainly composed of the genus Microcystis, which may contain species or strains in their community that can produce toxicity.

Figure 4.1 shows the different groups (phylum) of cyanobacteria. Cyanobacteria are a group among the many possible groups of bacteria. To simplify the graph, only the 20 most abundant groups were shown. In this sample, the group of cyanobacteria is in first position (relative position) followed by the group of proteobacteria.

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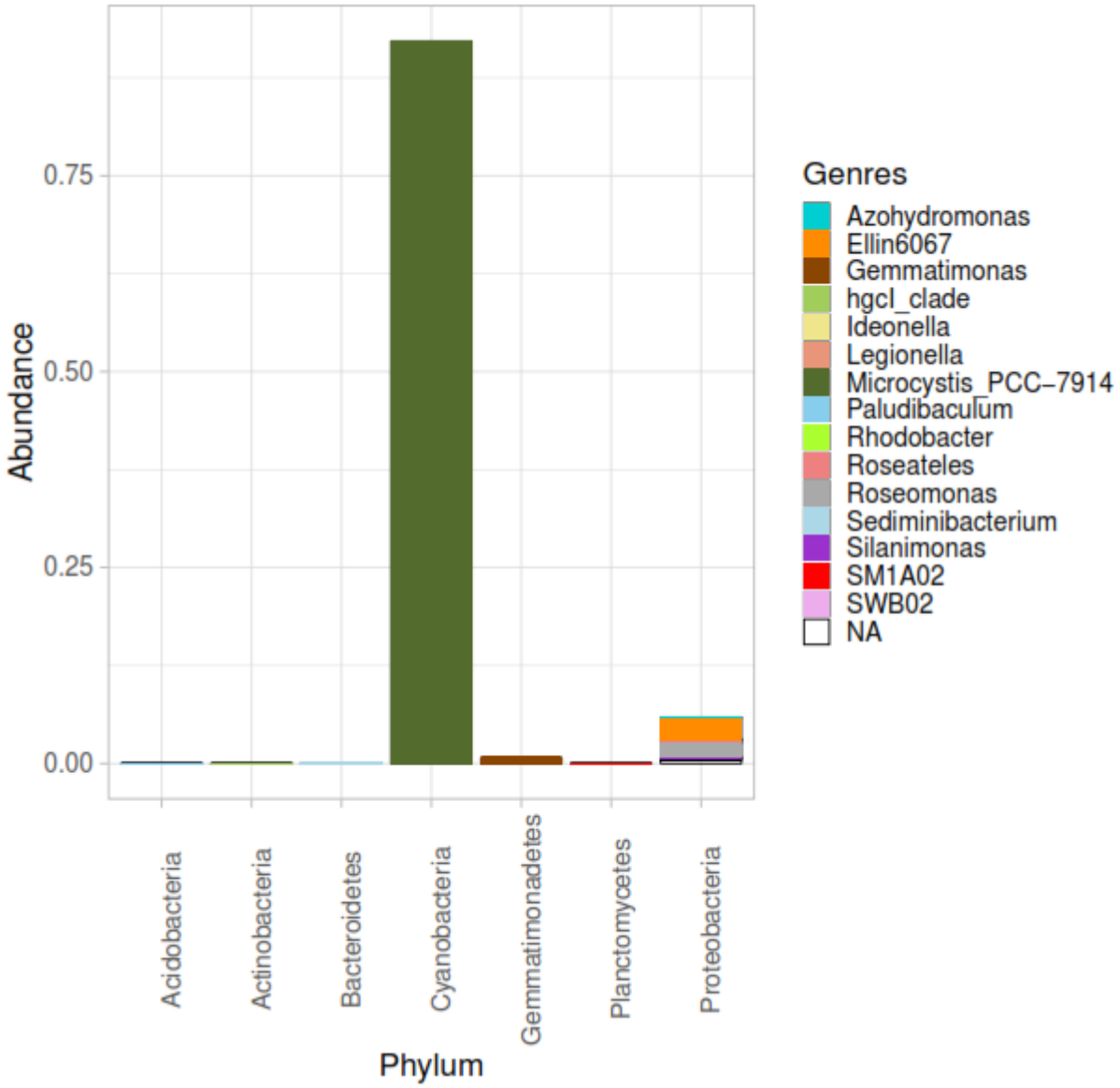
Figure 4.1 The 20 groups (phylum) of bacteria in the sample.



During the expansion of the group of cyanobacteria (Figure 4.2), the most dominant genus is Microcystis. This genus is known to contain strains that can produce toxins. The 16S result does not give any indication of the type of species or strain.

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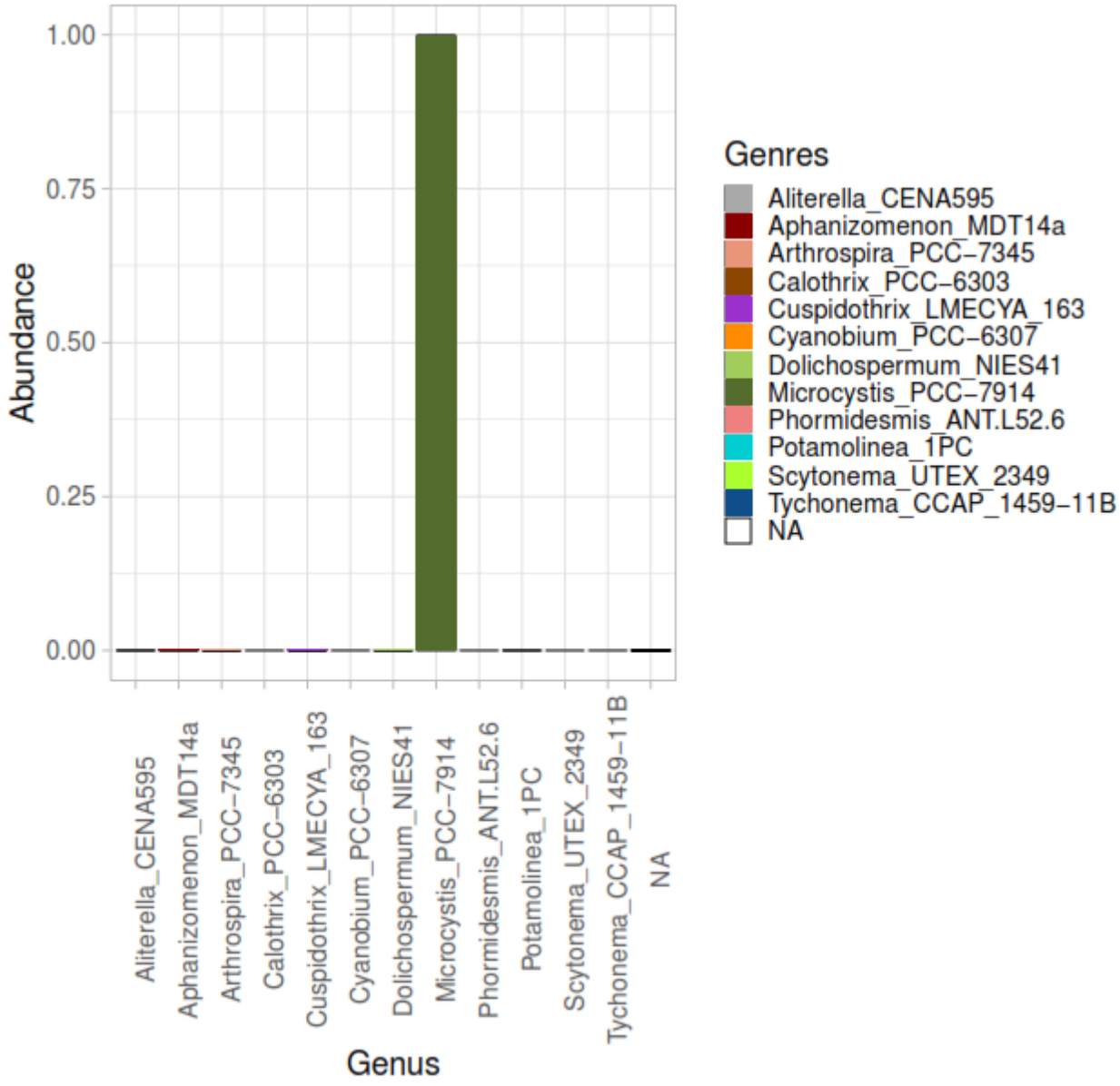
4.2 The 20 genera of bacteria found in the Cyanobacteria phylum.



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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), chapitreQ-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. Solliec, M. F. Bouchard and S. Sauvé, Toxins (2017), Toxins 2017, 9(3), 76.

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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 MMPB[2] method.

Appendix 2 : Cyanotoxins, abbreviations and types of toxic effects

Table 1. Cyanotoxins, abbreviation and types of toxic effects

Microcystines (MC)		
Mode of toxicity known to date: Known hepatotoxicity[1] (liver damage)		
Microcystine-RR	Microcystine -LA	[D-Asp3]-RR (dmMC-RR)
Microcystine -YR	Microcystine -LY	HtyR (MC- HtyR)
Microcystine -LR	Microcystine -LW	[D-Asp3]-LR (dmMC- LR)
Microcystine -WR	Microcystine -LF	HilR (MC- HilR)

Other cyanotoxins	**Toxicity mode known to date**
Anatoxine-a (ANA-A)	Neurotoxic (damage to the nervous system)
Homoanatoxine-a (HANA-a)	Neurotoxic
Anabaenopeptin-A (AP-A)	Cytotoxic (cell damage)
Anabaenopeptin-B (AP-B)	Cytotoxic
Cylindrospermopsine (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]

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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

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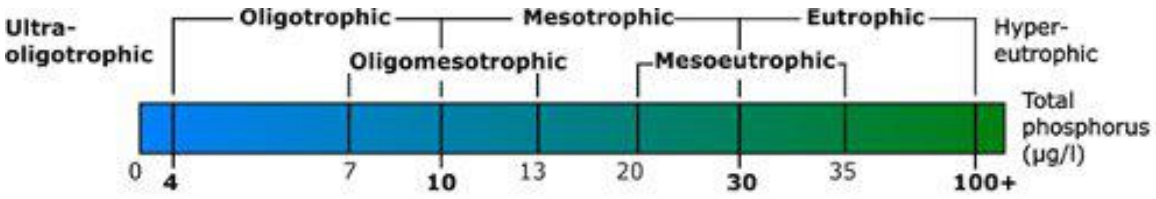
below. The trophic level classification diagram below is an indication of the state of the state point at the sampling point.

Diagram: The trophic level of lakes [10]



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 sample;
- 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 (CO_2) into the water and make it more acidic. In contrast, when they photosynthesize during the day, the CO_2 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 ($CaCO_3$) and is affected by surrounding soil, bedrock, vegetation and industrial waste. High alkalinity is not necessarily a sign

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of poor water quality. An acidification sensitivity level is considered high (alkalinity of <10 mg/L $CaCO_3$), average (10-20 mg/L $CaCO_3$) and low (> 20 mg/L $CaCO_3$).

- 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 16S[19]

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.

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