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POLYTECHNIQUE MONTRÉAL

affiliée à l'Université de Montréal

The effects of a coagulant and nutrients on cyanobacteria and cyanotoxins: a mesocosm study

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Thèse présentée en vue de l'obtention du diplôme de *Philosophiæ Doctor*

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en vue de l'obtention du diplôme de *Philosophiæ Doctor*

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DEDICATION

To my family and my friends, thank you for always being there for me,

To my husband, thank you for helping me get through this important milestone.

To my little one, thank you for coming into my life.

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RÉSUMÉ

La prolifération excessive de fleurs d'eau cyanobactériennes toxiques est un problème courant dans le monde entier et constitue une préoccupation pour la santé humaine. Le traitement de l'eau potable peut être affecté par la présence de concentrations élevées de cellules cyanobactériennes dans les sources d'approvisionnement en eau potable. Les coagulants peuvent-ils être utilisés pour éliminer rapidement les cellules cyanobactériennes et leurs toxines intracellulaires d'une source d'eau potable avant qu'elles n'entrent dans les stations de traitement ? On sait peu de choses sur l'effet des coagulants sur les cellules et les toxines cyanobactériennes ainsi que sur la communauté cyanobactérienne à court terme dans les sources d'eau. Pour répondre à cette question, la coagulation *in situ* a été étudiée dans des lacs avec des blooms en utilisant des expériences de mésocosme.

Au-delà de l'élimination des cyanobactéries, la gestion des plans d'eau pour contrôler les efflorescences cyanobactériennes est importante. L'enrichissement en nutriments est lié à la biomasse des cyanobactéries et à la production de toxines. Les solutions à long terme pour la gestion des nutriments sont importantes ; cependant, il est nécessaire d'étudier l'ajout de nutriments à court terme. Des ajouts de nutriments peuvent se produire pendant les événements de précipitations et l'étude de leur impact potentiel sur la croissance des cyanobactéries et la production de toxines est nécessaire afin d'améliorer notre compréhension des facteurs de l'apparition des fleurs d'eau toxiques dans les sources d'eau potable.

L'objectif principal de cette recherche était d'étudier les effets des nutriments et des coagulants sur les cyanobactéries et leurs toxines dans les sources d'eau grâce à la métagénomique. Deux sites avec des efflorescences cyanobactériennes récurrentes (Baie Missisquoi et Petit Lac Saint François) ont été sélectionnés pour les expériences. Les objectifs spécifiques étaient les suivants (1) déterminer l'efficacité de l'ajout de coagulants aux eaux de source pour l'élimination des cellules cyanobactériennes et des cyanotoxines, (2) déterminer la composition de la communauté cyanobactérienne pendant la coagulation et leur élimination selon les genres présents et (3) observer la réponse à court terme des cyanobactéries à l'ajout de nutriments.

La première partie de cette étude consistait à étudier l'élimination des cellules cyanobactériennes et des cyanotoxines avec un coagulant à base de sulfate ferrique ($Fe_2(SO_4)_3$) dans une eau de lac

présentant un bloom cyanobactérien. Avec une dose de 20 mgFe/L et 35 mgFe/L, plus de 51% et 96% des cellules totales, mesurées par le nombre taxonomique de cellules, ont été éliminées, respectivement. Des différences significatives ont été observées en ce qui concerne l'élimination des cellules cyanobactériennes totales et des espèces de cyanobactéries dominantes comme *Microcystis* et *Dolichospermum* entre les deux doses appliquées. Plus de 97% des toxines microcystines intracellulaires totales ont été éliminées pour les deux doses appliquées. Une élimination significative des toxines extracellulaires n'a pas été observée après la coagulation avec les deux doses.

Nous avons évalué la diversité des cyanobactéries et les changements de structure de la communauté cyanobactérienne après la coagulation par séquençage métagénomique shotgun. Les résultats ont montré que *Dolichospermum* et *Microcystis* étaient les genres prédominants au début des périodes d'échantillonnage dans la baie Missisquoi et le Petit lac Saint-François. La diversité et la richesse de la communauté cyanobactérienne n'ont pas changé de façon significative après la coagulation utilisant du $Fe_2(SO_4)3$ dans tous les mésocosmes de la baie Missisquoi et du Petit lac Saint-François. Le changement dans la composition des cyanobactéries a varié dans les deux sites après deux jours d'exposition. La présence de *Microcystis* était liée aux concentrations intracellulaires de microcystine et à l'azote dissous aux deux sites. *Synechococcus* était influencé par les paramètres d'azote total, d'azote dissous, de carbone organique dissous et de phosphore dissous.

La réponse des cyanobactéries et des cyanotoxines à l'ajout de nutriments a été étudiée. Après deux jours d'exposition (T48) suivant l'ajout d'azote, le nombre total de cellules taxonomiques, le nombre de cellules de *Dolichospermum* et d'*Aphanizomenon* ont diminué de façon significative dans les mésocosmes contenant du NH_4^+ par rapport aux mésocosmes témoins, tandis que le nombre total de cellules taxonomiques et le nombre de cellules du genre dans les mésocosmes contenant du NO_3^- n'ont pas changé de façon significative par rapport aux mésocosmes témoins. Un passage à *Microcystis* dans les mésocosmes avec ajout d'azote a été observé deux jours après l'ajout dans la baie Missisquoi. Il n'y a pas eu de changements significatifs dans les toxines intracellulaires des microcystines (MCs) totales entre les mésocosmes témoins et les mésocosmes avec l'ajout de NH_4^+ ou NO_3^- après deux jours d'exposition. Les MCs et MC-LR extracellulaires ont augmenté de manière significative dans les mésocosmes avec ajout de NH_4^+ ou NO_3^- après 48 heures par rapport

aux mésocosmes témoins. Les concentrations intra- et extracellulaires de microcystine étaient liées à l'apparition de *Microcystis*. Le NH_4^+ était associé à la présence de *Microcystis* 48 heures après l'ajout de N. Ainsi, la production de toxines à la suite de l'ajout d'azote se produira sur des échelles de temps associées à la croissance de la biomasse cyanobactérienne. Cette information aidera les opérateurs des stations de traitement à mieux anticiper l'arrivée de concentrations potentiellement plus élevées de cyanotoxines.

Bien que des solutions à long terme soient nécessaires pour réduire l'occurrence des cyanobactéries et des toxines dans les sources d'eau, cette recherche fournit de nouvelles données pour des options de traitement supplémentaires dans les sources d'eau pour l'élimination des cyanobactéries et de leurs toxines dans les lacs et les réservoirs.

ABSTRACT

The excessive proliferation of toxic cyanobacterial blooms is a common problem around the world and is a concern for human health. Processes in drinking water treatment plants may be impaired by the presence of high concentrations of cyanobacterial cells in source waters. Can coagulants be used to rapidly eliminate cyanobacterial cells and their intracellular toxins in a drinking water source before they enter treatment plants? Little is known about how coagulant affect on cyanbacterial cells and toxins as well as cyanobacterial community in the short term in source waters. To address this question, *in situ* coagulation was investigated in lakes with blooms using mesocosm experiments.

Beyond eliminating cyanobacteria, the management of water bodies for controlling cyanobacterial blooms is important. Nutrient enrichment is related to cyanobacteria biomass and toxin production. Long term solutions for managing nutrients is important; however, there is need to investigate proposed short term nutrient addition that can occur during storm events and their potential impact on cyanobacterial growth and toxin production to improve our understanding of the drivers of the arrival of toxic blooms in drinking water sources.

The main objective of this research was to study the effects of nutrients and coagulants on cyanobacteria and their toxins in source waters through metagenomics. Two sites with recurrent cyanobacterial blooms (Missisquoi Bay and Petit Lac Saint François) were selected for the experiments. The specific objectives were as follows: (1) determine the effectiveness of the addition of coagulants to source waters for the removal of cyanobacterial cells and cyanotoxins, (2) determine the cyanobacterial community composition during coagulation and varying removal according to the genera present and (3) observe the short-term cyanobacterial response to nutrient addition.

The first part of this study was to study the removal of cyanobacterial cells and cyanotoxins with a ferric sulfate ($Fe_2(SO_4)_3$) coagulant in lake water with a cyanobacterial bloom. With a dose of 20 mgFe/L and 35 mgFe/L, greater than 51% and 96% of total cells as measured by taxonomic cell counts were eliminated, respectively. Significant differences were observed with regards to the removal of total cyanobacterial cells and dominant cyanobacteria species as *Microcystis* and *Dolichospermum* between two applied doses. More than 97% of total intracellular microcystin

toxins were removed for both applied dosages. Significant removal of extracellular toxins was not observed after coagulation with both doses.

We assessed the cyanobacterial diversity and cyanobacterial community structure changes following coagulation by shotgun metagenomic sequencing. Results showed that *Dolichospermum* and *Microcystis* were the predominant genera at the beginning of the sampling periods in Missisquoi Bay and Petit Lac St. François, respectively. The diversity and richness of cyanobacterial community did not change significantly after coagulation of $Fe_2(SO_4)_3$ in all of the mesocosms in Missisquoi Bay and Petit Lac St. François. The change in the cyanobacterial composition varied across the two sites after two days of exposure. The presence of *Microcystis* was related to intracellular microcystin concentrations and dissolved nitrogen at both sites. *Synechococcus* was influenced by total nitrogen, dissolved nitrogen, dissolved organic carbon and dissolved phosphorus parameters.

Cyanobacteria and cyanotoxin response to nutrient addition was studied. After two days of exposure (T48) following nitrogen addition, the total taxonomic cell counts, *Dolichospermum* and *Aphanizomenon*'s cell counts significantly decreased in the mesocosms containing NH_4^+ as compared to the control mesocosms while total taxonomic cell counts, and genus's cell counts in NO_3^- mesocosms did not significantly change compared to control mesocosms. A shift to *Microcystis* in the mesocosms with nitrogen addition was observed two days after the addition in Missisquoi Bay where nitrogen was more limited as compared to Petit Lac Saint François based on nutrient ratios. There were no significant changes in intracellular toxins of total microcystins (ΣMCs) between control mesocosms and mesocosms with the addition of NH_4^+ or NO_3^- after two days of exposure. However, extracellular ΣMCs and MC-LR increased significantly in mesocosms with NH_4^+ or NO_3^- addition after 48 hours as compared to the control mesocosms. Intra- and extracellular microcystin concentrations were related to the appearance of *Microcystis*. NH_4^+ was associated with *Microcystis* presence 48 hours after the addition of N. Thus, toxin production following sudden nitrogen addition can occur on short time scales relevant to drinking water treatment plant operation. Examples of sudden inputs nitrogen include agricultural runoff during storm events. This information will help treatment plant operators better anticipate the arrival of potentially higher concentrations of cyanotoxins.

Although long term solutions are needed for reducing the occurrence of cyanobacteria and toxins in sources waters, this research provides new data for additional treatment options in source waters for the removal of cyanobacteria and their toxins in lakes and reservoirs.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANA	Anatoxin
ANA-a	Anatoxin a
ANOVA	Analysis of variance
AP-A	Anabaenopeptin-a
AP-B	Anabaenopeptin-b
ATRAPP	Algal Blooms, Treatment, Risk Assessment, Prediction and Prevention through Genomics Project
CYN	Cylindrospermopsin
CyanoHABs	Cyanobacterial harmful algal blooms
DN	Dissolved nitrogen
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DP	Dissolved phosphorus
D. spiroides	*Dolichospermum spiroides*
DWTS	Drinking water treatment sludge
$FeCl_3$	Ferric chloride
$Fe_2(SO_4)_3$	Ferric sulfate
HPLC	High performance liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
M. aeruginosa	*Microcystis aeruginosa*
M. flos-aquae	*Microcystis flos-aquae*
MB	Missisquoi Bay

MC-HilR	Microcystin-HilR
MC-HtyR	Microcystin-HtyR
MC-LA	Microcystin-LA
MC-LR	Microcystin-LR
MC-LW	Microcystin-LW
MC-LY	Microcystin-LY
MC-RR	Microcystin-RR
MC-total	Microcystin Total
MC-WR	Microcystin-WR
MC-YR	Microcystin-YR
N	Nitrogen
NH_4^+	Ammonia
NO_3^-	Nitrate
NO_2^-	Nitrite
P	Phosphorus
PAC	Polyaluminium chloride
PAX-18	Polyaluminium hydrochloride
PCA	Principal Component Analysis
PP	Particulate phosphorus
PLSF	Petit Lac Saint-François
RDA	Redundancy analysis
RNA	Ribonucleic acid
SEM	Scanning electron microscopy

SRP	Soluble reactive phosphorus
TOC	Total organic carbon
UHPLC-MS/MS	Ultra-high performance liquid chromatography coupled to tandem mass spectrometry
US	United States
WHO	World Health Organization

LIST OF APPENDICES

CHAPTER 1 INTRODUCTION

Harmful cyanobacterial blooms (HCBs) appear worldwide, and seasonally affect many Canadian surface waters. HCBs are a potential threat to drinking water, animal, and human health [1, 2]. The cost of managing HCBs can reach millions of dollars in countries such as the USA and Canada [3]. Driven by the need of municipalities and water quality authorities for HCBs treatment in Quebec, Canada, the Algal Blooms, Treatment, Risk Assessment, Prediction and Prevention through Genomics (ATRAPP) project was launched. Its first objectives were to assess the risk of toxicity in water sources and guide prevention and treatment strategies through a chemical-genomic diagnostic toolkit. Next, the project aimed to identify best treatment practices to prevent toxin production at the source, in drinking water treatment plants and in toxic sludge. Finally, a cost-benefit analysis was conducted of short-term solutions and a cost-sharing models for long-term solutions for improved water quality [4].

This study is one component of the ATRAPP project studying *in situ* treatment solutions via mesocosm experiments. Blooms in lakes were sampled and mesocosm experiments were conducted to explore environmental conditions (pH, temperature, nutrients) that could favour blooms and toxicity as well as *in situ* treatment options. Elsewhere, the addition of coagulants has been shown to be effective for managing HCBs by decreasing the availability of phosphorus (P) in the water column [5] but these treatment options have not been fully explored at short time scales.

Cyanobacteria, among the ancient organisms on the Earth, have a high ability to survive and proliferate in lakes and reservoirs all over the world [6, 7]. Nutrient loads and climate change contribute to the development and expansion of cyanobacterial blooms in a variety of aquatic habitats, from freshwater to marine ecosystems [8-11]. Harmful cyanobacteria blooms can produce toxins and kill animals in or near water [12]. There are four main groups of cyanotoxins: hepatotoxins, cytotoxins, neurotoxins and dermatotoxins. These toxins have been associated with human illnesses ranging from contact dermatitis to cancer [1, 13].

P is traditionally considered as primary nutrient that limits organism growth in freshwater ecosystems [14, 15]. An increase in P loading in water bodies can indeed cause the dominance of cyanobacteria in the phytoplankton community, especially for diazotrophic cyanobacteria such as *Dolichospermum* and *Aphanizomenon* that can live in water systems that are depleted in nitrogenous nutrients [14, 16, 17]. An increase of nitrogen (N) concentrations in eutrophic water

bodies may stimulate the growth of cyanobacteria and intensify the proliferation of algal blooms in surface waters [10, 18, 19]. In lakes and reservoirs around the world, total nitrogen (TN) was closely related to the formation of cyanobacteria blooms [20-23]. A monthly monitoring of mesocosm experiment was carried out to see if dissolved N enhances total algal abundance and hazardous cyanobacteria in hypereutrophic lakes [24, 25]. They found that nitrate (NO_3^-) promoted the growth of siliceous algae, while ammonium (NH_4^+) increased the growth of non-N_2-fixing cyanobacteria. These findings are consistent with studies that suggest that NH_4^+ may support long-term increases in cyanobacteria abundance in lakes [26-28]. Moreover, numerous studies indicated that NO_3^-, and NH_4^+ may promote the toxicity of cyanobacterial blooms, particularly microcystins toxin [14, 23, 27, 29-31].

Understanding bacterial community composition associated with cyanobacterial blooms in water under N addition is important. Bacteria play an important role in supporting cyanobacterial growth such as supplying vitamins, CO_2, regenerating nutrients, and removing toxic metals [7, 32, 33]. Therefore, high throughput sequencing has recently been applied to assess bacterial community composition. High throughput sequencing not only provides data on microbial communities but also identifies cyanotoxin biosynthesis genes [34-36]. In recent years, bacterial community composition under N addition in water was assessed by high throughput sequencing [32, 37, 38]. Several studies used high throughput sequencing of 16S rRNA gene amplicons to analyze bacterial community composition before, during and after nutrient (N and P) addition to aquatic microcosms over 20 days and found that bacterial community composition significant changed during the study period [39, 40]. Conversely, a lab scale study over a short-term (48 hours) showed the diversity of the bacterial community did not change significantly with the addition of N [37]. However, bacterial community structure associated with cyanobacterial blooms after N addition in short-term has not been studied in mesocosms *in situ*. To the best our knowledge, such results are currently not available and are needed to assess cyanobacterial and toxin production response times to events that bring large quantities of nutrients to water supplies.

Coagulation is a crucial tool for removing cyanobacterial cells and their intracellular toxins in drinking water treatment facilities [13]. In spite of the fact that traditional treatment facilities are imortant in supplying fresh drinking water for communities the world over, lakes or reservoirs remain important source waters for these plants [41]. Therefore, coagulant addition has also been

considered for in lake treament [42-46]. Coagulants such as ferric sulfate or alum chloride may reduce cyanobacterial cells and decrease the P concentration in water column [47, 48]. Iron-based coagulants are potentially safer than aluminium-based coagulants for treating in lakes and reservoirs [49].

Orihel *et al.* 2016 [5] conducted a mesocosm experiment to manage $FeCl_3$ in a hypereutrophic to suppress cyanobacteria blooms and their toxins over the long-term. Their results showed that Fe^{3+} addition reduced P accumulation in porewaters and the flux of P from sediments and reduced the dominance of cyanobacteria blooms and cyanotoxins. However, coagulant addition showed that extracellular toxins can be released into water during and after coagulation process at a lab scale [50-53]. Moreover, iron addition may enhance the abundance of cyanobacteria in eutrophic systems [54]. In recent year, several studies used high throughput sequencing to understand the change of bacterial community composition of cyanobacterial after coagulation and found that the bacterial community composition in raw water may influence the bacterial community composition in sludge [55-57]. It is unknown the fate of cyanobacterial cells and cyanotoxins as well as bacterial community composition after coagulation process in *in situ* scale in short-term, more specially, in blooms lakes, based on mesocosm experiments.

This thesis is divided into 8 Chapters and 1 Appendix. Chapter 1 is the **Introduction,** presenting general information about cyanobacteria, nutrients and coagulants. Chapter 2 presents a **Literature review** on cyanobacterial blooms, cyanotoxins, coagulation in lakes, nutrient addition and high throughput sequencing approaches. Chapter 3 consists of **Research objectives** and **Hypotheses**. Chapter 4 is the **Materials and Methods**, presenting the mesocosm experiment. Chapter 5 and 6 presents results as scientific articles in the Toxins Journals. Chapter 7 consists data to be published as an article titled **The effect of nitrogen on cyanobacteria and cyanotoxins**. Chapter 8 presents a **General Discussion** and **Conclusion**. The Appendix presents supplementary information.

CHAPTER 2 LITERATURE REVIEW

2.1 Cyanobacteria

Cyanobacteria are among the first species on the planet and they have played a key role in the release of oxygen into the atmosphere [6]. Cyanobacteria are photosynthetic prokaryotes and have the ability to synthesis chlorophyll *a* [58]. Most cyanobacteria also have the ability to synthesize the phycobilin and phycocyanin pigments, which give the organism's cells a green-blue colour when present in high concentrations, from which their popular name is derived - blue-green algae [58]. Cyanobacteria have the benefit of being the Earth's ancient microorganisms, having allowed a long time to evolve diversity and extremely effective eco-physiological adaptations thereby ensuring their survival and dominance in aquatic settings under both natural and human-caused environmental change. They now exist in fresh water, marine, terrestrial, and symbiotic habitats around the world, ranging from polar to tropical locations in the northern and southern hemispheres [8, 59-61].

Cyanobacteria are classified into three main orders: *Chroococcales*, which is comprised of unicellular or colony-forming cells, have the smallest cell sizes (~1 to 5 μm) and thus have a competitive advantages in low nutrient conditions; *Nostocales*, which contain filamentous forms with large sizes (>100 μm), have a ability to use atmospheric N and thus are favored in high P and low N concentrations; *Oscillatoriales* is a family of filamentous plants that lack heterocysts [62; references therein]. Most cyanobacteria are aerobic photoautotrophs, and photosynthesis is their primary source for energy metabolism. Water, carbon dioxide, inorganic compounds and light are required for cyanobacterial survival [13]. Cyanobacteria can live in a variety of environments by using anaerobic photosynthesis [63]. Moreover, several cyanobacteria genera can directly convert atmospheric N into NH_4^+ by using the nitrogenase enzyme and these N_2-fixing cyanobacteria are common among the filamentous genera (e.g. *Dolichospermum*) [64].

2.1.1 Cyanobacterial harmful algal blooms

Cyanobacterial harmful algal blooms have been widely known as cyanobacterial communities with high population densities in waterbodies under favourable environmental conditions. They may

form subsequent surface scums resembling clotted mats or paint-like slicks especially in eutrophic waters [8]. These cyanobacteria with high population abundances do not last long and die out rapidly after one or two weeks. However, they can quickly replace the previous blooms if conditions remain favorable. Thus, the blooms may last for up to several months [12, 65]. High density blooms can cause taste-odor problems and economic loss [8, 66]. Cyanobacterial blooms are frequently associated with eutrophication [9, 67, 68].

Cyanobacteria blooms can be nontoxic or have species that produce toxins or harmful metabolites [69]. Furthermore, cyanobacterial toxins may have a long-term effect on human heath as tumour promoters, they may cause liver damage, gastrointestinal problems, difficulty breathing or even death (in some rare cases) [66]. Several cyanobacterial species however, do not produce toxins but, can cause habitat loss and changes to food web interactions. Both forms types of blooms can cause hypoxia/anoxia and fish kills due to the decrease in oxygen during bloom die-off [8]. In addition, it showed that increase in NH_4^+ corresponded to the collapse of the cyanobacterial blooms [23, 70], and high level of NH_4^+ are harmful to the growth performance of fish [71].

Due to a variety of illnesses connected to recreational cyanobacteria exposure [72-74], the World Health Organization (WHO) guidelines propose three approaches [75]. Such as:

1) Waters containing 20,000 cyanobacterial cells/mL or 10 μg chlorophyll *a*/L (if cyanobacteria are dominant) have a low probability risk of harmful health impacts;
2) There is a moderate probability of risk of negative consequences from waters containing 100,000 cyanobacterial cells per mL or 50 μg chlorophyll *a*/L;
3) High probability of adverse effects from contact with and/or ingestion/aspiration of cyanobacteria at scum-forming densitites (>100,000 cells).

Numerous environmental elements have an impact on cyanobacterial blooms. These include light intensity, high nutrient concentration, water temperature, pH, carbon dioxide, physical characteristics of the water body (shape and depth) and climate change [59, 76-78]. N and P loads of anthropogenic inputs in water bodies [79, 80] play an important role in leading proliferation of harmful algal blooms [9]. For example, the Missisquoi Bay (Quebec, Canada) is prone to cyanobacterial blooms each summer as a result of crop fertilisation and spreading of animal manure

since a third of its watershed is drained by agricultural regions [81-83]. Moreover, the interannual intensity of cyanobacterial blooms in Lake Erie (Canada and USA) has been successfully predicted using external sources of P [84-86].

2.1.2 Cyanotoxins

Cyanotoxins are the products during the metabolite synthesis process. They may be within the cell (intracellular) or dissolved (extracellular) in the water column [87]. The key cyanobacterial genera known for their potential ability to produce a wide range of different toxic compounds include *Microcystis*, *Dolichospermum*, *Aphanizomenon*, *Cylindrospermopsis*, *Nodularia*, *Lyngbya*, *Nostoc* and *Planktothrix* [30, 88]. Cyanotoxins are classified into four main groups: hepatotoxins, cytotoxins, neurotoxins and dermatotoxins (Table 2.1).

Table 2.1 Classification of cyanotoxins. Adapted from [13, 66, 89]

Group	**Toxin**	**Cyanobacteria**
Hepatotoxins	Microcystins	*Microcystis*, *Dolichospermum*, *Nostoc*, *Planktothrix*, *Aphanizomenon*, *Merismopedia*, *Oscillatoria*, *Snowella*, *Gloeotrichia*
	Nodularins	*Nodularin*, *Nostoc*
Cytotoxins	Cylindrospermopsins	*Cylindrospermopsis*, *Dolichospermum*, *Aphanizomenon*, *Oscillatoria*, *Umezakia*
Neurotoxins	Anatoxin-a	*Dolichospermum*, *Microcystis*, *Oscillatoria*, *Aphanizomenon*, *Cylindrospermopsis*
	Anatoxin-a(S)	*Dolichospermum flos-aquae*, *Dolichospermum spiroides*
	Saxitoxins	*Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Planktothrix*
Dermatotoxins	Lyngbyatoxin	*Lyngbya*, *Schizotrix*, *Oscillatoria*

Hepatotoxins are toxins that can damage the liver. The hepatotoxins produced by cyanobacteria consist of microcystins, nodularins and cylindrospermopsins. Neurotoxins contain anatoxin-a, anatoxin-a(S) and saxitoxins have caused nerve synapse in mammals or animal poisonings [13].

Lyngbyatoxins cause severe dermatitis, as well as oral and gastrointestinal inflammation [13]. Moreover, they are one of the many potential causes of impairment of water quality in aquatic systems and may have impacts on biological communities [90].

In the past several decades, cyanobacterial blooms and cyanotoxins have been increasing, and microcystins are among the most frequently detected toxins [7, 91]. Microcystins-LR (MC-LR) is considered as one of the most widespread and toxic variants of the microcystins [7, 92]. The WHO has set a limit of 1 μg/L for MC-LR in drinking water as an acceptable concentration (cell-bound and extracellular), and it is recommended that bottle-fed infants and small children be provided with an alternative water source if concentration are greater than 3 μg/L [13]. According to *Guidelines for Canadian Drinking Water Quality*, a seasonal maximum acceptable concentration is 1.5 μg/L for total microcystins in drinking water, including young children [93]. It is important to note that the maximum recommended concentration in Quebec is 1.5 μg/L for MC-LR [94].

2.2 Nutrients

Nutrient input loading (N and P) is one of many environmental factors influencing the development of cyanobacterial blooms and cyanotoxins [76, 78]. Before the industrial era, concentrations and loads of N were frequently low and mainly originated from erosion and the leakage of their organic/inorganic forms [79, 95, 96]. Nowadays, it is predicted that a global average of N fertilizer surpluses will increase by 25% until 2050, and by up to 75% in Latin America [18, 19]. Consequently, there has been an increase of N levels in water bodies that has led to the widespread eutrophication of both inland and coastal waters [79, 80, 97]. In a survey of 2012, 63.1% of the 2058 global water bodies were found to be eutrophic in central Africa, estern Asia, and North America [20, 98, 99]. Eutrophication can cause of a change in lake, river, stream and/or coastal environments, with a shift from one dominated by benthic microalgal to a phytoplankton-dominated community [9, 100, 101].

2.2.1 Phosphorus (P)

P presents in aquatic environment in many forms including organic, water soluble inorganic, and insoluble inorganic forms of phosphorus. The water soluble inorganic form (PO_4^{3-}) is the most important of these different forms because it can be directly consumed by phytoplankton [102]. P

also exists in different forms in lake sediment such as metal complex salts and organic species [103, 104]. Concentrations of inorganic P in lake sediments are higher than in the water column and vary seasonally. Oxygen is a primary factor effecting the release/sorption P between sediments and overlaying water [23, 105]. Under aerobic conditions, PO_4^{3-} ion can bind with Fe(III) oxide in sediments, but during summer months as increasing of temperature and microbial degradation, oxygen level in sediments are gradually depleted. Therefore, PO_4^{3-} release from Fe(III) oxides as these compounds are reduced by anoxic conditions [23, 105].

Several cyanobacterial genera can grow and dominate in P limited condition because they have ecophysiological abilities to access P stored in lake sediments, even in aerobic conditions including high-affinity phosphate uptake system, the capacity for luxury uptake (luxury uptake is the storage P within the biomass in the form of polyphosphate), the production of polyphosphate enzymes to access sediment-bound P, storage capacity P and the control buoyancy in the water column [106-109]. For instance, *Microcystis* can grow in low-water column P conditions because it can regulate buoyancy using gas vesicles move down though the water column to take benthic P sources [23, 110].

Harke *et al.* 2016 [27] conducted a experiment of cyanobacterial populations under nutrient-controlled niche differentiation in western basin of Lake Erie (from the mouth of the Maumee River to the Bass Islands). They observed that *Dolichospermum* and *Planktothrix* are the dominant cyanobacterial populations under high-P conditions at the location that is closest to the mouth of the Maumee River. In contrast, within offshore low-P regions of the western of Lake Erie, *Microcystis* up-regulated genes associated with P scavenging and became dominant among the cyanobacterial population (*Microcystis*, *Dolichospermum* and *Planktothrix*). In conclusion, P enrichment can enhance growth of N_2-fixing cyanobacteria (*Dolichospermum* spp.) while lower P concentrations led to a greater dominance of non-diazotrophic cyanobacteria as *Microcystis*. Similar results have aslo been observed in the Baltic Sea and Lake Taihu, China [23, 62, 111]. *Microcystis* have a ability to grow and develop well under low P concentration conditions because of its ecophysiological traits [106].

2.2.2 Nitrogen (N)

N has played a critical role in bloom promotion [84, 112, 113]. Huang *et al.* 2020 [20] monitored 142 lakes and reservoirs in China during 2005 and 2018, and found that the lakes with the most severe harmful algal blooms were those with the highest total nitrogen (TN) concentrations. Likewise, a study of over 1000 lakes across the entire continental United States demonstrated that TN and temperature provide the best multiple linear regression model for predicting cyanobacterial biomass [21]. In addition, a recent study indicated that N is an essential element for *Microcystis* dominance of extensive algal blooms in the Bay of Quinte, Ontario, Canada [22].

Effects of N on cyanobacterial abundance and their toxicity in eutrophic water may depend on different chemical forms of N [18, 31, 114, 115]. Three forms of dissolved N occurs in the aquatic environment are ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) [116]. NH_4^+ and NO_3^- account for the majority of inorganic N sources in freshwater. More details on each form are described below.

2.2.2.1 Nitrate (NO_3^-)

NO_3^- accounts for the majority of N in oxidized form promoting eutrophication in many aquatic ecosystems [117]. Before entering the cyanobacterial cell, NO_3^- must be metabolised into NH_4^+ and thus, NO_3^- assimilation is more energy consuming than NH_4^+ by cyanobacteria with regards to their N requirement [28, 118, 119].

A field study to examine addition of NO_3^- (700 μg/L) on phytoplankton communities (diatoms, chlorophytes, and cyanobacteria) in a hypereutrophic reservoir in a weekly experiment from May to October showed that NO_3^- stimulates the growth of large diatoms under N-limitation [114]. Similarly, Donald *et al.* 2013 [24] assessed effect of NO_3^- (6 mg/L) on phytoplankton abundance and toxicity in a hypereutrophic lake from July to September though a mesocosm experiment. Results indicated that blooms of siliceous diatoms were promoted by NO_3^- while N_2-fixing cyanobacteria expressed little response to NO_3^- addition. In addition, previous study showed that non-N_2-fixing cyanobacteria as *M. aeruginosa* had the lowest growth rates with the addition of NO_3^- compared to NH_4^+ and urea in the lake water in lab scale [120].

2.2.2.2 Ammonium (NH_4^+)

NH_4^+ is one of the most commonly available N sources in freshwater [31, 121, 122]. NH_4^+ has long been thought to be the preferred form of N for phytoplankton uptake and cellular development [115, 123-126]. The assimilation of NH_4^+ for the cell is thought to be less energy expensive, and NH_4^+ is easier to transfer into the cell membrane than NO_3^- [127, 128].

Mesocosm studies present many examples of NH_4^+ effects on cyanobacteria abundance and toxicity in eutrophic freshwaters. For instance, several studies examined addition of NH_4^+ (6 mg/L) to naturally P-rich lake water though mesocosm experiments for 21 days in summer, and they observed that NH_4^+ selectively promotes the growth of non-N_2-fixing cyanobacteria such as *Microcystis*, *Planktothrix* and significantly increases microcystin toxins [24, 25]. It probably caused *Microcystis* to be the dominant in lake during summer [112, 117, 129] and *Microcystis* had also been demonstrated to have a strong affinity for NH_4^+ [23, 117, 130]. Similarly, Harke *et al.* 2016 [27] conducted *in situ* experimental addition of NH_4^+ (0.5 μM) on cyanobacterial populations in western Lake Erie (USA) and found that total cyanobacterial biomass and intracellular microcystins toxin significantly increased in response to NH_4^+. In addition, NH_4^+ may favor the increase of *Microcystis* blooms in Lake Erie (USA) during summer [26].

Over the course of 16 years, Swarbrick *et al.* 2019 [115] studied the effects of NH_4^+ on phytoplankton growth in a eutrophic lake. They discovered that the overall effect of NH_4^+ on natural phytoplankton communities was dependent on the community composition in the receiving aquatic ecosystems as well as the physico-chemical states at the time of NH_4^+. Results showed a suppression of phytoplankton by NH_4^+ when diatoms and cryptophytesare were abundant and water temperature and soluble reactive phosphorus concentrations (SRP) were low.

In contrast to field-based studies, laboratory studies desmonstrated that high concentrations of NH_4^+ (7 mg/L) suppressed the growth of *M. aeruginosa* and increased extracellular MCs levels in water [31]. Li *et al.* 2016 [131] also showed that *M. aeruginosa* growth in NH_4^+ cultures was inhibited when N concentrations were higher than 1.2 mgN/L. These finding are also consistent with the inhibition of cyanobacterial biomass (*Microcystis*) by high concetrations of NH_4^+ (7 mmolN/L) [119].

Table 2.2 Impact of N on cyanobacteria and cyanotoxins by mesocosm study

	Study site	Conditions	Period	Conclusions	Ref.
Mesocosm *in situ*	Nakdong River (Korea)	• Nutrient enrichment • Mean TN = 2.62 mg/L • Mean TP = 0.046 mg/L • NO_3^- added = 3; 6; 9; 12; 15 mg/L • PO_4^- added = 0.01; 0.02; 0.05; 0.1; 0.2 mg/L	10 days	• N stimulated cyanobacteria mostly *Microcystis aeruginosa* more than P • P addition did not stimulate cyanobacteria	Kim *et al.* 2020 [132]
Mesocosm *in situ*	Willow Creek Reservoir (Oregon, USA)	• Eutrophic • Mean TP = 30 µg/L • N added to achieve TN:TP > 75	3 months	• At TN:TP > 75, chlorophytes dominated the phytoplankton community while cyanobacterial biovolumes was significantly reduced and microcystisn was not detected	Harris *et al.* 2014 [133]
Mesocosm *in situ*	Wascana Lake (Saskatchewan, Canada)	• Hypereutrophic • Mean TP in lake = 175 µg P/L • NH_4^+ added = 6 mg N/L • NO_3^- added = 6 mg N/L	3 weeks	• Addition of NH_4^+ and urea favoured non-heterocystoud cyanobacteria and chlorophytes • NO_3^- promoted chlorophytes and some cyanobacteria • N_2-fixing cyanobacteria exhibited a little response to added N. • Added N also increased microcystin production by up 13 times	Donald *et al.* 2013 [25]

Table 2.2 Impact of N on cyanobacteria and cyanotoxins by mesocosm study (cont'd)

Lab scale	Lake Shenandoah (Virginia, USA)	• N added = 10 µM or 50 µM as NO_3^-, NH_4^+ and urea	48 hours	• At the phylum level, the dominant taxa were comprised of *Actinobacteria* and *Proteobacteria* • There was not a significant shift in the diversity of bacterial community in Lake Shenandoah with addition N sources	Reynoso *et al.* 2019 [37]
Lab scale		• *Dolichospermum flos aquae* (CPCC 67) • *Microcystis aeruginosa* (CPCC 300) • *Synechococcus* sp. • NO_3^-, NH_4^+, urea: 1; 3; 5; 7 mmol N/L	10 days	• Positive correlation between three species growth and highest concentration of NO_3^- • *D. flos-aquae* accumulated the lowest levels of NH_4^+ in the medium, whereas *M. aeruginosa* showed complete inhibition and the highest NH_4^+ • With highes concentration of urea (7 mmol N/L). *M. aeruginosa* displayed complete inhibition, *Synechococcus* sp. exhibited signs of impairment and *D. flos-aquae* appeared to be unaffacted	Errat *et al.* 2018 [119]

2.2.2.3 High throughput sequencing

In recent years, high throughput sequencing approaches have been used to evaluate the impact of N on the microbial community structure in water [32, 37, 38]. Microbiome diversity can be divided into two kind of measures, alpha and beta diversity. Alpha diversity measures the variability of species within a sample while beta diversity accounts for the differences in composition between samples [134].

Ren *et al.* 2013 [39] used high throughput sequencing of 16S rRNA gene amplicons to study the change of bacterial community composition following nutrient addition (N and P) to aquatic microcosms. The results showed that Actinobacteria, Polynucleobacter were the predominant phyla and the bacterial community composition significantly changed and decreased in size under different nutrient enriched conditions after 20 days. A recent study applied high throughput MiSeq sequencing to analyze the bacterioplankton community in outdoor and flow-through long-term mesocosms under N amendment and warming conditions [40]. Results showed that a combination of N enrichment and warming increased bacterioplankton beta diversity. It also found that the bacterial composition structure significantly shifted from Actinobacteria, Bacteroidetes, and Betaproteobacteria to Cyanobacteria under N enriched conditions [40].

A recent study assessed the bacterial community structure of shallow freshwater lakes (Virginia, USA) under N addition (NO_3^-, NH_4^+ and urea) using 16S rRNA amplicon sequencing at a lab scale [37]. Actinobacteria and Proteobacteria were the most predominant phyla and were not impacted by exposure to the various N treatments after 48 hours. Moreover, there was not a significant shift in the diversity of the bacterial community with the addition of N addition.

2.3 Source water treatment

Although water treatment plants are indispensable, various physical, chemical, and biological processes have been developed and used to remove and control algae in natural water systems [135]. These include algaecides (e.g. copper sulfate) [136], sediment capping agents [47], ultrasonication [137], hydrogen peroxide [138], and aeration [139].

2.3.1 Sonication

Ultrasound radiation in water can generate cavitation bubbles within the cyanobacterial cells, which collapse and cause localized temperatures and pressures to reach as high as 5000°C and 500 atmospheres of pressure [135, 140]. The buoyancy of cyanobacteria is destroyed in that extreme environment because it causes inhibition of photosynthesis, destruction of the cell membrane and free radical species production [141, 142].

2.3.2 Algaecides (e.g. copper sulfate)

Algaecides are chemical reagents applied to control slime mold, algae, fish pathogens, and mollusks in ponds canald and bodies of water [143]. The most well known algaecide is copper sulfate. It has been used since 1904 for control of noxious phytoplankton in surface waters [47, 136]. There are several concerns with regards to using algaecides in water, including increasing resistance of the targeted cyanobacteria to algaecides [144]. Some algaecides may also damage the cyanobacterial cell, causing the release of intracellular toxins and taste and odor compound into the water [144, 145].

2.3.3 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is considered as friendly photosensitizers that is applied for inactivation of bacteria, fungi, human cells or organic molecules [47]. It said that H_2O_2 may suppress cyanobacterial photosynthesis by inhibiting photosynthetic electron transfer and it is able to impair DNA, protein, and existing lipids in the cells [146, 147]. It observed that microcystin release in water may be prevented by H_2O_2 as a result of impairing transcription of the resonsible genes [138, 148].

2.3.4 Aeration

Water quality in lakes and reservoirs may be improved by applying aeration or an artificial mixing technique [139, 149]. Aeration mainly controls cyanobacterial blooms by reduce internal phosphorus release from sediments and by entraining the buoyant cyanobacteria in the deeper parts of the water body [139, 150].

Table 2.3 Several source water treatments studies

Treatment	Study site	Conditions	Period	Conclusions	Ref.
Sonication	Canoe Brook Reservoir (New Jersey, USA)	• Field scale • Reservoir: 2.9 million m^3 • Cyanobacteria (*Aphanizomenon*)	From spring to summer 5 months	• More than 90% reduction of algae based on cell concentration after more than a week sonication	Scheider *et al.* 2015 [141]
	Gyeryong-si Lake (Chungnam, Korean)	• Field scale • Pond: 9000 m^3 • Eutrophic • Cyanobacterial blooms	1.5 months	• Efficiently suppressed the cyanobacterial growth and Chl *a* were low at 10 ug/L compared to control concentration (20 – 87 µg/L) after 1.5 months	Anh *et al.* 2007 [151]
Aeration	Fish pond (Sichuan, China)	• Field scale • Pond: 6300 m^3 • Eutrophic	Two years	• Occurence of blooms was eliminated in the treated pond in second year • The relative abundance of Cyanobacteria in the treated pond were less than the control pond	Wang *et al.* 2021 [150]
	Shibianyu Reservoir (Shaanxi, China)	• Field scale • Reservoirs: 28.1 million m^3 • Eutrophic	30 days	• Increased DO in the bottom layers • Inhibit the release of internal nutrients • Low algal concentration around aerators	Ma *et al.* 2015 [152]

Table 2.3 Several source water treatments studies (cont'd)

Algaecides	Lake Rockwell (Ohio, USA)	• Lab scale • Cells concentration: 40.000 cells/mL • Copper sulfate • Dose: 0.125 mgCu/L, 0.25 mgCu/L, 0.5 mgCu/L	14 days	• Cyanobacteria population decreased highly with apply copper sulfate • Limited release of microcystin was observed for lowest dose	Crafton *et al.* 2021 [153]
	Lake Okeechobee (Florida, USA)	• Lab scale • Copper-based algaecides • Cells concentration: 6.10^6 cells/mL	9 days	• Total microcystins declined from 494 to < 10 ug/L within 9 days	Kinley-Baird *et al.* 2021 [154]
Hydrogen peroxide	Missisquoi Bay (Quebec, Canada)	• Mesocosm • H_2O_2: 10 and 20 mg/L	2 days	• H_2O_2 reduced total MCs concentration after 48 hours • Dominant species were more impacted by H_2O_2.	Vatani [138]
	Lake Koetshuis (Groninge, Netherlands)	• Field scale • Lake: 0.12 km^2 • H_2O_2: 2 mg/L	Single inject	• The cyanobacteria and microcystin collapsed by 99% within a few days. • Cyanobacterial abundance remained low for 7 weeks.	Matthijs *et al.* 2012 [155]

2.4 Coagulants

Numerous methods for removing cyanobacteria and cyanotoxins in water have been applied such as oxidation, adsorption, membrane filtration, etc. [34, 156, 157]. In drinking treatment plants, coagulation is one of most crucial steps for eliminating both cyanobacterial cells and intracellular cyanotoxins [52, 158-160]. Coagulation for cyanobacteria removal is impacted by numerous factors including size, morphology, charge, cyanobacterial concentration, algal organic matter [161-164]. Besides, the application of coagulant for treating lakes has been in practice for decades [43, 165, 166]. Different forms of coagulants have been used inluding polyaluminium chloride, aluminium sulfate ($Al_2(SO_4)_3$), ferric chloride ($FeCl_3$), and ferric sulfate ($Fe_2(SO_4)_3$). For treating lake water, iron-based coagulants have been selected as a more favorable coagulant than aluminium because it is considered to be a natural and safer binder of phosphate [49].

2.4.1 Ferric-based coagulants (Fe^{3+})

Fe^{3+} coagulants can eliminate cyanobacteria blooms in three ways: (1) reduce cyanobacteria cell abundance from the water column; (2) remove P from the water column; (3) prevent the release of P from the sediments [47, 167]. Compounds containing Fe^{3+} are more efficient in cyanobacterial coagulation than those containing Fe^{2+}·[47, 168].

Fe^{3+} coagulants have been used for the treament of lake and reservoirs worldwide since the 1960s [44, 46, 169-172]. Fe^{3+} coagulants have been used for treating cyanobacterial blooms for many years in other European countries such as Germany, Poland, Austria, England [46, 173-176]. In Canada, $FeCl_3$ coagulant was applied in Black Lake (Bristish Columbia) to control eutrophication and blue-green algae bloom from 1990-1992 [171]. In Québec, the addition of 217 tons $FeCl_3$ salt in Lake Heney in 2007 resulted a significant reduction in the concentration of total phosphorus (TP) and chlorophyll *a* during 2008 [177]. Orihel *et al.* 2016 [5] conducted a mesocosm experiment with a $FeCl_3$ amendent on cyanobacterial blooms in a hypereutrophic lake Nakamun (Canada). After ten months of monitoring, results showed that $FeCl_3$ addition significantly decreased P concentrations in the water column and blooms of phytoplankton significantly declined the following summer. In addition, microcystin from cyanobacterial blooms was reduced by $FeCl_3$ addition [5].

Moreover, Fe^{3+} coagulant has been demonstrated as an effective approach to remove toxin-producing cyanobacteria without causing damage to cells, and thus avoiding the release of intracellular toxin and other organic matter [57, 178]. For example, a dose of 100 mg/L $FeCl_3$ may considerably remove microcystins by 38.5%. The extracellular toxin concentrations grew slightly and then declined in the system with coagulation, while they increased to over 30 µg/L in the system without coagulation at a lab scale [179]. Similarly, Xu *et al.* 2016 [180] found that $FeCl_3$ coagulant did not damage cell integrity of *M. aeruginosa* during coagulation and following 10 days of storage. They concluded the large size and the high desity of $FeCl_3$ flocs most likely protected *M. aeruginosa* cell integrity.

Table 2.4 Cyanobacteria and cyanotoxins during coagulation with coagulant

	Cyanobacteria	Conditions	Coagulant	Conclusions	Ref.
Mesocosm *in situ*	Cyanobacteria communities	TP: 100 μg/L TN: 2.5 mg/L Chlorophyll-a: 50 μg/L	$FeCl_3$	• Fe-based treatment eliminated cyanobacterial blooms in eutrophic lakes and reservoirs. Fe-based treatment highly decreases the ratios of TN:TP, chlorophyll-a and microcystin.	Orihel *et al.* 2016 [5]
Lab scale	*Microcystis aeruginosa*	pH: 8.4 Density: 10^7 cells/mL	$FeCl_3.6H_2O$	• Extracellular of microcystin is released during the coagulation process. • There was no observation of the extracellular augmentation in the sludge.	Xu *et al.* 2016 [180]
Lab scale	*Microcystis aeruginosa*	Turbidity: 1.31 NTU DOC: 4.67 mg/L pH: 8.44 Density: 10^6 cells/mL	$FeCl_3.6H_2O$	• Cell integrity is not affected by $FeCl_3$ and the mechanical action during the coagulation process. • *M. aeruginosa* retained its cell integrity up to 10 days in the sludge before releasing several toxins into surrounding water.	Li *et al.* 2015 [179]
Full scale	Cyanobacteria communities	Density: 5.10^4 cells/mL TOC: 5.3 mg/L	PACl	• Coagulation process removed more than 96% of *Microcystis* and *Dolichospermum* • Cell accumlation in the sludge up to 31 times more than in raw water • Changes in community structure over time at phylum, genus and species in raw water, holding tank and sludge supernatant	Jalili *et al.* 2020 [55]
Ful scale	Cyanobacteria communities	Density: 7.10^5 cells/mL Turbidity: 19.8 NTU pH: 8 DOC: 5.3 mg/L MC-LR: 6.1 μg/L	Polyaluminum chloride (PACl)	• Coagulant process removed 86% of total cyanobacterial cells. • Reduced the total toxins to 2.1 μg/L of MC-LR.	Zamyadi *et al.* 2012 [181]

Moreover, previous study showed that selective removal of some species has been documented in drinking water treatment plants; their cells were poorly coagulated and not trapped efficiently in the sludge [158, 160, 182]. Therefore, this may be a serious concern as that species has the potential to produce the toxins. For instance, *Aphanizomenon* cells passed through and were not retained over the filter in drinking treatment plant in bloom season and it severely disrupted drinking water treatment. Another concern is the long term accumulation of potentially toxic cells in the sludge [160]. The addition of coagulant for lake treatment could preferentially remove cyanobacterial cells that form more spherical, dense colonies as compared to cyanoabacterial cells that forms filamentous colonies; however, this has yet to be investigated.

Recently, high throughput sequencing has been recently applied to study the microbial communities in response to coagulation. Pei *et al.* 2017 [57] studied the microbial structure in drinking water treatment sludge formed by different types of coagulants (including aluminum chloride ($AlCl_3$), $FeCl_3$, and polyaluminium ferric chloride (PAFC)) using 16S rRNA sequencing. Results showed that the most dominant phyla in sludge belonged to Cyanobacteria, Proteobacteria, Firmicutes, Bacteroidetes, Verrucomicrobia, and Planctomycetes. The relative abundance of the dominant genera *Microcystis*, *Rhodobacter*, *Phenylobacterium*, and *Hydrogenophaga* decreased after 4 days of storage because of the lack of light and oxygen in sludge, which led to an increase of the extracellular microcystin concentration. Moreover, the pathogen *Bacillus cereus* was higher in $AlCl_3$ sludge than in $FeCl_3$ and PAFC sludges [57].

Likewise, 16S rRNA sequencing was applied to microbial communities in drinking water treatment sludge from six different drinking water treatment plants in China [56]. They found that Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes, Verrucomicrobia, Planctomycetes, and Actinobacteria were the dominant phyla in sludge. In addition, toxic cyanobacteria *Microcystis*, *Planktothrix*, and *Cylindrospermopsis* and pathogens *Escherichia coli, Bacteroides ovatus* and *Prevotella copri* were detected in all sludge samples. Besides, the increase of these toxic cyanobacteria and pathogens were mainly associated with nutrients and Fe, respectively, in raw water [56].

A recent study investigated the diversity of cyanobacteria over two seasons in a drinking treatment plant before, during, and after the bloom using shotgun metagenomic sequencing by our fellow ATRAPP colleagues [55]. The results showed that cyanobacterial cells accumulated in the sludge

to concentrations higher than in the raw water because of coagulation-flocculation-sedimentation processes. Cyanobacterial communites in the sludge supernatant should not be recycled as it could potentially introduce cyanobacteria and cyanotoxins into the intake water. Accumulation of cyanobacteria species in sludge beds could be frequently observed in drinking treatment plant during intense blooms [34, 55, 160] that may lead interruption of treatment and reduce removal performance [160]. Therefore, application of coagulant for intense blooms ($<10^5$ cells/mL) in lake or reservoirs drinking water sources could be considered as a barrier to avoid the breakthrough of cyanobacterial cells and their toxins [181] into the plant where they could be more challenging to manage with a risk of toxin breakthrough to finished treated water.

2.5 Originality of study

Although mesocosms experiments have been conducted over many years to explore relationships between environmental factors and toxin concentrations, these have often focused solely on long-term scale or lab scale. There is a need to investigate proposed short-term nutrient addition solutions and their potetial impact on toxin concentration. Moreover, high throughput sequencing approaches are considered to gain insight into the change in the cyanobacterial community composition dynamics before and after addition of nutrients. Furthermore, short term dynamics are important to understand for drinking water treatment plant operation. Mesocosms offer a semi-controlled environment in the source water for understanding these dynamics.

The interest in treating lakes is as follows: Although traditional drinking treatment plants are indispensable for supplying fresh drinking water for communities the world over, lakes or reservoirs remain important source waters for these plants. The treatment processes in these plants may be impaired by eutrophication of their source waters containing high concentrations of nutrients in the water column as well as in the sediments [41]. So far, application of coagulants in lakes is considered for lake restoration. Source water treatment can also be considered as a form of pre-treatment before entering a drinking water treatment plant. The addition of ferric coagulant has been shown to to effective for managing HCBs by decreasing the availability of phosphorus in the water column [5]. The question is: can ferric coagulants be used to rapidly remove cells and intracellular toxins in the water column in a drinking water source during bloom season, and how the cyanobacterial community composition changes following coagulation. Moreover, a

combination of the microscopic and high throughput sequencing results can reveal a wide diversity of cyanobacterial and other bacterial communities in response to coagulation.

CHAPTER 3 RESEARCH OBJECTIVES, HYPOTHESES

3.1 Objectives and hypotheses

3.1.1 General objectives

The main objective of this research is study the effects of nutrients and coagulant on cyanobacteria and their toxins through taxonomic and metagenomics.

These specific objectives will mentioned below

3.1.2 Specific objectives, hypotheses

Specific objective	Hypothesis	Paper
Assess the efficiency of ferric sulfate ($Fe_2(SO_4)_3$) coagulant in removing cyanobacterial cells and cyanotoxins from lake water with cyanobacterial blooms on a short time scale.	Coagulation may efficiently remove cyanobacteria and cyanotoxin in short time in lake.	1
Determine whether some species of cyanobacteria may be selectively removed.	The addition of coagulants replicating lake treatments will preferentially remove cyanobacterial cells that form more spherical, dense colonies as compared to cyanobacterial cells that form filamentous colonies.	
Determine the differential impact of ($Fe_2(SO_4)_3$) on intra- and extra-cellular toxins.	Coagulants will lead to an increase in the proportion of extracellular toxins in water.	
Investigate the cyanobacterial community composition (at genus level) and diversity of cyanobacterial community following coagulation using high throughput sequencing.	Cyanobacterial community composition may change after coagulation.	2
Assess the effect of different N forms (NO_3^- and NH_4^+) on cyanobacterial cells and cyanotoxins from lake water with cyanobacterial blooms in short-term.	Nitrogen forms that are more reduced will lead to enhance cell growth and greater cyanotoxins and these effects can be observed within a short time frame representing a pulse of nutrients as can occur during rainfall-runoff events.	3
Evaluate the cyanobacterial community composition (genus) following N addition.	N addition may change the cyanobacterial community composition.	

CHAPTER 4 MATERIALS AND METHODS

4.1 Study sites

Two study sites were selected – Missisquoi Bay (MB) and Petit Lac St. François (PLSF) because they are both eutrophic surface waters that are prone to cyanobacterial blooms. MB is a shallow embayment of Lake Champlain, situated in southern part of Quebec (Canada; 45°02′23″ N; 73°04′41″ W; depth (Z) = 4.75 m) (Figure 4.1). PLSF is located in south-western Quebec (Canada; 45°32′16″ N; 72°02′11″ W; max depth (Z) = 1.8 m) (Figure 4.2).

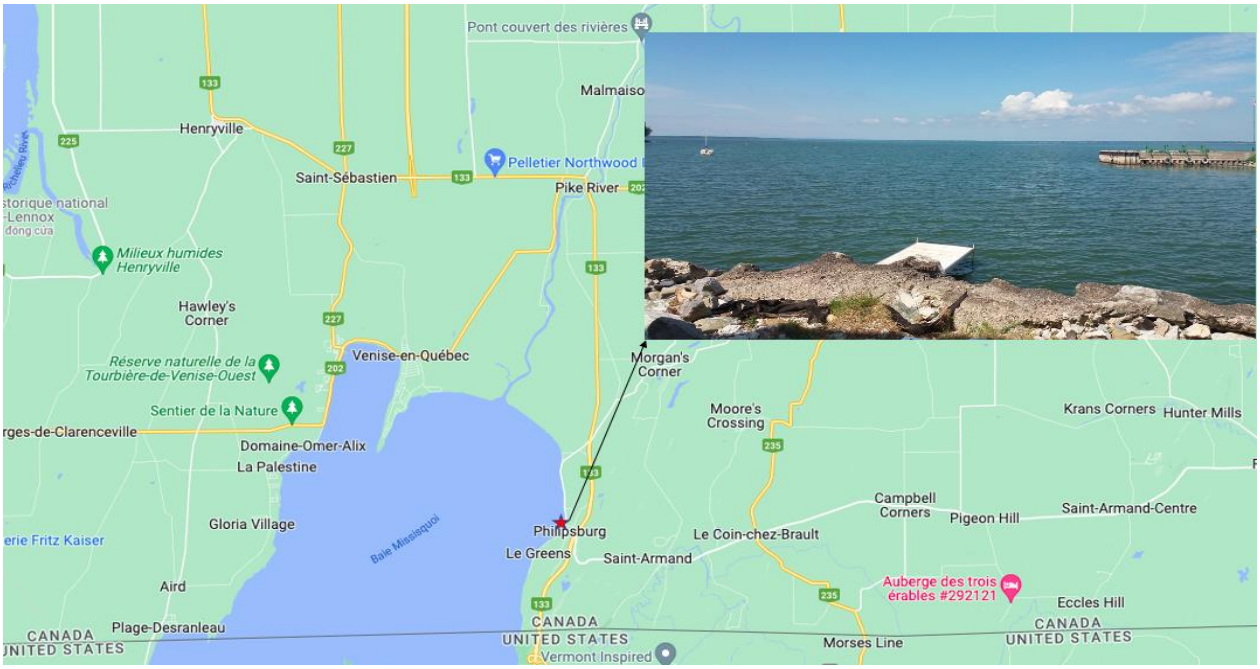

Figure 4.1 Study site in Missisquoi Bay

Excessive cyanobacterial blooms in Missisquoi Bay has increased in recent decades [81, 181]. Agricultural runoff contributes to sustaining the poor water quality of MB [70]. Particulate nitrogen and particulate phosphorus have been the nutrient fractions associated with blooms in MB [183].

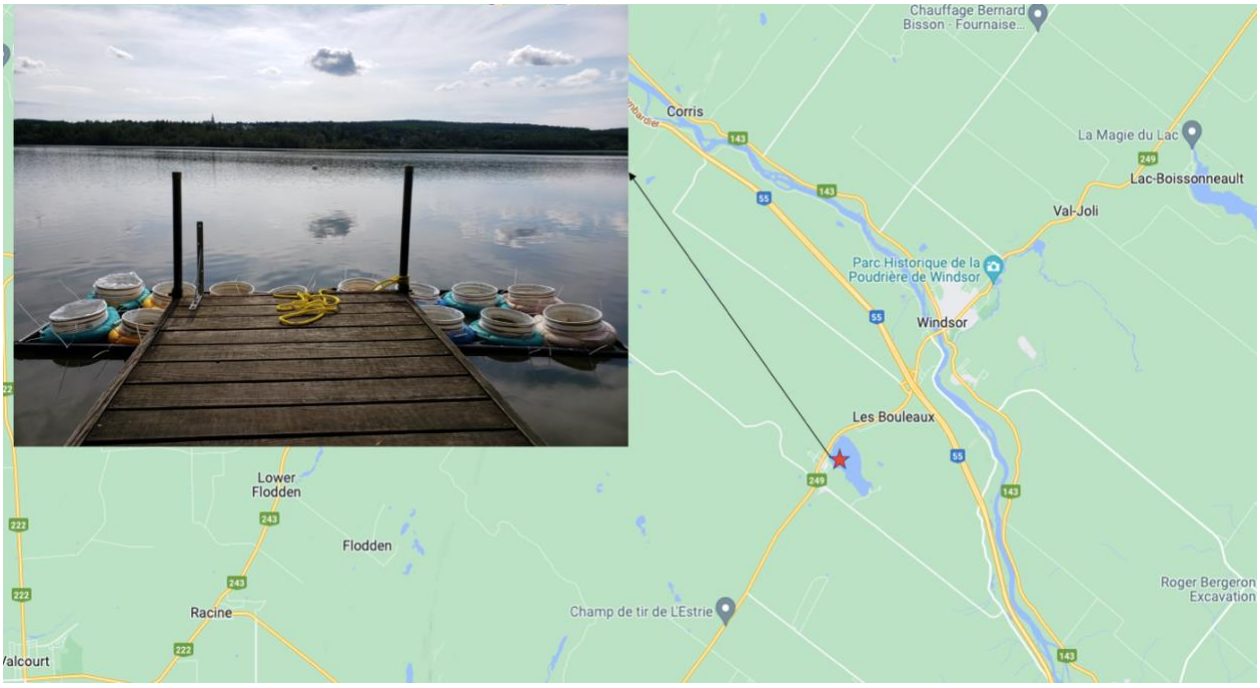

Figure 4.2 Study site in Petit Lac St. François

PLSF is a hypereutrophic lake, and its watershed is drained by agricultural region. N and P concentrations are high in this lake [29].

4.2 Mesocosm description

Mesocosm structures were designed based on the study of Wood *et al.* 2012 [184], and consisted of two parts, the floating ring and the impermeable plastic bag. The floating ring is a colour coordinated (to identify replicates) foam floating tube tied around one rigid plastic ring. This floating part supports the top of the polyethylene bags. Each bag can contain up to 21 L of water in the mesocosm. The polyethylene plastic bag is at 50 cm in deep. Mesocosms were grouped in a rectangle plastic frame, which connected with a dock. A dock was installed approximately 5 m from lake shore. The mesocosms floated at the surface and were not covered [185] (Figure 4.3).

Figure 4.3 A view of mesocosm installed in Missisquoi Bay (left), and a mesocosm photo (right)

4.3 Experiment

Surface water and cyanobacteria harvested from scums that the field site were mixed thoroughly in a large container containing the entire volume to be divided among mesocosms to achieve as homogeneous starting mixture as possible. To conduct the experiment, 6 mesocosms were sets of duplicates, then filled that mixed water of 21 L and were left unamended to serve as a control (control mesocosms) or amended with $Fe_2(SO_4)_3$ (20 mgFe/L and 35 mgFe/L) or nitrogen (NH_4^+ 700 µg/L and NO_3^- 500 µg/L). Sample was mixed immediately after addition of coagulant or nutrient. The mesocosms floated at the surface and were not covered [185].

Mix surface water and cyanobacterial scums in a large container

Filled that mixture into the mesocosms

Sampling before adding coagulant or nutrient (T0)

Filtration

Add coagulant or nutrient

Sampling after two days of exposure (T48)

Filtration

Figure 4.4 A schematic diagram of the experiment process

Samples were collected for taxonomic cell counts, metagenomic analyses, toxin analyses, and nutrients. Samples were filtered immediately at field sites and kept in dry ice box on the way to laboratory. Samples were analyzed at several responsible laboratories with associated methods through the ATRAPP project. Table 4.1 presents the experiment conducted in MB and PLSF in 2018 and 2019.

Table 4.1 List of experiment in 2018 and 2019

Site	Date	Experiment
	31 July – 02 August 2018	Nutrient
Missisquoi Bay	08 – 10 August 2018	Coagulant
	10 – 12 September 2018	Coagulant
	24 – 26 September 2018	Coagulant, Nutrient
	13 – 15 August 2019	Coagulant, Nutrient
	20 – 22 September 2019	Coagulant, Nutrient
Petit Lac St. François	26 – 28 June 2019	Coagulant, Nutrient
	24 – 26 July 2019	Coagulant, Nutrient
	05 – 17 August 2019	Coagulant, Nutrient
	09 – 11 October 2019	Nutrient

CHAPTER 5 ARTICLE 1: THE EFFECTS OF FERRIC SULFATE ($FE_2(SO_4)_3$) ON THE REMOVAL OF CYANOBACTERIA AND CYANOTOXINS: A MESOCOSM EXPERIMENT

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5.1 Abstract

Cyanobacterial blooms are a global concern. Chemical coagulants are used in water treatment to remove contaminants from the water column and could potentially be used in lakes and reservoirs. The aims of this study was to: 1) assess the efficiency of ferric sulfate ($Fe_2(SO_4)_3$) coagulant in removing harmful cyanobacterial cells from lake water with cyanobacterial blooms on a short time scale, 2) determine whether some species of cyanobacteria can be selectively removed, and 3) determine the differential impact of coagulants on intra- and extra-cellular toxins. Our main results are: *(i)* more than 96% and 51% of total cyanobacterial cells were removed in mesocosms with applied doses of 35 mgFe/L and 20 mgFe/L, respectively. Significant differences in removing total cyanobacterial cells and several dominant cyanobacteria species were observed between the two applied doses; *(ii)* twelve microcystins, anatotoxin-a (ANA-a), cylindrospermopsin (CYN), anabaenopeptin A (APA) and anabaenopeptin B (APB) were identified. Ferric sulfate effectively removed the total intracellular microcystins (greater than 97% for both applied doses). Significant removal of extracellular toxins was not observed after coagulation with both doses. Indeed, the occasional increase in extracellular toxin concentration may be related to cells lysis during the coagulation process. No significant differential impact of dosages on intra- and extra-cellular toxin

removal was observed which could be relevant to source water applications where optimal dosing is difficult to achieve.

5.2 Introduction

Cyanobacteria are well adapted to survive and proliferate in water bodies worldwide. Eutrophication and climate change are the main drivers of cyanobacterial blooms [59]. Many cyanobacterial genera are commonly associated with toxicity including *Microcystis*, *Planktothrix*, *Dolichospermum*, *Cylindrospermopsis*, *Nodularia*, and *Schizotrix* [91]. Cyanotoxins are the byproducts of metabolite formation process. They may appear within the cell (intracellular) or released into in the water column (extracellular). Cyanotoxins can be classified into several main groups: hepatotoxins (microcystins and nodularins), cytotoxins (cylindrospermopsins), neurotoxins (e.g. anatoxin-a), dermatotoxins (e.g. lyngbyatoxin), endotoxins (lipopolysaccharides) and other toxins [66, 186]. Microcystins are the most commonly detected hepatotoxins associated with cyanobacterial blooms worldwide [7, 187]. Several health impacts such as gastroenteritis and cutaneous reactions, have been identified [1]. Moreover, they are one of the many potential causes of water quality degradation in aquatic systems with potential consequences for biological communities [90].

Given the challenges that cyanobacteria pose to drinking water production, effective remediation practices and safety measures are needed to protect aquatic communities and human health. Coagulation is a widely used water treatment technology for effectively removing cyanobacteria and undissolved toxin compounds in drinking water treatment plants [181] and some of these compounds have been used to treat lakes affected by blooms. For instance, aluminium sulfate has been applied in the U.S.A. to reduce cyanobacterial biomass from water bodies and remove internal phosphate [188]. In 2008, the authors of [45] also used polyaluminium chloride (PAC) to remove cyanobacteria blooms of *Aphanizomenon flos-aquae* in Lake Rauwbraken (Berkel-Enschot, The Netherlands). In a eutrophic fishpond in the Czech Republic, a polyaluminium hydrochloride coagulant (PAX-18) was used to remove *Planktothrix agardhii* blooms [43]. However, at laboratory scales, several studies have shown that some conventional types of coagulants (for example polyaluminium chloride, aluminium sulfate, and ferric chloride) can cause cell lysis, resulting cell bound toxin release into the water during the coagulation process [50, 189, 190].

Among the conventional chemical coagulants, ferric based-coagulants (e.g., ferric chloride ($FeCl_3$), ferric sulfate ($Fe_2(SO_4)_3$)) have been used in natural water sources to effectively remove cyanobacterial cells [47, 188]. Previous studies have demonstrated that ferric coagulants can also reduce phosphorus concentrations in the water column and in the sediment, reducing the internal phosphorus loading from sediment and subsequently inhibiting cyanobacteria growth [5]. Iron supplementation, on the other hand, was thought to increase the abundance of cyanobacteria in eutrophic environments [54] and it was also considered as one of the inducing factors of the microcystin synthesis [191].

To the best of our knowledge, no short term outdoor mesocosm experiments with fresh blooms have been conducted to investigate the removal cyanobacterial cells and their toxins by ferric sulfate coagulants. Differences in the removal of cyanobacterial species has been observed in drinking water treatment, and it is unclear whether coagulants will selectively remove particular species [160]. There is a need to investigate the differential removal of cyanobacterial species in the water column. Although long term remediation efforts are critical for mitigating harmful cyanobacterial blooms, specific uses such as drinking or recreational waters require immediate attention. Therefore, our research aims to investigate the feasibility of the coagulation process in an in-situ scale, specifically, in cyanobacteria dominated lakes. The main objectives of this study are to: 1) assess the efficiency of ferric sulfate coagulant in removing harmful cyanobacterial cells, and their related toxins from lake water with cyanobacterial blooms on a short time scale, 2) determine whether some species of cyanobacteria are selectively removed, and 3) determine the differential impact of coagulants on intra- and extra-cellular toxins.

5.3 Results and discussions

5.3.1 Impact of ferric sulfate on cyanobacterial cell counts

5.3.1.1 Taxonomic cyanobacterial cell counts and composition

In the beginning of the experiment (T0), several genera such as *Dolichospermum* spp., *Microcystis* spp., *Aphanothece* spp., *Aphanocapsa* spp., *Aphanizomenon* spp., *Chroococcus* spp., *Coelosphaerium* spp., *Merismopedia* spp. And *Pseudanabaena* spp. were identified in Missisquoi Bay (MB) and Petit Lac Saint-François (PLSF) (Table A.1). The genera *Dolichospermum*,

Microcystis, *Aphanizomenon* were found in both control and coagulated mesocosms at both studied sites and they accounted for more than 70% of total taxonomic cell counts (Figure A.1). The initial average total taxonomic cell counts varied across the study sites and the experimental events, as shown in Figure 5.1. Most mesocosms at both MB and PLSF sites had average total taxonomic cell counts greater than 10^5 cells/mL. On 13 August 2019, the total taxonomic cell counts were recorded with more than 10^7 cells/mL in MB. These above-mentioned cyanobacteria genera frequently occurred at both study sites and varied in time with wide ranges of taxonomic cell counts, with *Microcystis*, *Dolichospermum*, and *Aphanizomenon* being the most common genera [29, 55, 181, 192].

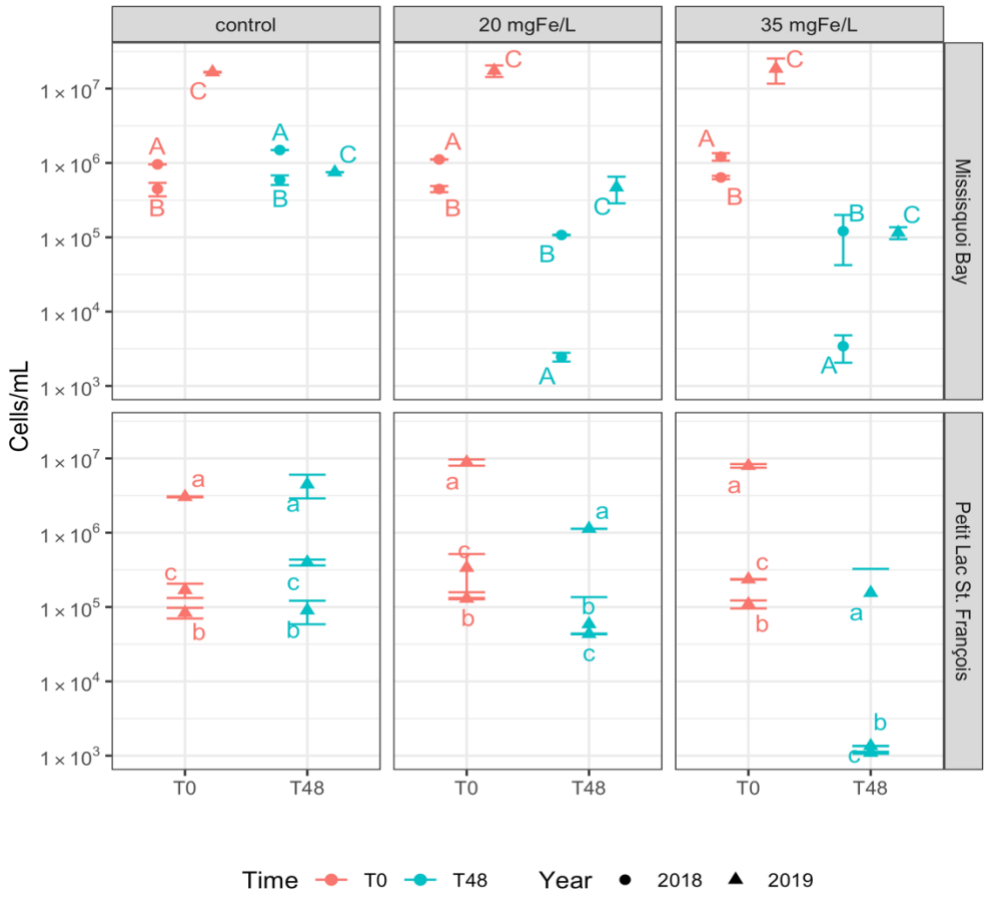

Figure 5.1 Total taxonomic cell counts in control, 20 mgFe/L, and 35 mgFe/L mesocosms (Mean ± standard deviation): A) 10–12 September 2018; B) 24–26 September 2018; C) 13–15 August 2019; a) 26–28 June 2019; b) 24–26 July 2019; c) 05–07 August 2019

Except for an incident in MB on 13 August 2019, *Microcystis* was a common genus in all of the mesocosms, with dominance or representative percentages as shown in Figure 5.2 and Figure A.1. The relative cell abundance of *Microcystis* (as measured by total taxonomic cell counts) ranged from more than 16.3% in MB (10 September 2018) up to 61.5% in PLSF (24 July 2019). *Microcystis aeruginosa* (*M. aeruginosa*) was a dominant species in MB while *Microcystis flos-aquae* (*M. flos-aquae*) was a dominant species in PLSF (Figure A.2). Similarly, *Dolichospermum* spp. was present in high relative abundance in almost all of our experiments with *Dolichospermum spiroides* (*D. spiroides*) most frequently observed in samples. Their relative abundance ranged from 3.16% in PLSF (24 July 2019) to more than 73% in PLSF (26 June 2019) (Figure A.2). In terms of biovolume, *Dolichospermum* was the dominant genus in all events at both sites with the exception of the event on 13 August 2019 where the biovolume of *Aphanizomenon* was more important (Figure A.5).

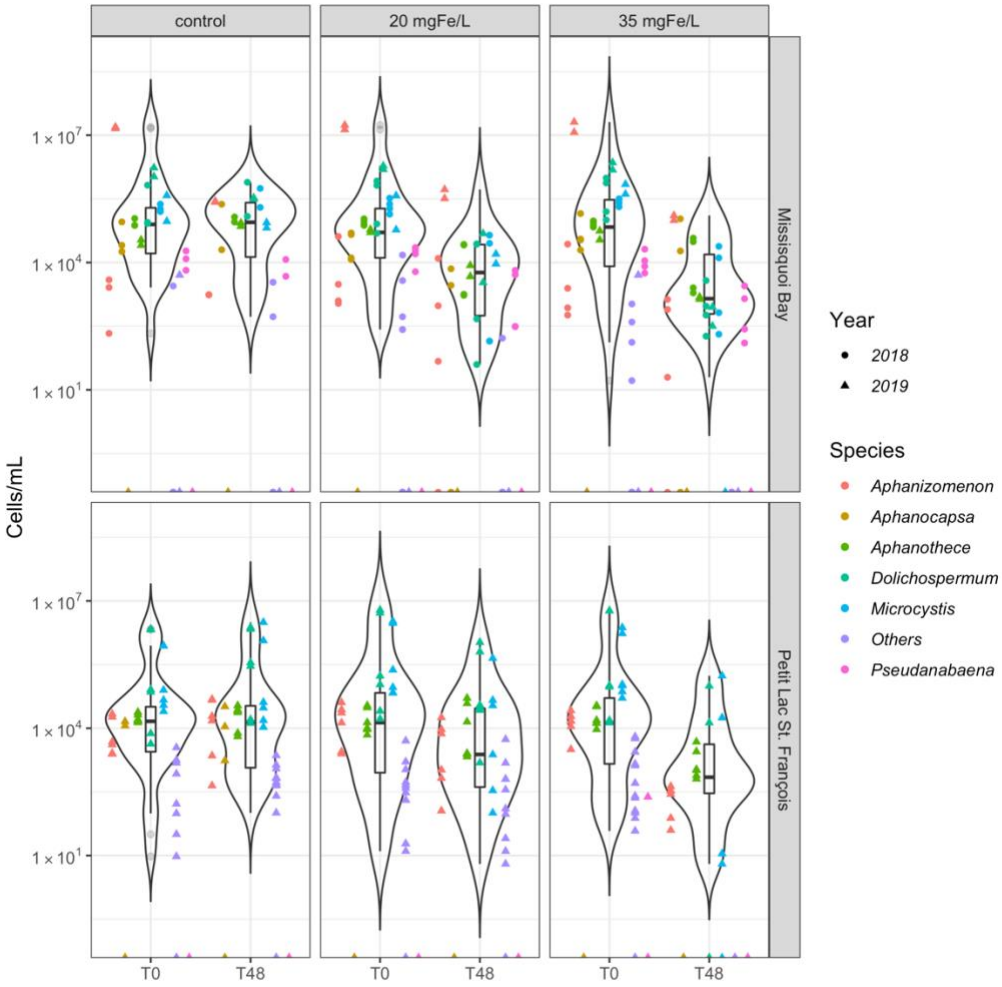

Figure 5.2 Violin plot shows the distribution of taxonomic cell counts at level genus in control, 20 mgFe/L, and 35 mgFe/L mesocosms. (Others include *Chroococcus*, *Coelosphaerium*, *Merismopedia*). The bottom and top of the box shows the lower and upper quartiles, the band in between them shows the median, whiskers show the minimum and maximum (excluding outliers) and circles show the outliers. Outliers are values more than 1.5 times the length of the interquartile range greater than the upper quartile or smaller than the lower quartile

Interestingly, within MB (on 13 August 2019), *Aphanizomenon flos-aquae* was the dominant species (more than 86% of total cell counts and 87% of total cyanobacterial biovolume) in all mesocosms (Figure A.2 and Figure A.5). Several studies have also indicated that *Aphanizomenon flos-aquae* was frequently identified as the dominant species in MB [160, 193]. That *Aphanizomenon* genus became dominant during this sampling event is likely due to a large

variability of dissolved phosphorus (DP). The DP concentration increased approximately 18 times in the water as compared to previous events (10 September 2018 and 24 September 2018) (Table 5.1). The historical total phosphorus (TP) monitoring data in Missisquoi Bay has also shown an increasing trend in TP concentrations in August and that additional TP could be from the sediment TP concentration at that time [194]. Increases in P-loading in water bodies can indeed cause the dominance of cyanobacteria in the phytoplankton community, especially for diazotrophic cyanobacteria such as *Aphanizomenon* spp. [14, 16, 17]. The *Aphanizomenon* genus might be adapted to environments with higher P concentrations having been sensitive to changes in ambient DP [195, 196].

Table 5.1 Environmental conditions of lake water samples in mesocosms (n = 6) at T0 (Mean ± standard deviation)

Parameters	Missisquoi Bay			Petit Lac St. François		
	(A) 10 September 2018	**(B) 24 September 2018**	**© 13 August 2019**	**(a) 26 June 2019**	**(b) 24 July 2019**	**(c) 05 August 2019**
Total cell counts (cells/mL)	998,183 ± 35,034	547,325 ± 16,578	17,408,158 ± 138,898	6,033,197 ± 316,425	109,193 ± 3578	235,723 ± 5986
Chlorophyll-*a* (RFU)	No data	No data	62.22 ± 1.03	3.97 ± 0.04	5.14 ± 0.06	6.27 ± 0.08
Phycocyanin (RFU)	No data	No data	93.43 ± 0.52	16.51 ± 1.54	1.77 ± 0.04	4.83 ± 0.13
pH	6.5 ± 0.08	6.4 ± 0.07	8.05 ± 0.24	8.08 ± 0.07	7.84 ± 0.03	8.01 ± 0.01
TDS (mg/L)	101 ± 0.00	100 ± 0.00	98.00 ± 0.00	122.50 ± 0.71	116.00 ± 0.00	115.00 ± 0.00
Temp (°C)	21.8 ± 0.01	18.7 ± 0.12	26.89 ± 0.21	25.96 ± 0.49	25.14 ± 0.12	24.43 ± 0.24
TOC (mg C/L)	15.22 ± 0.25	5.55 ± 0.07	885.00 ± 33.34	175.00 ± 9.50	11.10 ± 0.38	10.76 ± 0.13

Table 5.1 Environmental conditions of lake water samples in mesocosms (n = 6) at T0 (Mean ± standard deviation) (cont'd)

DOC (mg C/L)	7.50 ± 0.05	5.00 ± 0.00	19.34 ± 1.67	9.80 ± 0.11	9.83 ± 0.13	11.34 ± 1.58
TN (mg N/L)	5.55 ± 0.65	2.75 ± 0.08	7.84 ± 2.95	12.85 ± 0.57	1.11 ± 0.05	1.29 ± 0.008
TP (µg P/L)	360.51 ± 0.01	292.11 ± 13.28	4336.92 ± 74.65	723.55 ± 24.97	110.38 ± 1.89	131.78 ± 7.71
DN (mg N/L)	0.45 ± 0.004	0.52 ± 0.009	2.66 ± 0.12	0.73 ± 0.08	0.60 ± 0.02	0.50 ± 0.02
DP (µg P/L)	17.05 ± 0.07	17.99 ± 0.45	302.17 ± 12.98	139.06 ± 3.35	23.92 ± 4.91	42.86 ± 1.13

Aphanothece clathrata was also observed in some events at both sites (MB, 10 and 24 September 2018; PLSF 24 July and 05 August 2019) (Figure A.2). Small numbers of the *Pseudanabaena* genus was found in MB in 2018 and it was not present at either MB or PLSF in 2019. The *Aphanocapsa* genus was occasionally observed in MB in 2018, while it appeared only once in a control mesocosm in PLSF on 24 July 2019 (Figure A.1). Taxonomic cell counts of "Other" include the genera *Chroococcus*, *Merismopedia*, and *Coelosphaerium* were extremely small and were negligible compared to the dominant genera (Figure 5.2). "Other" genera accounted for less than 1% of total taxonomic cell counts as well as total cyanobacterial biovolume (Figure A.1 and Figure A.5).

5.3.1.2 Removal of cyanobacterial cells

In the control mesocosms, after two days of exposure (T48), the average total taxonomic cell counts did not decrease in all of the control mesocosms in MB in 2018 and PLSF in 2019, except for MB on 15 August 2019 (Figure 5.1). The cyanobacterial genus composition was similar to that observed at T0, as demonstrated in Figure A.1 and Figure A.2. In the natural environment, cyanobacteria in high numbers may be maintained for up to a week or two [12, 65]. For the event on 15 August 2019, total taxonomic cell counts suddenly decreased by more than 90% in the control mesocosm in MB (Figure 5.1), and *D. spiroides* became a dominant species in the control mesocosm

accounting for 43.1% of total taxonomic cell counts, followed by *Aphanizomenon flos-aquae* (36.2%) (Figure A.2). Since the total cell abundance in MB (13 August 2019) exceeded 10^7 cells/mL at T0, the decomposition process would release CO_2 and organic acids into the water body leading to a decrease in pH [196, 197]. A decrease in pH (<7.1) (Table A.7) may cause growth suppression of *Aphanizomenon flos-aquae* in the control mesocosms [198]. Moreover, a decrease in genus *Aphanizomenon* may be caused by the appearance of *Synechococcus* in the blooms [199].

In contrast, the overall cell concentration at T48 in the ferric-sulfate-coagulated mesocosms decreased considerably in all coagulant treated mesocosms, dropping down from around 10^6 or 10^5 to 10^5 or 10^3 cells/mL (MB September 2018 and PLSF July and August 2019) or from 10^7 or 10^6 to 10^5 or 10^4 cells/mL (MB August 2019 and PLSF June 2019) (Figure 5.1). Mesocosms exposed to 35 mgFe/L coagulant had a total cell count reduction of more than 98% when compared to T0 (Table A.2). Mesocosms exposed to 20 mgFe/L showed a decrease in cell count ranging from 71% in MB on 26 September 2018 to more than 85% in other treated mesocosms, except for the PLSF (26 July 2019) mesocosm with the lowest decrease (approximately 51% reduction) (Table A.2).

During two study periods (2018 and 2019), taxonomic cell counts of *Dolichospermum* spp., *Microcystis* spp., *Aphanizomenon* spp., and *Pseudanabaena* spp. decreased in the mesocosms containing 20 mgFe/L and 35 mgFe/L at both sites (Figure 5.2). Other species (*Chroococcus*, *Merismopedia*, and *Coelosphaerium*) were removed to below detection limits for both doses in mesocosms in MB in 2018 and 2019; however, they were stable in the lower dose mesocosm and removed to below detection limits in the higher dose mesocosm in PLSF in 2019 (Figure 5.2). The dose of 35 mgFe/L was more effective than the dose of 20 mgFe/L for removing total taxonomic cell counts and individual dominant cyanobacteria at both study sites (Figure 5.1, Table A.2 and Table A.3). In June and July 2019, in PLSF, *Aphanothece* spp. was increased and *Coelosphaerium* spp. were less removed after coagulation in mesocosms with the dose of 20 mgFe/L (Table A.2).

Coagulation of cyanobacteria, especially mixed-species bloom samples, may be harder than other pollutants as they are living organisms and complex interactions exist among species. Most drinking water research has focused on coagulation process performance for total cyanobacterial cells rather than studying the efficiency of removing individual cyanobacterial species. However, in some cases, the less effectively removed cyanobacteria can impact drinking water quality after

treatment. A previous study reported that *Aphanizomenon* cells were poorly coagulated in a drinking treatment plant and passed through water treatment [160]. The impact of cyanobacterial physical and chemical characteristics on coagulation processes has been studied, and it was found that variable surface charges or allogenic organic matter (AOM) may interfere and prevent agglomeration [182, 200-203]. Moreover, some types of cell morphologies can prevent close contact of cells or cell liberation from flocs by cell motility [201, 204, 205]. Types and doses of coagulants also impact coagulation processes. Higher cell removal efficiency by using ferric coagulant in comparison to alum for the removal of *M. aeruginosa* has also been observed [206]. Flocs of ferric coagulant with a large size and high density could increase separation performance by sedimentation [180]. The pH values at studied sites ranged between 6 and 8 (Table 5.1) wherein cyanobacterial cells easily combine with ferric-based coagulants [182, 207].

A considerable change in cyanobacterial species composition was detected in several events in the coagulated mesocosms after 48 h (Figure A.1 and Figure A.2). *Aphanothece clathrata* became a dominant species in both coagulated mesocosms on 12 September 2018 in MB and in the higher dose mesocosm in July and August 2019 in PLSF. In contrast, there was negligible change in cyanobacterial relative abundance on 15 August 2019 in MB and on 28 June 2019 in PLSF.

Application of coagulants for intense blooms (>10^5 cells/mL) in lake or reservoir drinking water sources may help to prevent the accumulation and breakthrough of cyanobacteria cells and their related toxins [181]. However, coagulation in the water source has the potential to lead to lower cell counts at the water intake and reduce cyanobacterial cell buildup in the clarifier sludge bed [208]. Therefore, treatment plant processes need to be carefully adjusted during the algal bloom season. For example, preozonation of raw water before coagulation may also help cell removal during clarification [208].

Principal component analysis (PCA) was performed to evaluate the relationship between environmental conditions and cyanobacterial cell counts in MB and PLSF (Figure A.7). No relationship was found between dominant genus and the environmental conditions in the beginning of study period at both sites. Overall, the PCA plot for PLSF showed that the genus *Aphanothece* was associated with total dissolved solid (TDS), pH, temperature, total nitrogen (TN), total phosphorus (TP) and total organic carbon (TOC) in mesocosm with dose of 35 mgFe/L but not 20

mgFe/L at T48. A relationship was observed between these above-mentioned parameters with genera *Aphanothece* and *Dolichospermum* in mesocosms with doses of 20 and 35 mgFe/L at T48 in MB.

5.3.2 Impact of ferric sulfate on intracellular cyanotoxins

5.3.2.1 Intracellular cyanotoxin concentration and composition

At the time of experiment (T0), twelve intracellular microcystins (MCs) including MC-RR, MC-YR, MC-HtyR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF, [Asp^3]MC-RR and [Asp^3]MC-LR, anatoxin-a (ANA-a), cylindrospermopsin (CYN), and some rarely monitored cyanopeptides (anabaenopeptin A (AP-A) and anabaenopeptin B (AP-B)) were detected (Figure 5.3 and Figure A.3). The concentration of total intracellular microcystins (intracellular ΣMCs) at T0 varied across the sites and study periods but was greater than 10^3 ng/L with the highest quantity of more than 10^5 ng/L documented in PLSF on 26 June 2019 (Figure 5.3). The initial concentration of intracellular AP-A was higher than 10^3 ng/L in MB on 13 August 2019 (Figure 5.4). Several studies found intracellular toxins including MC-LR, MC-YR, MC-LY, MC-RR, MC-LW, MC-LA, CYN in MB, and MC-LR, MC-LA, [Asp^3]MC-LR in PLSF [29, 181, 209].

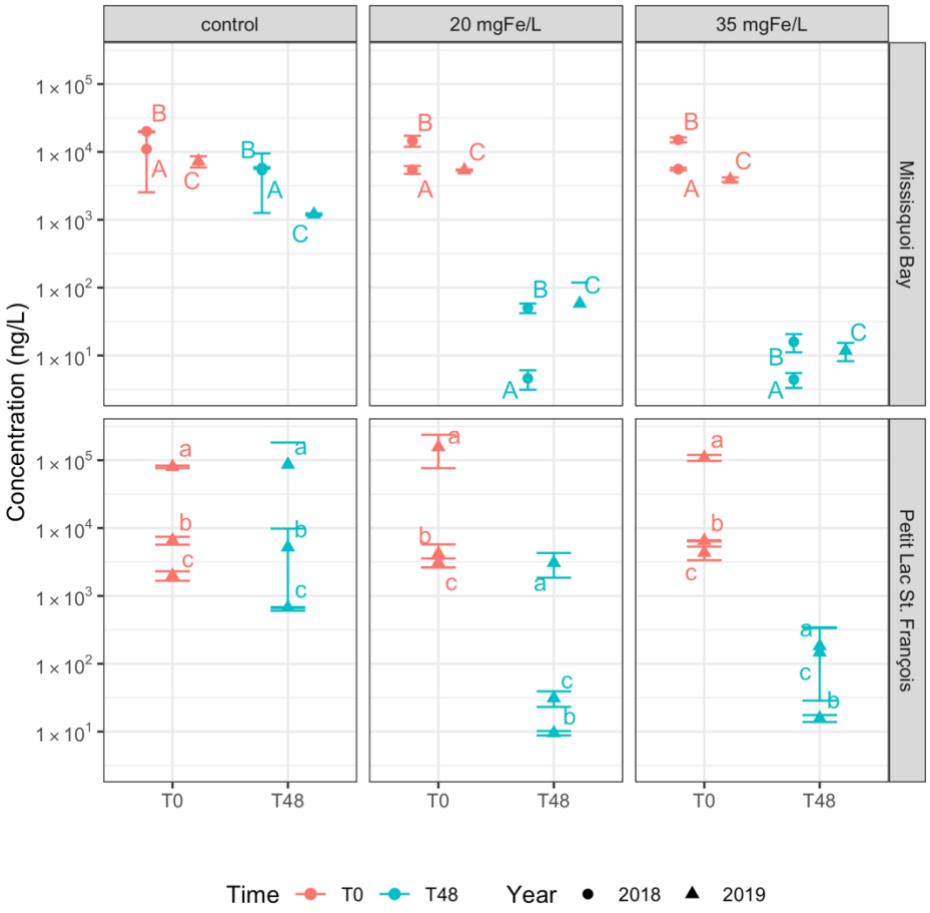

Figure 5.3 Concentration of total intracellular microcystins in control, 20 mgFe/L, and 35 mgFe/L mesocosms (Mean ± standard deviation): A) 10–12 September 2018; B) 24–26 September 2018; C) 13–15 August 2019; a) 26–28 June 2019; b) 24–26 July 2019; c) 05–07 August 2019

Except for one sampling event in MB on 13 August 2019, MC-LR was frequently detected, accounting for at least 40% and up to 75% (count on total intracellular toxin concentration) in all mesocosms (Figure A.3). While the percentage of MC-RR was between 17%–23% in all sampling events in MB, MC-LY accounted for around 20% to 27% of total intracellular toxin concentration in most PLSF sampling events. A small amount (less than 3%) of MC-LA was observed on 10 September 2018 and 13 August 2019, and up to 14% of total intracellular toxin concentration during 24 September 2018 sampling event in MB. AP-B was also detected in MB, ranging from 5% (10 September 2018) to 9% (13 August 2019) of total intracellular toxin concentration. In MB

on 13 August 2019, the individual toxin with highest concentration was AP-A representing nearly 50% of total intracellular toxin concentration, followed by MC-LR and MC-RR (around 20%). Other MC's relative abundance were negligible (Figure A.3).

Those detected were toxins associated with the cyanobacteria taxa at T0 in both MB and PLSF [91]. A significant correlation between intracellular microcystin and toxigenic genera such as *Dolichospermum* and *Microcystis* was found after sampling a series of 22 lakes in southern Québec [210]. Previous studies indicated that the maximum intracellular cyanotoxins concentration was related to amounts of potentially toxic cyanobacterial increase sing in the water body, which is consistent with our findings [192]. For instance, the highest intracellular toxin ΣMCs, which occurred during the event on 26 June 2019, coincided with the predominance of *Microcystis* and *Dolichospermum* and the highest total taxonomic cell counts. The opposite trend was observed on 05 August 2019, where the total taxonomic counts were the lowest.

5.3.2.2 Intracellular cyanotoxin removal

In the control mesocosms, the concentration of intracellular ΣMCs did not change considerably after 2 days except for MB on 13 and 15 August 2019, where its concentration was reduced up to 90% (Figure 5.3). Furthermore, the composition of cyanobacterial toxins including MC-LR, MC-RR, MC-LY and AP-A remained stable after two days (Figure A.3). In MB in August 2019, AP-A concentrations also decreased by 90%, although it remained the toxin with the highest concentration, followed by MC-LR (14% of total intracellular toxin concentration) and MC-RR (12%). A reduction in the concentration of intracellular ΣMCs and AP-A in MB was probably due to a decrease in total taxonomic cell counts.

MCs retained in intact cells may persist for several months and may increase under conditions which are most favorable for their growth [13]. Several studies have indicated that the dominance of toxigenic strains and the subsequent increase in microcystins production were affected by environmental factors, such as temperature, light, intensity, pH, nutrient concentration (NO_3^-, NH_4^+, PO_4^{3-}, Fe^{2+}), salinity, CO_2 concentration, and turbidity [13, 211, 212].

On the contrary, in all mesocosms with ferric sulfate addition, the concentration of intracellular ΣMCs remarkedly decreased by more than 97% for the two applied doses (Figure 5.3 and Table A.4). Ferric sulfate is also efficient in removing individual microcystins such as MC-LR, MC-LY,

MC-RR and MC-LA (Figure 5.4 and Table A.4). In that table, we also observed a remarkable reduction in the concentration of intracellular AP-A (80%–99% of reduction) and AP-B (90%–99% of reduction) in MB. From a statistical perspective, there is no significant difference in removing intracellular ΣMCs and individual intracellular cyanotoxins between two applied dosages (Table A.5).

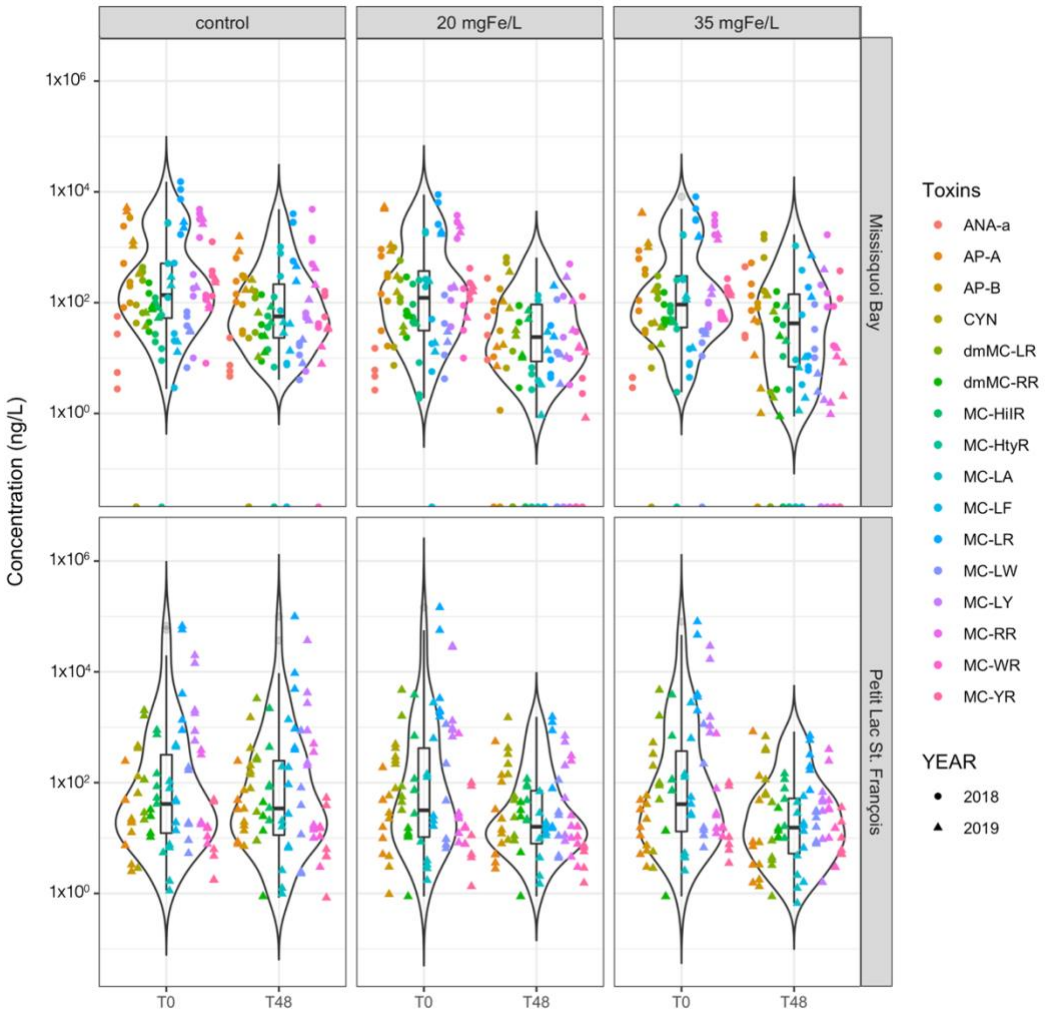

Figure 5.4 Violin lot shows the distribution of intracellular toxin concentration of individual cyanotoxins in control, 20 mgFe/L, and 35 mgFe/L mesocosms in Missisquoi Bay (MB) and Petit Lac St. Francois (PLSF). The bottom and top of the box show the lower and upper quartiles, the band in between them shows the median, whiskers show the minimum and maximum (excluding outliers) and circles show the outliers. Outliers are values more than 1.5 times the length of the interquartile range greater than the upper quartile or smaller than the lower quartile

A coagulation process is one of the most important steps for the removal of cyanotoxins because the majority of cyanobacterial toxins naturally exist intracellularly and are retained within the cell [13]. However, several toxins are frequently found extracellularly (i.e., cylindropermopsin) in lake water or culture medium [213, 214]. Our results for intracellular toxins may also include toxins adsorbed into particulate matter and the cells themselves in the water column. Microcystins toxin can be retained on natural suspended solids in rivers and reservoirs [13]. Several studies have indicated conventional coagulation is effective (up to 98%) for removing intracellular microcystin at the lab-scale [157, 215].

5.3.3 Effect of ferric sulfate on extracellular cyanotoxins

5.3.3.1 Extracellular cyanotoxin concentration and composition

The concentration of extracellular ΣMCs at the time of experiment may vary within the same location. For instance, at the MB experiment site, an extremely high concentration of extracellular ΣMCs, around 115 μg/L, was observed in July 2010 [181] while lower concentrations, ranging from 50 to 191 ng/L, were recorded from June to September 2017 [55]. In PLSF, the concentration of ΣMCs was high, approximately 1.7 μg/L, in June 2010 and lower, around 35 ng/L, in the summer 2017 [29]. In our research, the initial concentration of extracellular ΣMCs was higher than 10^2 ng/L with highest value (more than 10^3 ng/L) documented in PLSF on 26 June 2019 (Figure 5.5). We also observed an initial extracellular concentration of more than 10^2 ng/L of AP-A in MB on 13 August 2019 (Figure 5.6).

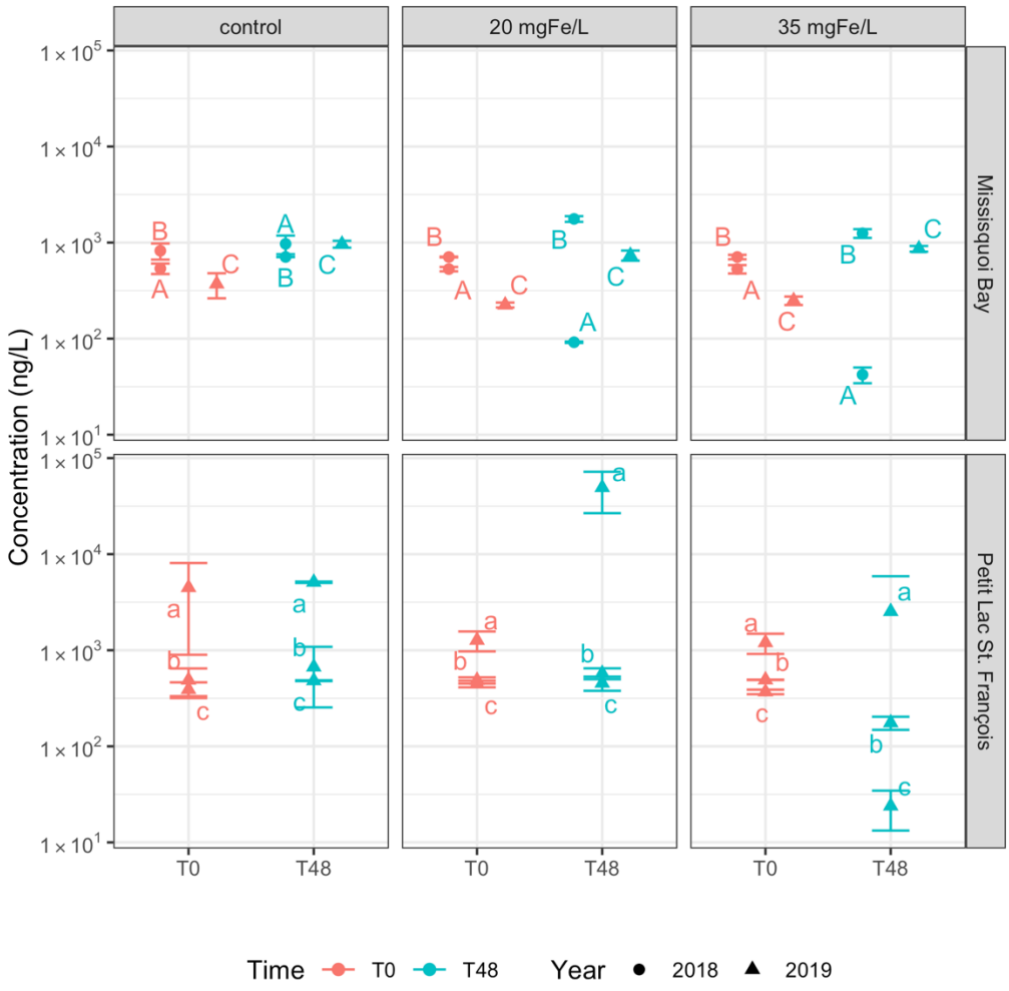

Figure 5.5 Concentration of total extracellular microcystins in control, 20 mgFe/L, and 35 mgFe/L mesocosms (Mean ± Standard deviation). A) 10–12 September 2018; B) 24–26 September 2018; C) 13–15 August 2019; a) 26–28 June 2019; b) 24–26 July 2019; c) 05–07 August 2019

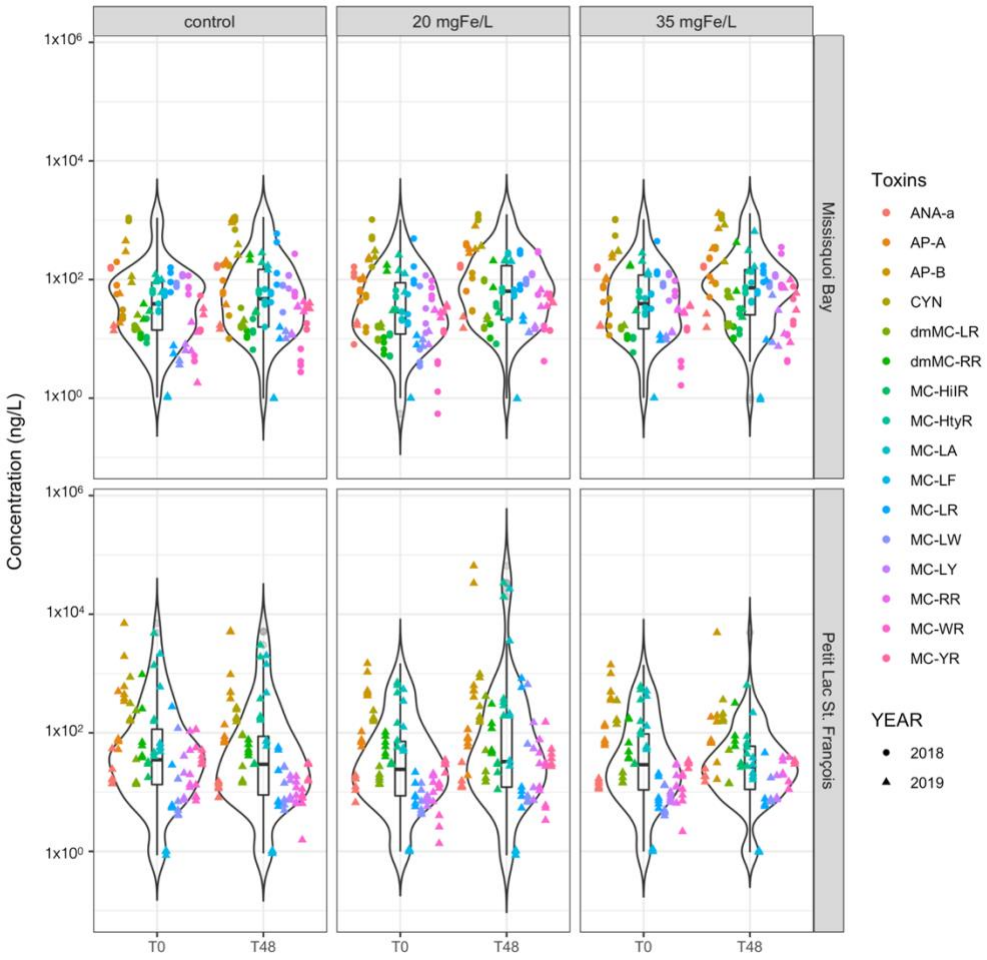

Figure 5.6 Violin plot shows the distribution of extracellular toxin concentration of individual cyanotoxins in control, 20 mgFe/L, and 35 mgFe/L in Missisquoi Bay (MB) and Petit Lac St. François (PLSF). The bottom and top of the box show the lower and upper quartiles, the band in between them shows the median, whiskers show the minimum and maximum (excluding outliers) and circles show the outliers. Outliers are values more than 1.5 times the length of the interquartile range greater than the upper quartile or smaller than the lower quartile

In Figure A.4 we describe the percentages of all extracellular cyanotoxins observed in our experiments. In control and coagulated mesocosms, while MC-LR was dominant at the PLSF site, representing 75%–90% of total extracellular toxin concentration in almost all events in PLSF, it was also dominant or significant at the MB site, accounting for at least 11% (10 September 2018) and up to 37% (24 September 2018) of the total extracellular toxin concentration. CYN toxin accounted for 40% to 67% (10 September 2018) and around 12%–55% (13 August 2019) of the

total toxin concentration. In addition, relative abundance of AP-A toxin accounted for around 13% in MB on 24 September 2018 while it occasionally appeared in the control mesocosms on 12 September 2018 and 13 August 2019.

5.3.3.2 Extracellular Cyanotoxins Removal

After 48 h, in the control mesocosms, we observed a trend of either a slight increase in the concentration of extracellular ΣMCs in MB (2018 and 2019), or a negligible change in PLSF (2019) (Figure 5.5). Concentrations of individual cyanotoxins after 48 h are presented in the Figure 5.6. The change in the relative abundance of individual cyanotoxins is documented in Figure A.4. In MB, the relative abundance of AP-A remained unchanged on 12 September 2018, but markedly decreased in two other experiments (26 September 2018, and 15 August 2019). In term of relative abundance, extracellular CYN decreased from 67% to 35.5% on 12 September 2018. An increase in the relative abundance of MC-LR and MC-RR was observed in MB in 2018 and 2019. In PLSF, the relative abundance of MC-LR decreased remarkably while MC-LY increased highly on 26 July 2019 (Figure A.4). Overall, MC-LR remained as a representative toxin in MB and PLSF.

The stability of extracellular toxin concentration over a wide range of temperatures and pH in water has been observed [216-219]. For instance, a previous study examined the stability of variant MCs, ANA-a, CYN, AP-A and AP-B during short-term storage at the lab-scale by inoculating a mixture of these toxins into surface water with no detectable cyanotoxin levels collected in PLSF and samples were stored at ambient temperate (20°C). Results of that paper showed that extracellular toxins MC-LR, CYN and ANA-a changed negligibly while MC-LA, MC-LY, MC-RR, AP-A and AP-B slightly decreased in surface water after two days [220]. Moreover, it also indicated that microcystins degraded more slowly in mixtures compared to individual toxins [221].

In the coagulated mesocosms, an increase in the concentration of extracellular ΣMCs was observed at both MB (26 September 2018, and 15 August 2019) and PLSF (28 June 2019) in coagulated mesocosms (Figure 5.5). Besides, AP-A, AP-B and CYN toxins also increased after 48 h (Figure 5.6). The increase in the concentration of the total extracellular toxins in coagulated mesocosms after treatment was likely due to cell lysis and desorption of cyanotoxins from sediments or particulate matter. Several laboratory experiments have shown that following coagulation, cyanobacterial cells are lysed, and their toxins are released into the water [51-53, 222]. Moreover,

extracellular cyanotoxins were found not just in the water column but also in sediments and other organic debris [221, 223]. As a result, cyanotoxins may desorb and be released into aqueous water in these locations where toxins accumulate, causing their concentration in water to rise [223, 224].

In contrast, a decreasing trend of concentration of extracellular ΣMCs was observed in MB (12 September 2018) and PLSF (26 July and 07 August 2019) (Figure 5.5) for coagulated mesocosms which could be related to biodegradation and adsorption [225]. However, in this study, biodegradation was not observed in the control mesocosms. In this case, the reduction in extracellular ΣMCs is probably more associated to adsorption on to the sediment at the bottom of the coagulated mesocosms. It was discovered that cyanotoxins including CYN, MC-RR, MC-LF and MC-LW showed high adsorption (36.4% and 72.6%) in sandy sediments [223]. There is no significant difference between control and coagulated mesocosms as well as two applied doses (20 and 35 mgFe/L) on removing extracellular toxins (Table A.6).

5.4 Conclusions

- *Dolichospermum*, *Microcystis*, and *Aphanizomenon* were the dominant genera throughout the experimental period. *Aphanothece* spp. and *Coelosphaerium* spp. were also documented at some events;
- Total cyanobacterial cells were efficiently removed, with more than 96% and 51% removal in mesocosms with applied dose of 35 mgFe/L and 20 mgFe/L, respectively. Significant differences in removing total cyanobacterial cells and several dominant cyanobacterial genera were observed between applied doses;
- Both applied doses of ferric sulfate help to efficiently remove *Dolichospermum*, *Microcystis*, and *Aphanizomenon*. *Aphanothece* and *Coelosphaerium* had a lower removal rate in mesocosms with a dose of 20 mgFe/L in PLSF;
- Intracellular microcystins (MC-RR, MC-YR, MC-HtyR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF, [Asp^3]MC-RR and [Asp^3]MC-LR), anatoxin-a (ANA-a), cylindrospermopsin (CYN), anabaenopeptin A (AP-A) and anabaenopeptin B (AP-B) were detected throughout the experiment;

- Ferric sulfate effectively removes detected intracellular cyanotoxins but not extracellular cyanotoxins. More than 97% removal of total intracellular microcystins were achieved for both applied doses;
- Different dosages of ferric sulfate have almost the same effectiveness in removing intra- and extra-cellular cyanotoxins meaning that source water treatment will not be highly sensitive to suboptimal dosing.

5.5 Materials and Methods

5.5.1 Study Site Description

Petit Lac St. François (PLSF) and Missisquoi Bay (MB) were selected as study locations because they are both eutrophic lakes that are prone to cyanobacterial blooms during growing season. PLSF is located in south-western Quebec (Canada; 45°32′16″ N; 72°02′11″ W; max depth (Z) = 1.8 m). PLSF is relatively small, shallow lake with approximately 50% agricultural land use in its catchment. Phosphorus and dissolved organic carbon concentrations were found to be high [23]. MB is a shallow embayment of Lake Champlain, situated in southern part of Quebec (Canada; 45°02′23″ N; 73°04′41″ W; depth (Z) = 4.75 m). This site was chosen because of frequent occurrence of intense cyanobacterial blooms [55, 192]. The presence of cyanobacteria in this bay poses a threat to drinking water treatment and recreational activities [160, 181].

5.5.2 Mesocosms Experiments Description

The mesocosm experiments were conducted over 6 different cyanobacterial bloom events, September 2018 and from June to August 2019 (Table 5.1) and each experiment lasted for 48 h. The mesocosm design followed the study of Wood [184], including the series of treatments and controls as described below. Cylindrical low-density polyethylene bags of 21 L mesocosms were suspended by floats on the shore of Lake Champlain and PLSF, Québec, Canada. Surface lake water and cyanobacteria harvested from scums at the field site were pooled and mixed thoroughly in a large (200 L) container to achieve as homogeneous starting mixture as possible. To conduct the experiment, 6 mesocosms were sets of duplicates, then filled with that mixed water of 21 L and were left unamended to serve as a control (control mesocosm) or amended with 2 doses of the ferric sulfate coagulant: 20 mgFe/L (mesocosm 20) and 35 mgFe/L (mesocosm 35). Samples were mixed

immediately after addition of coagulant. The mesocosms floated at the surface and were not covered. All of the environmental conditions are presented in Table 5.1.

5.5.3 Preparation and Application of Chemical Coagulant

Standard liquid grade ferric sulfate $Fe_2(SO_4)_3$ with Fe content 12.2% was purchased from Kemira Water Solution, Inc. (Canada). Two different doses of ferric sulfate coagulant (20 mgFe/L and 35 mgFe/L) were administered in a mesocosms. The dosages were chosen based on previous study [160].

5.5.4 Sampling and Filtration Procedure

Sterilized high density 1-L polypropylene bottles were used to collect water samples from center of each mesocosm before adding the coagulant (T0) and after the addition of the coagulant at 48 h (T48). Water in mesocosm was mixed thoroughly before pouring into sterilized 1-L bottles at T0 while mixing was avoided to take the supernatant water at T48. For taxonomic samples, water samples were taken in 40 mL glass vials and preserved in Lugol's iodine solution. Subsamples were taken for cyanotoxin, total organic carbon (TOC), dissolved organic carbon (DOC) and specific nutrients: total nitrogen (TN), total phosphorus (TP), dissolved nitrogen (DN), dissolved phosphorus (DP).

Subsamples of 120 mL were filtered on hydrophilic polypropylene GHP, 0.45 µm filters (PALL, Mississauga, ON). After filtration, the filter for intracellular toxin (sorbed to particulate matter or cell-bound) was placed in a petri dish (Millipore Sigma, Oakville, ON) while the filtrate for extracellular toxin (dissolved toxin) was transferred into 125 mL amber Nalgene™ polyethylene terephthalate glycol (PETG) bottles (Thermo Scientific, Mississauga, ON). Samples for TOC, TN, and TP were filled directly. The DOC samples were filtered by a pre-rinsed (1 L MilliQ water) 0.45 µm membrane filters (PALL, Mississauga, ON). A filter membrane 0.45 µm, 47 mm (Millipore Sigma, Oakville, ON) was used for subsample of DN/DP. Toxin and nutrient samples were taken in duplicate.

Toxin followed by storage at −25 °C. TN/TP/TOC/DOC samples were stored at 4°C until analysis. Taxonomic samples were stored in a dark place at room temperature.

5.5.5 Analysis Methods

Taxonomic identification was achieved by using inverted microscopy in a Sedgwick-Rafter chamber with 40× magnification (Leica Microsystems GmbH, Wetzlar, Germany) [160, 226, 227], at the Université du Québec à Montréal's (UQAM) Biological Sciences Department.

A total of 17 cyanobacterial toxins were tested with 12 microcystins (MC-RR, MC-YR, MC-HtyR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF, [Asp3]MC-RR and [Asp3]MC-LR), anatoxin-a (ANA-a), cylindrospermopsin (CYN), anabaenopeptin A (AP-A) and anabaenopeptin B (AP-B). Total microcystins samples were detected via a Lemieux oxidation step and an online solid phase extraction (SPE) and salting coupled to ultrahigh performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). The limit of detection was 0.5 ng/L and accuracy was in the range of 93%–110%, deemed satisfactory. An online solid phase extraction ultra-high-performance liquid chromatography high-resolution mass spectrometry (SPE-UHPLC-HRMS) was developed for those multi-toxins with a limit of detection between 8 and 53 ng/L and recoveries ranging from 81% to 113%. More detail in [228, 229].

The TOC and DOC were measured on a OI Instrument, Aurora 1030 according to method 415.1 [230]. TN and DN were analyzed on a Lachat, Quickchem 8500 based on the method 353.2 and the method 350.1 [231]. Standard method numbered 365.3 and 365.1 [232] were applied for phosphorus and phosphate with machine of Astoria-Pacific, Astoria 2. TOC/DOC and specific nutrient samples were analyzed at Groupe de Recherche Interuniversitaire en Limnologie, University of Montréal.

An online YSI EXO2 water-quality multi-probe (YSI, Yellow Springs, Ohio, USA) was fitted with total algae sensor (chlorophyll-a/phycocyanin) was applied in this paper to measure phycocyanin, chlorophyll a, pH, total dissolved solid (TDS) and temperature [160, 181].

5.5.6 Statistical Analysis

Statistical analysis was performed by R (R Core Team, 2020). All of the plots in this paper were generated by ggplot2 package in R (https://ggplot2.tidyverse.org, accessed on 12 March 2021). Principal component analysis (PCA) was performed to evaluate the impact of environmental conditions on total cell counts and cyanobacterial species in MB and PLSF, it is with the vegan

(https://cran.r-project.org/package=vegan, accessed on 10 October 2021) and missMDA (https://cran.r-project.org/package=missMDA, accessed on 22 April 2021). Statistical tests were normalited by the Shapiro-Wilk test in mvnormtest package (https://cran.r-project.org/package=mvnormtest, accessed on 03 July 2021) and one-way ANOVA (Analysis of variance) test. Statistical significance was set at a 0.05 *p*-value cut-off ($p < 0.05$). Imputation of missing value is conducted by using the left-censored imputation method in MICE (https://cran.r-project.org/package=mice, accessed on 05 July 2021) and QGCOMP packages (https://cran.r-project.org/package=qgcomp, accessed on 20 June 2021).

CHAPTER 6 ARTICLE 2: SHOTGUN METAGENOMIC SEQUENCING TO ASSESS CYANOBACTERIAL COMMUNITY COMPOSITION FOLLOWING COAGULATION OF CYANOBACTERIAL BLOOMS

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6.1 Abstract

The excessive proliferation of cyanobacteria in surface waters is a widespread problem worldwide, leading to the contamination of drinking water sources. Short- and long-term solutions for managing cyanobacterial blooms are needed for drinking water supplies. The goal of this research was to investigate the cyanobacteria community composition using shotgun metagenomics in a short term *in situ* mesocosm experiment of two lakes following their coagulation with ferric sulfate ($Fe_2(SO_4)_3$) as an option for source water treatment. Among the nutrient paramenters, dissolved nitrogen was related to *Microcystis* in both Missisquoi Bay and Petit Lac St. François while the presence of *Synechococcus* was related to total nitrogen, dissolved nitrogen, dissolved organic carbon, and dissolved phosphorus. Results from the shotgun metagenomic sequencing showed that *Dolichospermum* and *Microcystis* were the dominant genera in all of the mesocosms in the beginning of the sampling period in Missisquoi Bay and Petit Lac St. François, respectively. Potentially toxigenic genera such as *Microcystis* were correlated with intracellular microcystin concentrations. A principal component analysis showed that there was a change of the cyanobacterial composition at the genus level in the mesocosms after two days, which varied across

the studied sites and sampling time. The cyanobacterial community richness and diversity did not change significantly after its coagulation by $Fe_2(SO_4)_3$ in all of the mesocosms at either site. The use of $Fe_2(SO_4)_3$ for an onsite source water treatment should consider its impact on cyanobacterial community structure and the reduction of toxin concentrations.

6.2 Introduction

Cyanobacteria are among the most ancient organisms on Earth, and they are present in aquatic environments worldwide [12, 233]. When intense blooms appear, cyanobacteria can produce large quantities of toxins. The increasing frequency and intensity of toxic cyanobacterial blooms have affected wild animals, domestic animals, aquatic ecosystems and human health [12, 181]. Microcystins (MC), nodularin (NOD), cylindrospermopsin (CYN), and anatoxin-*a* (ANTX-*a*) are frequently found in freshwater blooms, while microcystins are the most well-known and reported toxins [91].

Given the harmful nature of cyanobacterial blooms for recreational and drinking water uses, various types of treatments have been proposed, including their coagulation, oxidation, and adsorption with powdered activated carbon [34, 156, 181]. The effectiveness of coagulation on cyanobacterial cells and cyanotoxin removal has been studied [55, 181, 234] and it has been demonstrated to be effective for the removal of cyanobacterial cells and cell-bound toxins [181]. However, cyanobacterial cells may be lysed and their toxins released into the supernatant during the drinking water treatment sludge storage processes [50]. Several studies have applied high-throughput sequencing to investigate the microbial diversity and community structure during coagulation [56, 57]. For example, a recent study applied high-throughput sequencing to microbial communities in sludge from six different drinking water treatment plants, and the authors of this found that there was a relationship between the toxic cyanobacteria and the pathogens in the sludge resulting from raw water with nutrients and iron (Fe) [56]. Similarly, Pei *et al*. 2017 [57] studied the microbial structure in drinking water treatment sludge that was formed by different types of coagulants, including $AlCl_3$, $FeCl_3$, and polyaluminium ferric chloride (PAFC). Their results indicated that after four days of storage, the relative abundance of the dominant genera *Microcystis*, *Rhodobacter*, and *Phenylobacterium* decreased, thus leading to an increase in the quantity of extracellular microcystin and organic matter [57].

Shotgun metagenomic sequencing has been used to understand the cyanobacterial diversity in sludge and the risk of toxin release after conventational drinking water treatment processes [55]. This study showed that there was an accumulation of cyanobacterial cells, as well as potential cell growth, in the sludge from the raw water influx as a result of coagulation and sedimentation in the drinking water treatment process. If the cyanobacterial communites in the sludge supernatant are then recycled, the cyanobacteria and cyanotoxins could thereby be introduced to the intake water. The sludge cyanobacterial communites were positively correlated with the total nitrogen (TN), total phosphorus (TP), particulate phosphorus (PP), and total organic carbon (TOC) [55].

Understanding the cyanobacterial composition structure before and after coagulation occurs will offer an insight into the mechanisms of coagulation and provide a stronger basis for predicting treatment efficacy. Most of the studies on this subject have been conducted at the laboratory scale; however, the cyanobacterial communities might be dynamically affected by various environmental conditions. With the exception of the study that was conducted by Le *et al.* 2021 [185], where the cyanobacterial fate after the coagulation in the mesocosms was assessed using taxonomic cell counts, there have been no studies that have been conducted on the exploriation of the cyanobacterial communities in water after the coagulation in fresh blooms in the source water. Such information can be used to guide source water treatment options. In order to improve the understanding of the cyanobacterial communities subsequent to source water coagulation, this study aims to: 1) investigate the composition structure of the cyanobacteria using shotgun metagenomics sequencing in *in situ* mesocosms before and after coagulation of the cyanobacterial blooms over a short time period; 2) compare the shotgun metagenomics sequencing result with the microscopic taxonomic cell counts; 3) determine the relationship between the environmental parameters and the cyanobacterial communities that are associated with cyanotoxins before and after their coagulation with ferric sulfate ($Fe_2(SO_4)_3$).

6.3 Results and Discussions

6.3.1 Cyanobacterial Community Composition Assessed by Shotgun Metagenomics Sequencing

The cyanobacterial communities in the mesocosms were studied at the beginning (T0) and after two days (T48) of the study period by using shotgun metagenomic sequencing at the genus level in Petit Lac St. François (PLSF) in 2019 and Missisquoi Bay (MB) in 2018 and 2019. The number of reads were normalized by their relative abundance (Figure 6.1). The metagenomic results were validated for the experiments on August 08-10, 2018, and August 13-15, 2019, in MB; on July 24-26, 2019, and August 05-17, 2019, in PLSF.

In MB, *Dolichospermum* was the dominant genus (approx. 25% - 30% of the total relative abundance) and *Microcystis*, *Nostoc*, *Fischerella*, and *Calothrix* were the representative genera in all of the mesocosms at T0 on August 08, 2018, and August 13, 2019 (Figure 6.1A,B, respectively). These results are in accordance with those of several previous studies which indicated that *Dolichospermum* was a cyanobacterial genus that was frequently found in the blooms in MB [34, 160, 181, 183]. After two days, on August 10, 2018, there was no change in the relative abundance in any of the mesocosms in comparation with the initial composition (Figure 6.1A). This observation is in corresponds with that of a previous report, thereby demonstrating the stability of the cyanobacterial community composition at the genus level after two days of oxidation with H_2O_2 (20 mg/L) and $CuSO_4$ (2 mg/L) in the control mesocosms and the mesocosms with an oxidant in Petit Lac St. François [138].

However, a remarkable change in the cyanobacterial composition at the genus level was observed in both the control and the coagulated mesocosms at T48 on August 15, 2019, in MB (Figure 6.1B). The most abundant genus shifted from being *Dolichospermum* to being *Synechococcus* (approx. 45% of the total relative abundance) and *Microcystis* (38.7% of the total relative abundance) in the control and coagulant mesocosms, respectively. This shift can probably be attributed to the marked decrease of the *Dolichospermum* cell count (greater than 97% reduction) in all of the mesocosms (Table B.1). The loss of *Dolichospermum* biomass in the control mesocosms from August 13 to August 15 in 2019 may be the result of a drop in pH from 8 to 6 which occurred (Table B.4 and

Table B.5), thereby potentially interfering with the cyanobacterial growth, in general [235]. This lower pH level could contribute to the dominance of *Synechococcus* in the control mesocosms because it can flourish at pH 6.5 [236, 237]. However, we did not observe such an abundance of *Synechococcus* in the mesocosms at the doses of 20 mgFe/L and 35 mgFe/L, thereby implying that the *Synechococcus* cells were well captured by the coagulation process. *Microcystis* became the dominant genus, although both *Dolichospermum* and *Microcystis* were largely removed (>86%) in the coagulated mesocosms on August 15, 2019 (Table B.1). This suggests that cell survival and re-growth might have occurred during the two days of the sludge storage process [55]. The competition between the *Microcystis* and *Dolichospermum* strains at the laboratory scale was investigated and it was found that *Microcystis* significantly inhibited the growth of *Dolichospermum* [34].

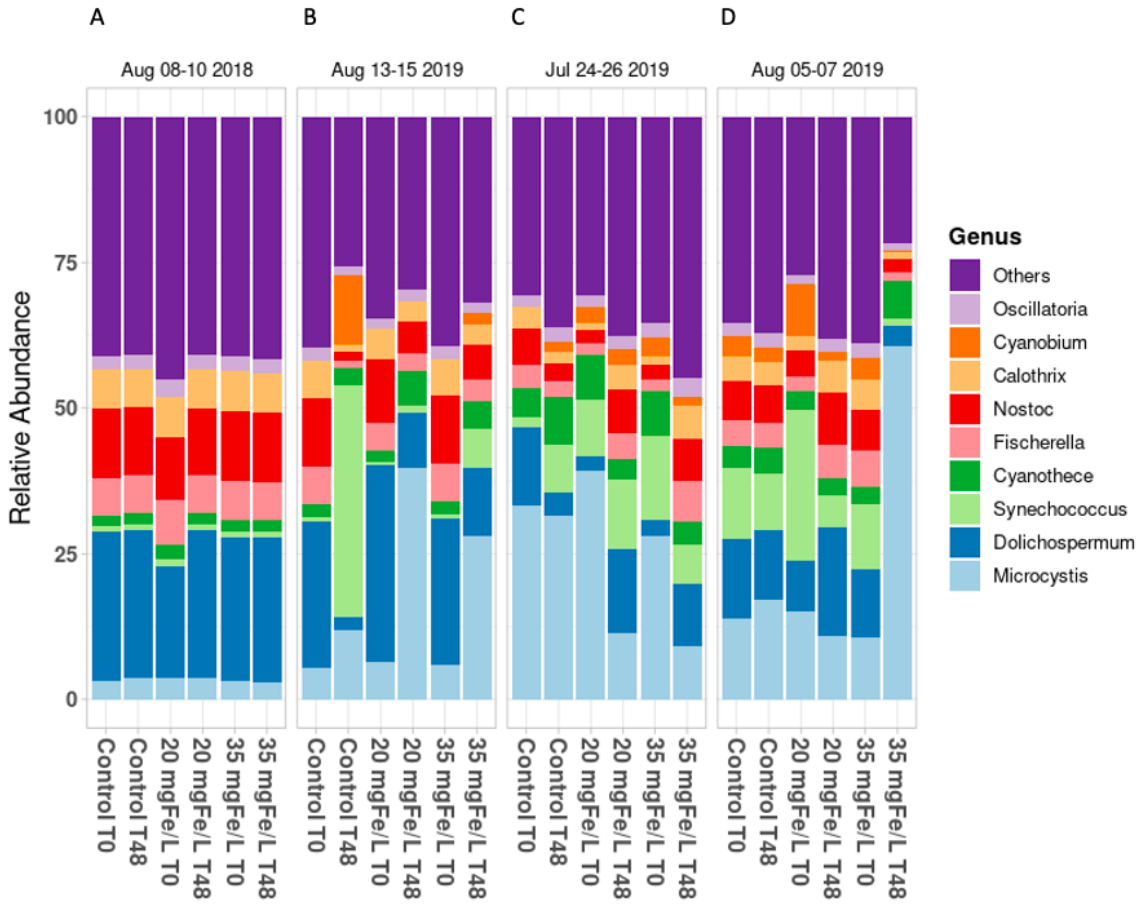

Figure 6.1 Average cyanobacterial relative abundance at genus level in Missisquoi Bay (A,B) and Petit Lac St. François (C,D)

In PLSF, at T0, *Microcystis* was the predominant genus, accounting for 30-37% of total relative abundance that was documented on 24 July 2019, while the highest cyanobacterial relative abundances consisted of *Dolichospermum* (9-10.5%), *Synechococcus* (8-12%) and *Microcystis* (10-12.5%) on August 05, 2019 in all of the mesocosms (Figure 6.1C,D). The relative abundance of the dominant genera remained stable in the control mesocosms at T48 on July 26 and August 07 in 2019 in PLSF. In the mesocosms tha had the addition of 20 mgFe/L and 35 mgFe/L, the relative abundance of *Microcystis* decreased to 11.5% and 10%, respectively at T48 on July 26, 2019 (Figure 6.1C). This is coherent with the trends that were observed in the taxonomic cell count results on July 26, 2019, in our previous study [185]. On August 07, 2019, the reduction of 100% of the *Dolichospermum* in the taxonomic results (Table B.1) may be the result of a notable increase in the relative abundance of *Microcystis* in the mesocosms that had 35 mgFe/L added to them (Figure 6.1D).

The results of the microscopic taxonomic cell counts and shotgun metagenomics sequencing do not completely correspond with each other in this study (Figure 6.1, Figure B.1 and Figure B.2). *Dolichospermum* and *Microcystis* were detected by both of the approaches, but *Aphanocapsa*, *Aphanothece*, *Coelosphaerium*, *Merismopedia*, and *Chroococcus* were detected only by microscopic taxonomic cell counts. Moreover, *Aphanizomenon* was identified to be predominant by the taxonomic cell counts, while the metagenomics test found that *Dolichospermum* was the most dominant genus at the beginning of the sampling period in all of the mesocosms on August 13, 2019 (Figure 6.1B and Figure B.2). These observations correspond with previously reported results findings wherein the microscopic taxonomic cell counts and high-throughput sequencing do not completely match [34, 55].

The limitations of microscopic and shotgun metagenomics approaches may provide an explanation for the differences in the structure of community composition that were found between these methods. Small-sized and morphologically similar species may escape their detection by microscopy [36, 238]. The significant morphological deformation of cyanobacteria species by the coagulation or the oxidation process may also hinder the ability to identify these cells [34, 55, 180]. Shotgun metagenomics (high-throughput sequencing) is a powerful tool for microbial species or community detection and classification to observe the microbial dynamics through water treatment processes [55]. A wide variety of taxonomic and functional classifier pipelines have been

developed and are available to make the analysis of metagenomic sequencing more accessible than they ever have been before. However, the accurate performance of these analyses may be hindered by the process of DNA extraction [238, 239], frequent taxonomy database changes over time, and the number of false positive taxa predictions [240].

6.3.2 Impact of Coagulation on Cyanobacterial Richness and Diversity

6.3.2.1 Principal Component Analysis on the Relative Abundance of Cyanobacterial Community

To gain an insight into the changes in the cyanobacterial community composition dynamics before and after the coagulation in MB and PLSF we performed a principal component analysis (PCA), the results of which are shown in Figure 6.2 and Figure B.3.

In MB, PC1 and PC2 explained 89.4% and 6.12%, respectively, of the variation in the cyanobacterial community composition among the samples (Figure 6.2). The cyanobacterial composition at the start of the study period (T0) on August 08, 2018, and August 13, 2019, was clustered in the blue circle and the orange circle, respectively (Figure 6.2A), and comprised of mainly *Dolichospermum*, as shown in Figure 6.2B, which supports the result that are shown in the bar graph (Figure 6.1A,B). Our result is in accordance with a previous paper which reported that the community composition did not vary considerably from year-to-year in Missisquoi Bay [183]. After 48 hours, on August 10, 2018, and August 15, 2019, there were two different trends in the cyanobacterial composition in the mesocosms. On the one hand, in all of the mesocosms, the cyanobacterial composition clustered in the same blue circle, which is similar to the initial conditions that were observed on August 10, 2018 (Figure 6.2A). On the other hand, the cyanobacterial composition displayed a progressive shift in both the control and coagulated mesocosms on August 15, 2019. *Synechococcus* became more abundant in the control mesocosms (Figure 6.2A,B), which is in agreement with the results that are shown in the bar graph (Figure 6.1B). For the mesocosms with doses of 20 and 35 mgFe/L, the cyanobacterial community composition shifted mainly from being dominated by *Dolichospermum* to being dominated by *Microcystis* dominance (Figure 6.2A,B). This result is in agreement with the recently reported results of the changes in the community structure at the genus level that were monitored in the

samples that were retrieved from raw water and sludge supernatant after a coagulation process in a drinking water treatment plant [55].

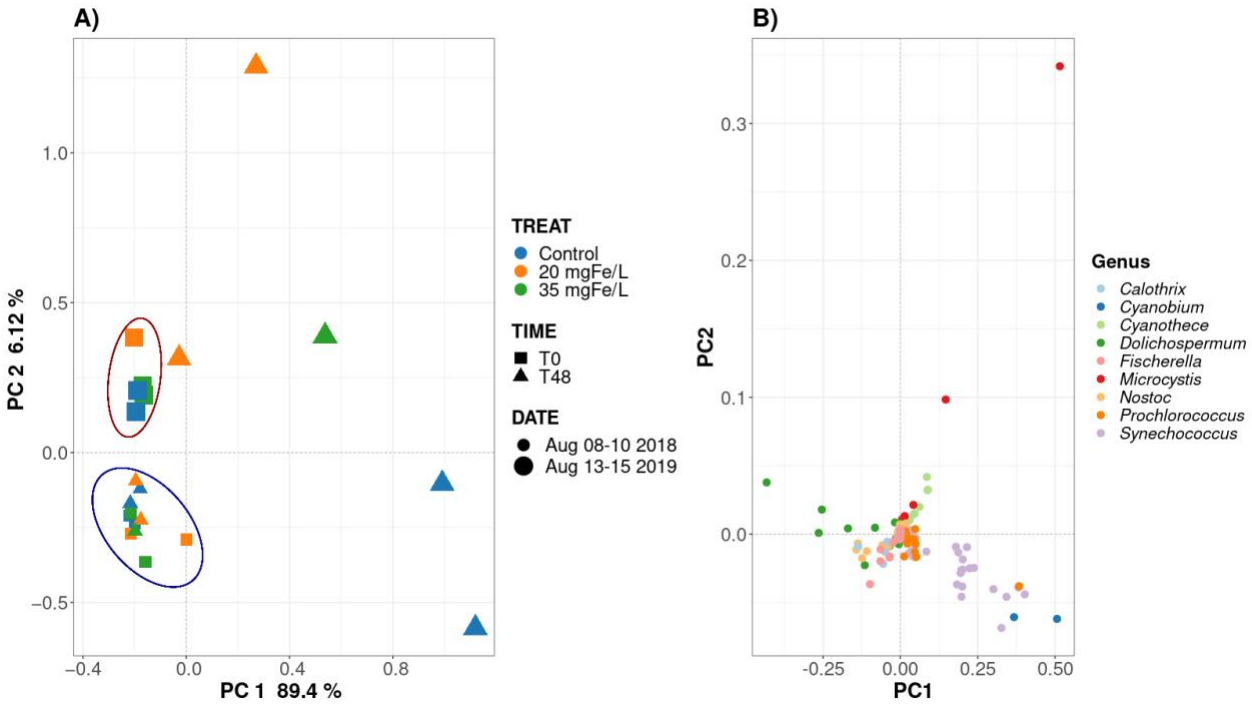

Figure 6.2 Principal components analysis (PCA) of the normalized relative abundance of cyanobacteria community composition in control, 20 mgFe/L and 35 mgFe/L mesocosms with respect to genus abundance in Missisquoi Bay. (a) PCA analysis of cyanobacterial community following coagulation; (b) Data plotted following the genus-level classification

Figure B.3 presentes the PCA analysis of the cyanobacterial community composition following its coagulation using different doses which were performed in PLSF. PC1 and PC2 explained 54.0% and 41.6% of the variation in the cyanobacterial community composition among the samples, respectively. The cyanobacterial composition at T0 on July 24, 2019, and August 05, 2019, were clustered separately into two groups (black and orange, respectively), thus implying that the cyanobacterial communities were distinct (Figure B.3A). At T0 of the study period, on July 24, 2019, in all of the mesocosms, *Microcystis* was the predominant genus, while on August 05, 2019, cyanobacterial composition comprised mostly of *Synechococcus*, *Dolichospermum*, and *Microcystis* (Figure B.3A,B). After 48 hours, on July 26, 2019, the cyanobacterial community composition was similar to that if the initial community composition in the control mesocosms,

whereas it experienced a substantial shift from *Microcystis* to *Synechococcus*, and *Dolichospermum* in the mesocosms containing doses of 20 and 35 mgFe/L (Figure B.3B), thereby confirming the results that are shown in the bar graph (Figure 6.1C,D). Similarly, after 48 hours, in the control mesocosms on August 05, 2019, the cyanobacterial composition was similar to that of the inital composition. A similar trend was observed in the mesocosms that had a dose of 20 mgFe/L, while the cyanobacterial composition changed in the mesocosms containing the dosage of 35 mgFe/L.

The PCA analysis was also utilized to evaluate the relationship between the cyanobacterial community and the cyanotoxins (Figure B.4). A relationship between the total intracellular microcystin (ΣMCs) and the intracellular toxin MC-LR with the genus *Microcystis* which was detected through metagenomics was found at T0 and T48 in all of the mesocosms. This is consistent with our previous observation using taxonomic cell counts where we found that the highest ΣMCs occured in the periods when *Microcystis* was dominant [185]. It also suggests that the potentially toxigenic genera that were correlated with the microcystin concentrations were *Microcystis* and *Dolichospermum* in a study of 22 lakes in southern Quebec [210]. In addition, a recent study observed that $Fe_2(SO_4)_3$ can remove more than 93% of the *Microcystis* cells with the a dose of 35 mgFe/L in a mesocosm experiment [185]. Therefore, the application of $Fe_2(SO_4)_3$ may be considered to be an efficient onsite barrier against toxin-producing cyanobacteria when it is added to the source water before it enters a drinking water treatment plant.

6.3.2.2 Effect of Coagulation on Cyanobacterial Community Richness and Diversity

To assess the effect of coagulation on the cyanobacterial community richness and diversity, the species richness and Shannon indices were utilized (Figure 6.3). The richness index is used for estimating the number of different species that are present in an environment and the Shannon index is used for measuring the homogeneity in the abundance of the different species in a sample [134, 241].

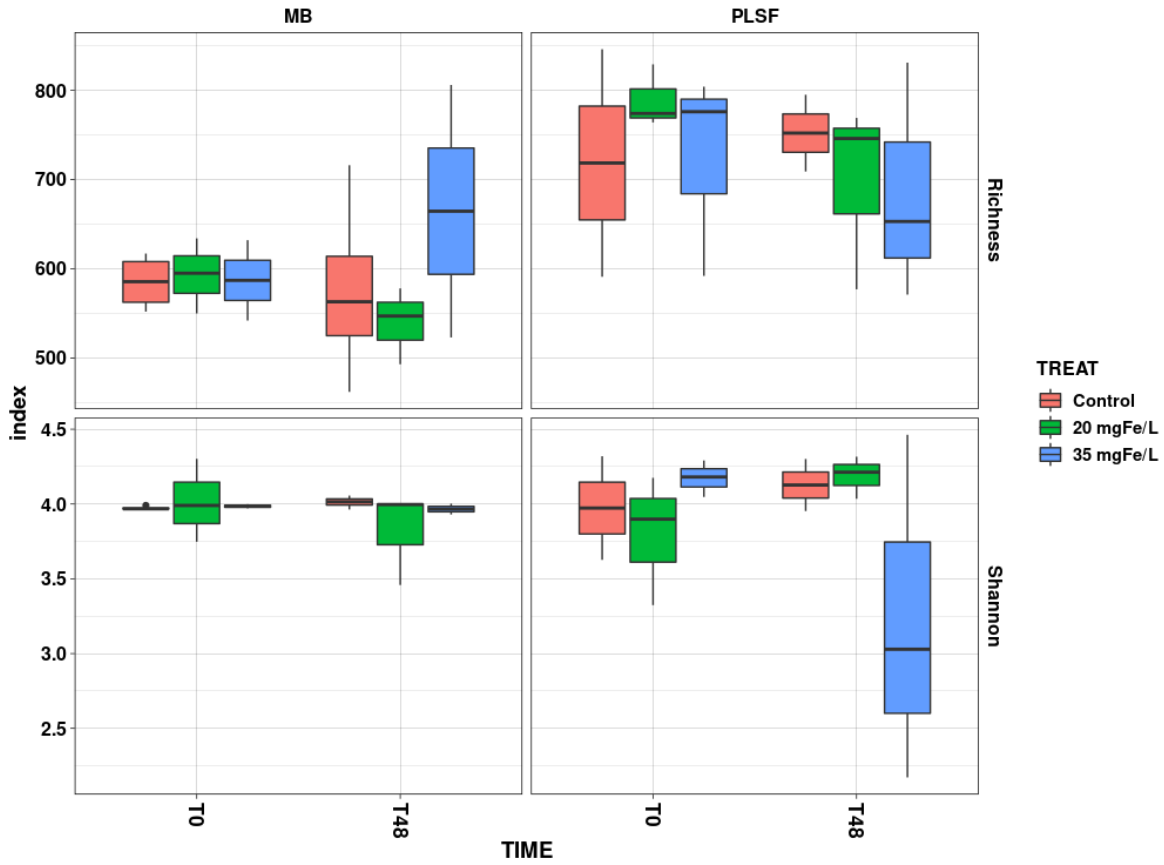

Figure 6.3 Evaluation of cyanobacterial richness and diversity in control, 20 mgFe/L and 35 mgFe/L mesocosms in Missisquoi Bay and Petit Lac St. François after two days of treatment. The bottom and top of the box shows the lower and upper quartiles, the band in between them shows the median, whiskers show the minimum and maximum.

In MB, the species richness and Shannon indices did not significantly change in any of the mesocosms after 48 hours of their exposure (Table B.8). The average species richness index decreased in the control mesocosms and in the mesocosms containing 20 mgFe/L, while an opposite trend was observed in the mesocosms containing 35 mgFe/L at T48 (Figure 6.3). This could be attributed to the reduction in the dominant species with a high abundance, such as *Dolichospermum* spp. and *Microcystis* spp., and this could potentially increase the genus richness [183, 242]. Furthermore, a prior study demonstrated that a dose of 35 mgFe/L was more efficient than a dose of 20 mgFe/L was in reducing the total cell counts and certain dominant genera such as *Dolichospermum*, *Microcystis*, and *Aphanizomenon* [185]. The Shannon index dropped slightly

following the administration of 20 mgFe/L, while it remained almost stable in the control and mesocosms with 35 mgFe/L (Figure 6.3).

Similarly, there was no significant change in the species richness and the Shannon indices between T0 and T48 in all of the mesocosms in PLSF (Table B.8). The decrease in the species richness index was observed in the coagulated mesocosms, while it increased slightly in the control mesocosms (Figure 6.3). The decline in the species richness index could be the result of the presence of a genus that could no longer be identified [34]. The Shannon index at T0 (approx. 4) showed that there was a slight change in the diversity profile at T48 (around 4.2) in the control and the 20 mgFe/L mesocosms. In contrast, the Shannon index decreased in the mesocosms with 35 mgFe/L when it was compared with that of the control.

6.3.3 Impact of Environmental Conditions on Cyanobacterial Community Composition in the Mesocosms Before and After Coagulation

To assess the impact of the environmental conditions on the cyanobacterial communities in MB and PLSF. A redundancy analysis (RDA) was used to evaluate the relationship between the environmental parameters (Table B.3, Table B.4, Table B.5, Table B.6, and Table B.7) and the cyanobacterial communities. The total nitrogen (TN), dissolved nitrogen (DN), dissolved phosphorus (DP), and dissolved organic carbon (DOC) exerted significant effects ($\rho < 0.05$) on the community profiles in MB (Figure 6.4).

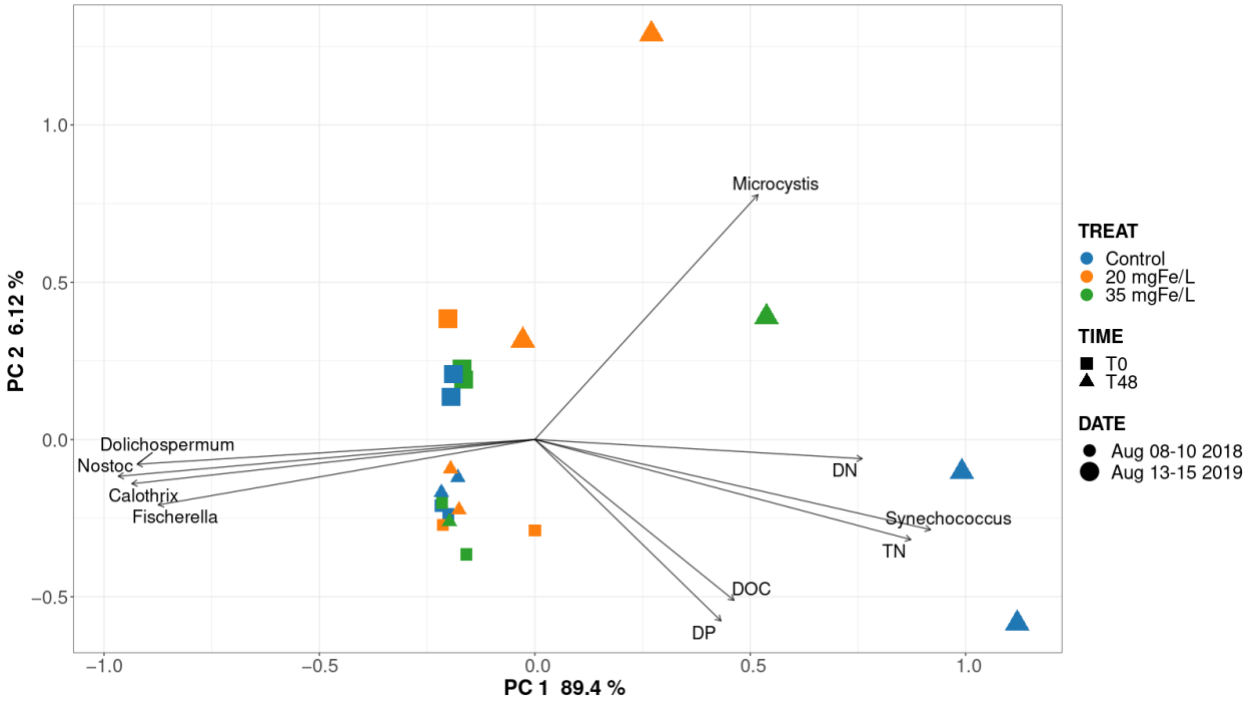

Figure 6.4 Redundancy analysis (RDA) of environmental parameters with respect to cyanobacterial communities in the control, 20 mgFe/L and 35 mgFe/L mesocosms in Missisquoi Bay. Only significant parameters ($\rho < 0.05$) are shown

In MB in 2018 and 2019, PC1 and PC2 represented 89.4% and 6.12% of the variation, respectively (Figure 6.4). A relationship between the environmental conditions and the cyanobacterial community composition was not observed in any of the mesocosms from August 08 and 10, 2018. The result corresponds with that of previous study which reported that these nutrients (TN, DN, TP, and DOC) did not affect the cyanobacterial community in the supernatant water after coagulation [55]. In contrast, TN, DN, DOC, and DP had a strong impact on the *Synechococcus* in the control mesocosms, while DN contributed to the presence of *Microcystis* in the coagulated mesocosms at T48 on August 15, 2019, in MB.

In PLSF, PC1 and PC2 represented 89.4% and 6.12% of the variation, respectively (Figure B.5). A clear association was observed between the DN and *Microcystis* in the control mesocosms on July 24 and 26, 2019, in the mesocosms with 20 and 35 mgFe/L at T0 on July 24, 2019, and in the mesocosms with 35 mgFe/L on August 07, 2019, where *Microcystis* was the dominant genus. In contrast, the DN was negatively related to *Dolichospermum* at both sites. Therefore, the persistence

of *Dolichospermum* in the mesocosms could be the result of its environmental resistance [55]. Our result is in accordance with those of previous studies, reporting that the DN may lead to the dominance of *Microcystis* [23, 27, 29].

6.4 Conclusions

- The shotgun metagenomic sequencing method is a more robust method that is used identify cyanobacterial communities when it is compared with the microscopic methods.
- In the beginning of the sampling period, *Dolichospermum* was the predomiant genus in Missisquoi Bay, while *Microcystis* was a common genus in all of the mesocosms in Petit Lac St. François.
- The change in the cyanobacterial composition at the genus level in the mesocosms after two days varied across the studied sites and over the sampling time. Therefore, the choice to use $Fe_2(SO_4)_3$ as an onsite source-control treatment should be made while considering its impact on the cyanobacterial community structure.
- *Synechococcus* may be satisfactorily removed in coagulated mesocosms.
- The intracellular microcystin concentrations were strongly associated with the presence of *Microcystis*.
- The cyanobacterial community richness and diversity did not change significantly after the coagulation by $Fe_2(SO_4)_3$ in any of the mesocosms at either of the sites.
- The dissolved nitrogen content was related to *Microcystis* in both Missisquoi Bay and Petit Lac St. François, while *Synechococcus* was influenced by the total nitrogen, dissolved nitrogen, dissolved organic carbon, and dissolved phosphorus contents.
- The source water coagulation process is a potential option for removing toxin-producing cyanobacterial species and intracellular toxins from the water column.

6.5 Materials and Methods

6.5.1 Study Sites Description

Missisquoi Bay (MB) and Petit Lac St. François (PLSF) were selected as study sites because of the frequent presence of cyanobacterial blooms [29, 81]. MB is located in southern Quebec (Canada; 45°02´23´´ N; 73°04´41´´ W). It is shallow and eutrophic, with a surface area of 77.5 km^2, an average depth of 2.8 m, and its groundwater and runoff sources are from predominantly agricultural lands via its tributary rivers [243, 244]. MB's tributaries have high turbidity and excessive concentrations of nitrogen and phosphorus [192, 243]. PLSF is located in south-western Quebec (Canada; 45°32´16´´ N; 72°02´11´´ W; max depth (Z) = 1.8 m). This lake is relatively small, shallow, with half of its watershed being occupied by agricultural land use, and there being high concentrations of phosphorus, resulting in frequent cyanobacterial blooms [29].

6.5.2 Description of the Mesocosm Experiments

The mesocosms were designed according to Wood's work in [184]. A mixture of raw lake water and cyanobacterial bloom scum was used to ensure that there were homogeneous initial conditions within the mesocosms. Sets of duplicate mesocosms (n = 6) were then filled with that mixed water of 20 L and were left unamended to serve as a control, or amended with two doses of the ferric sulfate coagulant: 20 mgFe/L and 35 mgFe/L. The samples were mixed immediately after the addition of the coagulant [185]. Seven mesocosm experiments were initiated with the appearance of cyanobacterial blooms (Table B.1 and Table B.2).

6.5.3 Preparation and Application of Chemical Coagulant

Standard liquid grade $Fe_2(SO_4)_3$ with 12.2% Fe content was purchased from Kemira Water Solution, Inc. (Varences, QC, Canada). Two different doses of $Fe_2(SO_4)_3$ coagulant (20 mgFe/L and 35 mgFe/L) were selected based on a previous study [185].

6.5.4 Sampling and Filtration Procedure

At the beginning (T0) (before adding $Fe_2(SO_4)_3$) and after two days (T48) of the sampling period, the water in the mesocosms was collected in 1 L sterilized high-density polyethylene (HPDE) bottles. For phytoplankton enumeration, the samples were taken in 40 mL glass vial and preserved

in Lugol's iodine solution. At the sampling site, a 50 mL sample was immediately filtered through a 0.22 μm filter (Millipore Sigma, Oakville, ON, Canada) in sterile conditions that were created with a burner stand. The filters were immediately stored with dry ice during their transfer to the lab where they were frozen at -80°C for a metagenomic analysis. Samples of 120 mL were filtered on hydrophilic polypropylene; 0.45 μm filters (PALL, Mississauga, ON) were used for the toxins. Samples for the total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) were aliquoted without being filtrated. Dissolved organic carbon (DOC) samples were filtered using a 0.45 μm filter membrane (PALL, Mississauga, ON, Canada). A 0.45 μm filter membrane (Millipore Sigma, Oakville, ON, Canada) was used for subsamples of dissolved nitrogen (DN), dissolved phosphorus (DP), and soluble reactive phosphorus (SRP). Genomic samples were taken in triplicate while nutrient samples were taken in duplicate. More detail are available in [185].

Samples for taxonomic cell counts were stored at room temperature in the dark. TN/TP/TOC/DOC samples were stored at 4 °C and DN/DP/SRP samples were kept at -25 °C until the analysis was conducted.

6.5.5 Sample Analysis

6.5.5.1 Taxonomic Cell Counts

Taxonomic cell counts were performanced by using an inverted microscope with a Sedgwick-Rafter counting chamber at 40X magnification (Leica Microsystems GmbH, Wetzlar, Germany) according to [160, 226, 227].

6.5.5.2 Nutrient Analysis

Standard methods 353.2 and 350.1 were used for analyzing nitrogen [231]. TOC and DOC were measured based on method 415.1 [230]. Standard method numbers 365.3 and 365.1 [232] were used for phosphorus and phosphate analysis.

Phycocyanin, chlorophyll-*a*, pH, dissolved oxygen (DO), and temperature were measured using a YSI EXO2 water quality multi-parameter sonde (YSI Inc., Yellow Springs, Ohio, USA) [160, 181].

6.5.5.3 Toxin analysis

The total microcystins samples were analyzed via a Lemieux oxidation step and an online solid phase extraction (SPE) and salting which were coupled with ultrahigh performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). The accuracy was in the range from 93 to 100%, and it was deemed satisfactory if it was in this range, and the limit of detection was 0.5 ng/L. More details can be found in [185].

6.5.5.4 DNA Extraction, Metagenomics Preparation and Bioinformatics Analysis

The sample filters were kept in 5 mL Falcon® tubes (VWR, USA) at -80° C and were used for DNA extraction. Total nucleic acid was extracted using this filter by RNeasy® PowerWater® Kit (Qiagen Group, Germantown, MD, USA). 200 μL of nuclease-free water and 5 μL of TATAA Universal DNA spike II (TATAA Biocenter AB, Gothenburg, Sweden) were filtered to quantify extraction yields. Dithiothreitol (DTT) was injected into RNeasy® PowerWater® Kit solution PM1 buffer to prevent formation of disulfide bonds protein residuals. Total nucleic acid was eluted with 60 μL nuclease-free water and 30 μL was used for DNA. After resuspending in 60 μL of nuclease-free water, each DNA was quantified with a Qubit® v.2.0 fluorometer (Life Technologies, Burlington, ON, Canada). To perform the pyrosequencing (Roche 454 FLX instrumentation with Titanium chemistry), 30 μL of DNA was sent to the Génome Québec Innovation Centre for sequencing.

An Illumina NovaSeq 6000 S4 (Illumina Inc., San Diego, CA, USA) was used for sequencing DNA libraries. A homemade bioinformatic pipeline was applied for analysis of paired-end raw reads of 150 base pairs (bp). The raw reads were trimmed to ensure their quality, which was firstly performed using SolexaQA v3.1.7.1 using the default parameters [245]. Next, the trimmed reads that were shorter than 75 nt were analysed. Artificial duplicates were removed using an in-house script based on the screening of identical reads of 20 bp. FragGeneScan-Plus v3.0 was used to predict gene fragments based on the trimmed high-quality reads [246]. Then, predicted fragments of protein were clustered at 90% similarity using cd-hit v4.8.1 [247]. More details are available in [34].

6.5.6 Statistical Analysis

Statistical analysis was performed using R statistical software (R Core Team, 2020). Taxanomic data were normalized by the centred log-ratio transformation using easyCODA (v.0.34.3) [248]. The alpha diversity metrics (Richness and Shannon) was analyzed using vegan package (v.2.6-2) [249], and the similarly matrices were calculated based on the Euclidean distance. The differences between the groups in alpha diversity were determined using Kruskal-Wallis test. Redundancy analysis (RDA) was performed to evaluate the impact of environmental conditions on cyanobacterial community in MB and PLSF, with the vegan package (https://cran.r-project.org/package=vegan, assessed on 10 June 2022).

CHAPTER 7 ARTICLE 3: THE EFFECT OF NITROGEN ON CYANOBACTERIA AND CYANOTOXINS

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7.1 Abstract

Toxic cyanobacterial blooms are acconcern across the globe. Nutrients are among the numerous factors that trigger cyanobacterial blooms and the production of cyanotoxins. Many jurisdictions regulate phosphorus loads to control eutrophication while nitrogen regulations are often based on ammonia toxicity. The aim of this study was to assess the effect of nitrogen on cyanobacteria and cyanotoxins though a short term mesocosm experiment. To achieve this, mesocosms were installed in situ in two lakes (Missisquoi Bay and Petit Lac St. François) and received 700 µg/L of NH_4^+ or 500 µg/L of NO_3^-. Total taxonomic cell counts, *Dolichospermum* and *Aphanizomenon*'s cell counts significantly decreased in the mesocosms containing NH_4^+ as compared to the control mesocosms while total taxonomic cell counts, and genus' cell counts in NO_3^- mesocosms did not significantly change compared to control mesocosms after two days following nitrogen addition. A shift to *Microcystis* in the mesocosms with nitrogen addition was observed two days after the addition in Missisquoi Bay where nitrogen was more limited as compared to Petit Lac St. François based on nutrient ratios. There were no significant changes in intracellular toxins of total microcystins (ΣMCs) between control mesocosms and mesocosms with the addition of NH_4^+ or NO_3^- after two days of exposure at both sites. However, there was significant increase of extracellular ΣMCs and MC-LR in mesocosms with NH_4^+ or NO_3^- addition after 48 hours as compared to control mesocosms. Intra- and extracellular microcystin concentrations were associated with *Microcystis*. *Microcystis* presence was associated with NH_4^+ 48 hours after the

addition of N at both sites. Thus, toxin production following sudden nitrogen addition can occur on short time scales relevant to drinking water treatment plant operation. This information will help treatment plant operators better anticipate the arrival of potentially higher concentrations of cyanotoxins.

7.2 Introduction

Harmful cyanobacterial blooms are becoming more common in surface waters around the world [250]. Several toxin producing cyanobacterial genera are of concern in water supplies. The most common cyanobacterial toxins include hepatotoxins (microcystins and nodularins), neurotoxins (e.g. anatoxin-*a*), cytotoxins (cylindrospermopsins), dermatotoxins (e.g. lyngbyatoxin) [66, 186] and microcystins, which are among the most frequently measured toxins produced by *Microcystis*, *Planktothrix* and *Dolichospermum* [7, 251]. Cyanobacterial toxins are a potential threat to aquatic organisms and human health [1].

Nitrogen (N) is one of the environmental factors influencing the growth of cyanobacterial blooms and cyanotoxins [76]. N has played an important role in bloom formation, particularly on non-N_2-fixing cyanobacteria, such as *Microcystis* and *Planktothrix* [23, 84]. In previous studies, the impact of different forms of N on cyanobacterial abundance and cyanotoxins has been evaluated [24, 133]. In a mesocosm study, the addition of nitrate (NO_3^-), and ammonium (NH_4^+) in hypereutrophic water bodies increased the abundance of total phytoplankton and increased microcystin production by up to 13-fold in 21 days [24]. Similarly, another study found that when N was added to a reservoir in Oregon (United States) through large-scale *in-situ* mesocosms during a three-month period, the concentration of microcystin was higher [133]. A recent study conducted mesocosm bioassays to determine the effect of different concentration of N (3, 6, 9, and 15 mg/L) and P (0.01, 0.02, 0.05, 0.1 and 0,2 mg/L) on algal growth in 10 days. The results indicated that nitrogen treatment stimulated cyanobacteria (mostly *Microcystis aeruginosa*) more than phosphorus. However, general nutrient limitation (P or N) will always play a role in a given environment and nutrient ratios can provide insight into the potential for bloom formation [132].

Recently, high throughput sequencing has been applied to evaluate the impact of nitrate (NO_3^-) and ammonium (NH_4^+) on the microbial community structure of freshwater [37] and showed no

significant shift in the diversity of the bacterial community following the addition of NO_3^- and NH_4^+ [37].

To our best of knowledge, no *in-situ* mesocosm experiment using fresh blooms have been conducted to investigate the short term effects of NO_3^- and NH_4^+ on cyanobacterial cells and their toxins. Although long term effects of nutrients on blooms and toxin production have been well established, there is a need for data on shorter time scales needed for management of drinking water sources that must continuously respond to fluctuations of water quality in real time. Furthermore, no study has looked at the differences in cyanobacterial community assessed by using microscopic and shotgun metagenomic techniques after NH_4^+ and NO_3^- addition. In this context, the primary goals of this research were to determine the effects of NH_4^+ and NO_3^- on cyanobacterial community composition, and their related toxins on a short time scale replicating a sudden increase of nitrogen that could occur as the result of the addition of stormwater with agricultural runoff or municipal effluents. Drinking water treatment plants are operated continuously and such information is needed for the short-term prediction of bloom toxicity in source waters affected by cyanobacterial blooms. In many jurisdictions, including Québec, regulations to limit the eutrophication of water bodies focus predominantly on phosphorus and nitrogen is regulated primarily for its toxicity without considering overall loads. This research also broadly aims to provide data in support of decision making with regards to nitrogen regulation in drinking water sources.

7.3 Materials and Methods

7.3.1 Study sites

Two selected sites were the Petit Lac St. François (PLSF) and Missisquoi Bay (MB). PLSF is located in south-western Quebec (Canada; 45° 32´ 16´ N; 72° 02´ 11´ W; max depth (Z) = 1.8 m). The PLSF lake is relatively small, shallow and half of its watershed is occupied by agricultural areas, resulting in excessive concentrations of phosphorus and cyanobacterial blooms [29]. Missisquoi Bay is a eutrophic and shallow (max. depth (Z) = 4.75 m) embayment of Lake Champlain in Southern Québec (Canada; 45° 02´ 23´ N; 73° 04´ 41´ W) [192]. Because of high nutrient loads from agricultural activities in the region's watersheds, cyanobacterial appearance is frequent and of concern [252, 253].

7.3.2 Nutrient Addition Experiments

We conducted *in-situ* mesocosm experiments over eight different cyanobacterial bloom events and each experiment lasted for 48 hours (Table C.1 and Table C.2). The mesocosm structure was based on the study of [184] and the experimental procedure was based on the study of [185]. To begin the experiment, lake water and fresh cyanobacteria scums were harvested at the field sites, pooled and mixed thoroughly in a large container to achieve homogeneity. Then, a total of six mesocosms (including duplicates) were filled with a mixture of 21 L. Mesocosms were left unamended to serve as a control (control mesocosms), added with nitrate (NO_3^- 500 µg/L), or ammonium (NH_4^+ 700 µg/L).

7.3.3 Preparation and Application of Nutrients

Concentration of NO_3^- and NH_4^+ were monitored when blooms appeared in July, August, September and, October in 2017 to prepare for the experiment in MB and PLSF in 2018 and 2019 (Table 7.1).

Table 7.1 Concentrations of nitrogen in surface water in Missisquoi Bay and Petit Lac St. François in 2017. Adapted from [55]

Site	**Date**	**NO_2^- + NO_3^-** **µg N/L**	**NH_3 + NH_4^+** **µg N/L**
MB	27 July 2017	120	130
	25 August 2017	80	90
	05 September 2017	110	80
	27 October 2017	130	70
PLSF	01 August 2017	20.68	26.27
	12 September 2017	16.33	22.07

Based on the concentrations of $NO_2^- + NO_3^-$ and $NH_3 + NH_4^+$ monitored in MB and PLSF in 2017 (Table 7.1), NO_3^- (500 µg N/L) and NH_4^+ (700 µg N/L) were added to the mesocosms to ensure their final mixed concentrations were higher than their initial concentrations (i.e. prior to adding nitrogen to the mesocosms). The final concentrations of NO_3^- and NH_4^+ after addition were in the range that enhance the cyanobacterial growth following reference [254, 255].

7.4 Sampling, Filtration Procedure

7.4.1 Sampling Procedure

Water samples were collected from the center of each mesocosm using two 1 L sterilized high-density polyethylene (HPDE) bottles before adding the nutrients (KNO_3 and NH_4Cl) at time zero (T0), and after the addition of the nutrients at 48 hours (T48). Samples were used for analyzing genomic, taxonomy, toxin (intracellular and extracellular), and nutrients including total nitrogen (TN), total phosphorus (TP), dissolved nitrogen (DN), dissolved phosphorus (DP), oxidized nitrogen ($NO_3^- + NO_2^-$), ammonia and ammonium ($NH_3 + NH_4^+$), Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP).

7.4.2 Filtration Procedure

For genomic analyses, a subsample of 50 mL was filtered though a 0.22 µm membrane (Merck Millipore, Ireland) in sterile conditions. Samples were filtered immediately at field sites and kept on dry ice box on the way to the laboratory. The filter membrane was kept in a 50 mL falcon tube (VWR, USA) and frozen at -80^{0}C prior to analysis.

For toxins, a subsample of 120 mL was filtered on a hydrophilic polypropylene (GHP) 0.45 µm membrane (PALL, USA). A petri dish (Millipore SAS, France) was used to keep the filter (for intracellular toxins) and the filtrate (for extracellular toxins) was kept in a 125 mL amber Nalgene™ polyethylene terephthalate glycol (PETG) bottle (Thermo Scientific Nalgene, Waltham, MA, USA). Toxins samples were filtered immediately at field sites and kept on dry ice on the way to the laboratory. They were stored at -25°C prior to analysis.

For enumeration of cyanobacteria, water samples were taken in 40 mL vials and preserved in Lugol's iodine solution. TN and TP were collected in 120 mL HPDE bottles. A 0.45 µm membrane

(Merck Millipore, Ireland) was used for NO_3^- + NO_2^-, NH_3 + NH_4^+, SRP and DN/DP. TN/TP/DN/DP were stored at 4°C, NO_3^- + NO_2^-, NH_3 + NH_4^+, SRP were frozen at -25°C prior to analysis.

7.5 Sample analysis

7.5.1 Taxonomic Cell Counts

Taxonomic identification and counts were performed by using inverted microscopy in a Sedgwick-Raffer chamber 40x magnification (Leica Microsystems GmbH, Wetzlar, Germany) [160, 185, 226].

7.5.2 Cyanotoxins

Samples were quenched, filtered and directly analyzed though solid phase extraction (SPE) and desalting coupled to ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). The online sample loading volume of 5 mL was achieved at a flow rate of 2500 µL/min into a Thermo Hypersil Gold aQ column (C18, 20 mm × 2 mm, 12 µm particle size) hereafter named on-line SPE column. After sample loading, analytes were injected into to a Thermo Hypersil Gold column with C18 selectivity (100 mm × 2.1 mm, 1.9 µm particle size), hereafter named analytical column at 500 µL/min. Then, samples were analyzed for total microcystins by UHPLC-MS/MS. The method limit of quantification was 0.5 ng/L. Standards were purchased from Enzo Life Science (Farmingdale, NY, USA). Accuracy proved satisfactory in the range of 93 – 110%. More details are provided in [185, 228].

7.5.3 Nutrients

Method 415.1 was used for analyzing TOC and DOC [230]. Total nitrogen, dissolved nitrogen, nitrate, nitrite and ammonia were analyzed according to Standard Methods 353.2 and 350.1 [231, 256]. Standard Method 365.3 was applied for total and dissolved phosphorus and soluble reactive phosphorus [232].

The sampling, filtration and analysis procedure followed a method described elsewhere [185].

7.5.4 DNA Extraction, Metagenomics Preparation and Sequencing

The filters frozen for DNA extraction were kept in 5 mL falcon tubes (VWR, USA) at -80°C. Total nucleic acid was extracted from this filter by RNeasy PowerWater Kit (Qiagen Group, Germantwon, MD, USA). A volume of 200 μL of nuclease-free water and 5 μL of TATAA Universal DNA spike II (TATAA Biocenter AB) were injected to filter for quantify extraction yields. Dithiothreitol (DTT) was injected into RNeasy PowerWater Kit solution PM1 buffer to prevent formation of disulfide bonds protein residuals. Total nucleic acid was eluted with 60 μL nuclease-free water and 30 uL was used for DNA. After resuspended in 60 μL of nuclease-free water, DNA was quantified with a Qubit v.2.0 fluorometer (Life Technologies, Burlington, ON, Canada). For performing pyrosequencing (Roche 454 FLX instrumentation with Titanium chemistry), 30 μL of DNA was sent to the Genome Quebec Innovation Centre for sequencing [257].

An Illumina NovaSeq 6000 S4 was used for sequencing DNA libraries. A homemade bioinformatic pipeline was applied for analysis of Paired-end raw reads of 150 base pairs (bp). Raw reads trimming quality was first performed using SolexaQA v3.1.7.1, default parameters [245]. Next, trimmed reads shorter than 75nt were analysed. Artificial duplicates was removed using an in-house script based on the screening of identical leading 20 bp. FragGeneScan-Plus v3.0 was applied to predict gene fragment based on the trimmed high-quality reads [246]. Then, predicted fragments of protein were clustered at 90% similarity using cd-hit v4.8.1 [247]. More details are in [34, 55, 185].

7.5.5 Statistical Analysis

Statistical analysis was performed by R (R Core Team, 2020). All of the plots in this paper were generated by ggplot2 package in R (https://ggplot2.tidyverse.org, accessed on 01 March 2022). Principal component analysis (PCA) was performed to evaluate the impact of environmental conditions on cyanobacterial community and cyanotoxins in MB and PLSF, it is with the vegan (https://cran.r-project.org/package=vegan, accessed on 10 April 2022). Statistical tests were determined using Kruskal-Wallis test. Statistical significance was set at a 0.05 *p*-value cut-off ($p < 0.05$). Imputation of missing value is conducted by using the left-censored imputation method in

MICE (https://cran.r-project.org/package=mice, accessed on 05 May 2022) and QGCOMP packages (https://cran.r-project.org/package=qgcomp, accessed on 20 May 2022).

7.6 Results and Discussions

7.6.1 The concentration of various forms of nutrients

7.6.1.1 Initial conditions (T0), before adding nitrogen

Nitrogen fraction (dissolved organic nitrogen (DON), ammonia and ammonium ($NH_3 + NH_4^+$), total Kjeldahl nitrogen (TKN), oxidized nitrogen ($NO_3^- + NO_2^-$), total nitrogen (TN), dissolved nitrogen (DN)) and phosphorus fraction (soluble reactive phosphorus (SRP), dissolved organic phosphorus (DOP), dissolved phosphorus (DP), particulate P (PP), total P (TP)) were measured for the samples taken in all mesocosms in the start of sampling period (T0) on July 31 and September 24 in 2018, August 13 and September 20 in 2019 in Missisquoi Bay (MB) (Table C.1) and on June 26, July 24, August 05 and October 09 in 2019 in Petit Lac St. François (PLSF) (Table C.2).

In MB, in both 2018 and 2019, the concentrations of TN and TP in the mesocosms were higher in mid-summer and lower in early fall (Table C.1). On July 31, 2018, TN and TP reached 5.8 mg N/L and 1311 µg P/L, respectively, before dropping to 2.82 mg N/L and 337 µg P/L on September 24, 2018. Similarly, TN and TP concentrations peaked at 8.89 mg N/L and 3101 µg P/L on August 13, 2019 before rapidly declining to 0.59 mg N/L and 780 µg P/L on September 20, 2019 (Table C.1). At T0, the most dominant N and P fractions in both studied sites were TKN and PP, respectively (Table C.1).

A different trend in TN and TP was observed at PLSF, wherein the concentrations of TN and TP in the mesocosms were lower in mid-summer and higher in early fall and early summer (Table C.2). TN concentrations were exceptionally high on June 26, 2019 (11.45 mg N/L) and October 09, 2019 (30.0 mg N/L), but they were significantly lower on July 24, 2019 (1.28 mg N/L) and August 05, 2019 (1.12 mg N/L) (Table C.2). TKN and DON were the most prevalent N fractions found, while $NO_3^- + NO_2^-$ and $NH_3 + NH_4^+$ levels were lower. The high concentration of TKN suggested that the primary forms of N were organic [70]. PP was the most common form of P. In the months of June, July, August, and October of 2019, the TP followed the same trends as the TN. The concentration of TP was high on June 26, 2019 (670 µg P/L), then declined on July 24, 2019

(112 µg P/L), remained lower on August 05, 2019 (137 µg P/L), before increasing again on October 09, 2019 (1550 µg P/L) (Table C.2). In both MB and PLSF, a linear association between TKN and PP with total cyanobacterial biomass ($R^2 \geq 0.98$) was observed (Figure C.1, Figure C.2, Figure C.3, and Figure C.4). During cyanobacterial blooms, the organic and particulate fractions of N and P fractions have been linked with cyanobacterial biomass rather than the amount of bioavailable compounds in water [70].

We also looked at the ratios of several N and P components in the mesocosms at T0 in MB and PLSF (Table C.1 and Table C.2), focusing on the TN:TP ratio as a general indicator and the DIN:SRP ratio as a measure of readily accessible nutrients [70]. In MB and PLSF, the TN:TP ratio ranged between 0.76 to 8.37 and 8.17 to 19.35, respectively. A low N:P ratio (<30) has been shown to promote the growth of cyanobacterial blooms [133, 258] and TN:TP < 22 can indicate N-limitation in fresh water [70, 259]. However, organic and particulate forms of N and P from cyanobacterial cells can influence TN and TP concentrations [70] and, therefore the ratio TN:TP was a byproduct of the presence of cyanobacterial blooms rather than the explanation in this study. In PLSF, the DIN:SRP ratio was in the range of 2 to 3 on July 24 and August 05 in 2019 and higher on June 26 (13.56) and October 09 (9.86) in 2019. The DIN:SRP ratio in MB was low on July 31, 2018 (4.45), August 13, 2019 (2.87) and September 20, 2019 (0.76), whereas it was higher on September 24, 2018 (24.8). DIN:SRP ratios of less than 10:1 have previously been shown to suggest significantly N-limiting circumstances that favour the growth and proliferation of N_2-fixing cyanobacteria [70, 260].

7.6.1.2 After two days of exposure (T48)

As expected, in MB and PLSF, the ratio of TN:TP in all of the mesocosms increased compared to the initial conditions but remained lower than 30 after two days following the addition of N (Table C.3, Table C.4, Table C.5, Table C.6, Table C.7, and Table C.8). The concentration of $NO_3^- + NO_2^-$ and $NH_3 + NH_4^+$ were higher at T48 comparing than at T0.

In MB, at T48, the DIN:SRP ratio was stable in the control mesocosm (Table C.3) but higher than its initial conditions in the mesocosms adding NH_4^+ 700 µg/L (8.13 – 118.19) and NO_3^- 500 µg/L (27.72 – 49.32) (Table C.5 and Table C.7). Similarly, it showed the same trend in PLSF (Table C.6 and Table C.8). In general, the DIN:SRP ratio changed from less than 13 (nitrogen limited

conditions) that favors the growth of N_2-fixing cyanobacteria at T0 to greater than 50 (phosphorus limited conditions) that enhances non-N_2-fixing-cyanobacteria at T48 in the mesocosms containing nutrients in both studied sites and is discussed further in Section 7.6.3.

As planned, there was a significant change in NH_4^+ concentration in the mesocosms adding NH_4^+ 700 µg/L comparing in the control mesocosms at T48 in MB. In PLSF, both NH_4^+ and NO_3^- concentration changed significanly in the mesocosms adding NH_4^+ 700 µg/L and NO_3^- 500 µg/L, respectively (Table C.9). Thus, the quantity of N added to the mesocosm was sufficient to change the concentrations in amended mesocosms.

7.6.2 The impact of NH_4^+ and NO_3^- on Total Taxonomic Cell Counts

Figure 7.1 shows the total taxonomic cell counts in the PLSF and MB mesocosms at the start of the sampling period (T0) and after 48 hours (T48).

In both PLSF and MB, the initial average taxonomic cell counts fluctuated among the experimental events but were greater than 10^4 cells/mL in all mesocosms (Figure 7.1). In MB, on July 31, 2018, *Dolichospermum* was the most numerous genus in all mesocosms, accounting for almost 85 % of total taxonomic cell counts (Figure C.5). On September 24, 2018, the dominant genera were more diverse including *Dolichospermum* (35.1% of total taxonomic cell counts), *Microcystis* (23.2%), and *Aphanothece* (25.1%) in all of the mesocosms. Interestingly, *Aphanizomenon* and *Aphanothece* were the most abundant genera on August 13 and September 20, 2019, respectively (Figure C.5). Our findings were in line with a short-to-long-term study of cyanobacterial dynamics in MB assessed by taxonomy cell counts and shotgun metagenomics [34, 55, 185].

Dolichospermum and *Microcystis* were the most common bloom forming cyanobacteria in all PLSF mesocosms at T0 (Figure C.6). We found that *Dolichospermum* was dominant on June 26, 2019 and October 09, 2019 (greater than 73% of total taxonomic cell counts), whereas *Microcystis* dominated on July 24, 2019 and August 05, 2019 (around 50-75% of total taxonomic cell counts) (Figure C.6). Our findings were consistent with prior findings that *Dolichospermum* peaked in early spring and late autumn, but *Microcystis* dominated during summer season in eutrophic lakes [261, 262]. For both the July 24, 2019 and August 05, 2019 experiments, *Aphanizomenon* (5-10% of total cell counts) and *Aphanothece* (10-25%) also showed up as representative genera.

After two days of exposure (T48), changes were observed with regards to total taxonomic cell counts in control mesocosms and mesocosms containing NO_3^- 500 µg/L or NH_4^+ 700 µg/L (Figure 7.1). A Krusal-Wallis statistical test was used to show differences in change (increase or decrease) of total taxonomic cell counts and species cell counts between the control mesocosms and mesocosms adding NO_3^- 500 µg/L or NH_4^+ 700 µg/L after two days of exposure (*p*-value <0.05 is significant). Total taxonomic cell counts, *Dolichospermum* and *Aphanizomenon*'s cell counts significantly changed in the mesocosms containing NH_4^+ as compared to the control mesocosms (Table C.10). In contrast, total taxonomic cell counts, and other genera's cell counts in NO_3^- mesocosms did not significantly change when compared to control mesocosms (Table C.10).

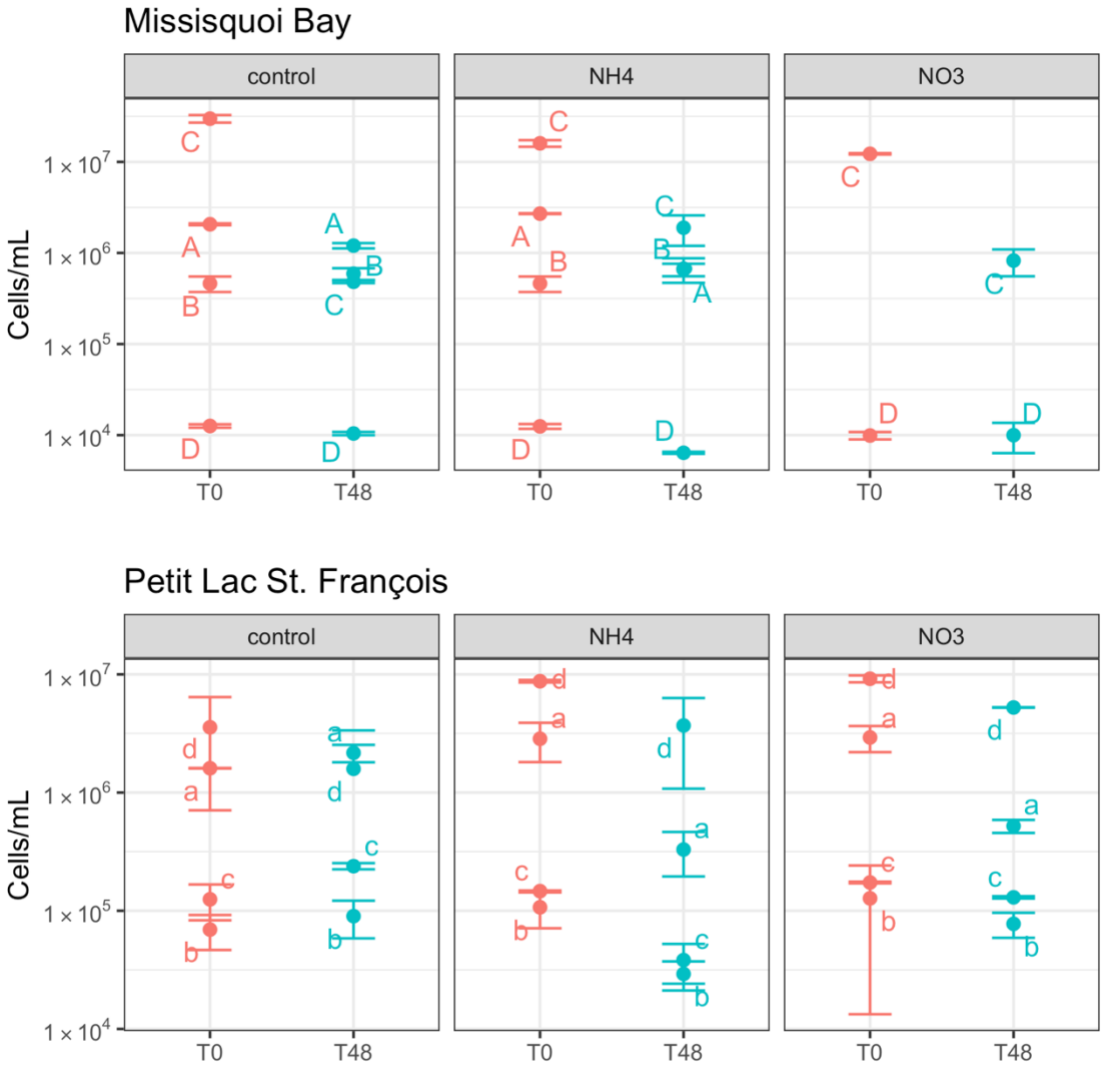

Figure 7.1 Total taxonomic cell counts in the control, NO_3^- (500 µg N/L) and NH_4^+ (700 µg N/L) mesocosms (Mean ± standard deviation). A) July 31-August 02, 2018, B) September 24-26, 2018, C) August 13-15, 2019, D) September 20-22, 2019; a) June 26-28, 2019; b) July 24-26, 2019; c) August 05-07, 2019; d) October 09-11, 2019. Data of mesocosms NO_3^- at event A and B were excluded because of unexpected conditions.

The relative cell abundance of individual cyanobacterial species remained stable in all mesocosms after 48 hours except for mesocosms containing NH_4^+ on June 28, 2019 in PLSF where *Microcystis* became the dominant genus in PLSF (Figure C.6). In MB, *Microcystis* became dominant in the mesocosms containing NO_3^- on August 15, 2019 (Figure C.5). These observations are in agreement

with several previous studies reporting that adding NO_3^- or NH_4^+ to lakes can promote *Microcystis* proliferation on a long-term time scale [18, 27, 31, 114, 115]. In addition, multiple laboratory investigations have shown that adding NH_4^+ and NO_3^- to the *Microcystis* species can help them develop faster [31, 119]. Our previous results showed that the *Microcystis* genus in particular was positively correlated with intracellular microcystin concentrations by mesocosm experiments [257].

Others have investigated the effects of different types of nitrogen on phytoplankton abundance in eutrophic waters, and discovered that NH_4^+ increased the abundance of non-N_2-fixing cyanobacteria (e.g. *Microcystis*) while NO_3^- favoured the formation of siliceous algae [24, 25]. In addition, *Microcystis* blooms took place in the presence of high concentrations of NH_3 in the water column [23, 70]. A recent study was conducted to investigate the effect of five different nitrogen (3, 6, 9, 12, and 15 mg/L) concentrations on phytoplankton growth through a mesocosm experiment. The result showed that *Microcystis* spp. in the N-treated mesocosms were higher than in the control especially in the higher doses after 10 days, but its concentration decreased slightly in both control mesocosms and mesocosms adding N after two days of exposure [132].

Despite the fact that NH_4^+ is a good source of N for cyanobacterial growth, concentrations of NH_4^+ greater than 0.08 mmol N/L have been demonstrated to suppress cyanobacterial growth, due to toxicity [119, 263-265]. In our study, six samples of $NH_4^+ + NH_3$ in the mesocosms adding NH_4^+ at T48 was higher than 0.089 mmol N/L (Table C.5 and Table C.6), then it could be a reason for observing the total cell counts declined in the mesocosoms adding NH_4^+ comparing to the control mesocosms in several events. Moreover, NH_4^+ is more biovailable to cyanobacterial growth at lower concentrations [117].

7.6.3 The Impact of NH_4^+ and NO_3^- on Cyanobacterial Relative Abundance Assessed by Shotgun Metagenomic Sequencing

Cyanobacterial community composition assessed by comparative metagenomics reads levels of genus during sampling periods is presented in Figure 7.2 and Figure 7.3. Results passing quality standards for DNA sequencing were available for September 24-26, 2018 and August 13-15, 2019 in MB and June 26-28, July 24-26 and August 05-07 in 2019 in PLSF.

Based on high throughput sequencing data, analysis of the relative abundance of the cyanobacterial community at the genus level in MB revealed *that Microcystis* (approximately 55 % of total relative abundance) and *Dolichospermum* (25 % of total relative abundance) were the dominant genera at T0 on September 24, 2018 (Figure 7.2). Our findings are consistent with prior research that found *Dolichospermum* and *Microcystis* dominated the cyanobacterial community in MB [34, 55, 181, 192]. After 48 hours of exposure (on September 26 2018), the cyanobacterial relative abundance remained stable in all mesocosms. In contrast, the experiment beginning on August 13, 2019 (Figure 7.2) showed shifts to *Microcystis* in mesocosms with nutrient addition whereas the shift was most notable to *Synechococcus* in the control mesocosm. Previous investigations in MB documented *Synechococcus* alternating before, during or after *Dolichospermum* and *Microcystis* blooms [55]. *Microcystis* has been associated with toxin production at Missisquoi Bay [257] and toxin production is further discussed in Section 7.6.4.

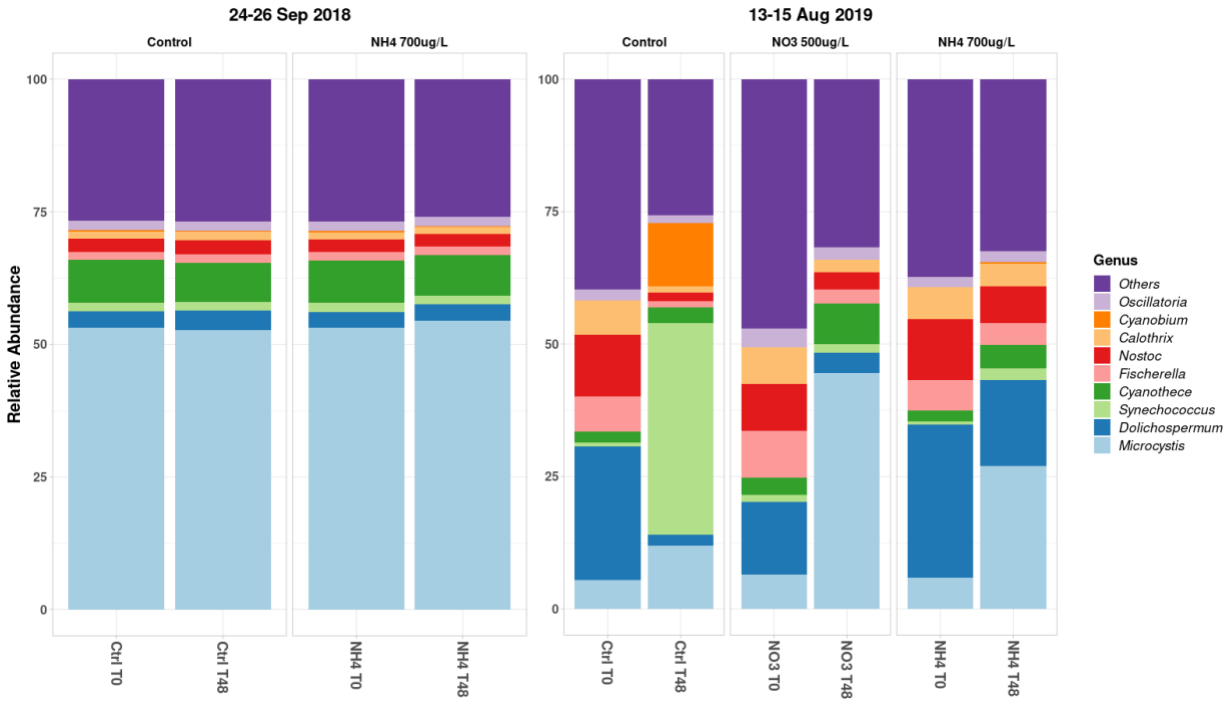

Figure 7.2 Relative abundance cyanobacterial community at genus level in Missisquoi Bay

In PLSF, at T0, *Microcystis* was the predominant genus accouting for more than 30-50% of total relative abundance at T0 on June 26, 2019 and July 24, 2019 while *Synechococcus* (13.5% total relative abundance), *Microcystis* (12.5%) and *Dolichospermum* (12.4%) were the predominant

genera on August 05, 2019 (Figure 7.3). After 48 hours of exposure, the relative abundance of cyanobacteria with regards to community composition had changed in all mesocosms depending on the sampling date. For example, at T48, *Microcystis* remained dominant and their relative abundance increased in all mesocosms on June 28, 2019. On August 07, 2019, the relative abundance of *Microcystis*, *Synechococcus* and *Dolichospermum* did not markedly change in the control mesocosms while the abundance of *Microcystis* was higher in the mesocosms with added nutrients. Interesly, *Synechococcus* became dominant in the mesocosms with added NO_3^- on July 26, 2019. This shift could be the result of random variability. *Synechococcus* can ulitize various nitrogen sources for growth and there was a no correlation between *Synechococcus* abundance and inorganic nutrient concentrations (NO_3^-, NO_2^-, NH_4^+ and PO_4^-) [266-268]. The change of cyanobacteria composition in the control mesocosms after two days were in agreement with other reported results on the changes in cyanobacterial composition and variation of the cyanobacterial community in the lake [34, 269].

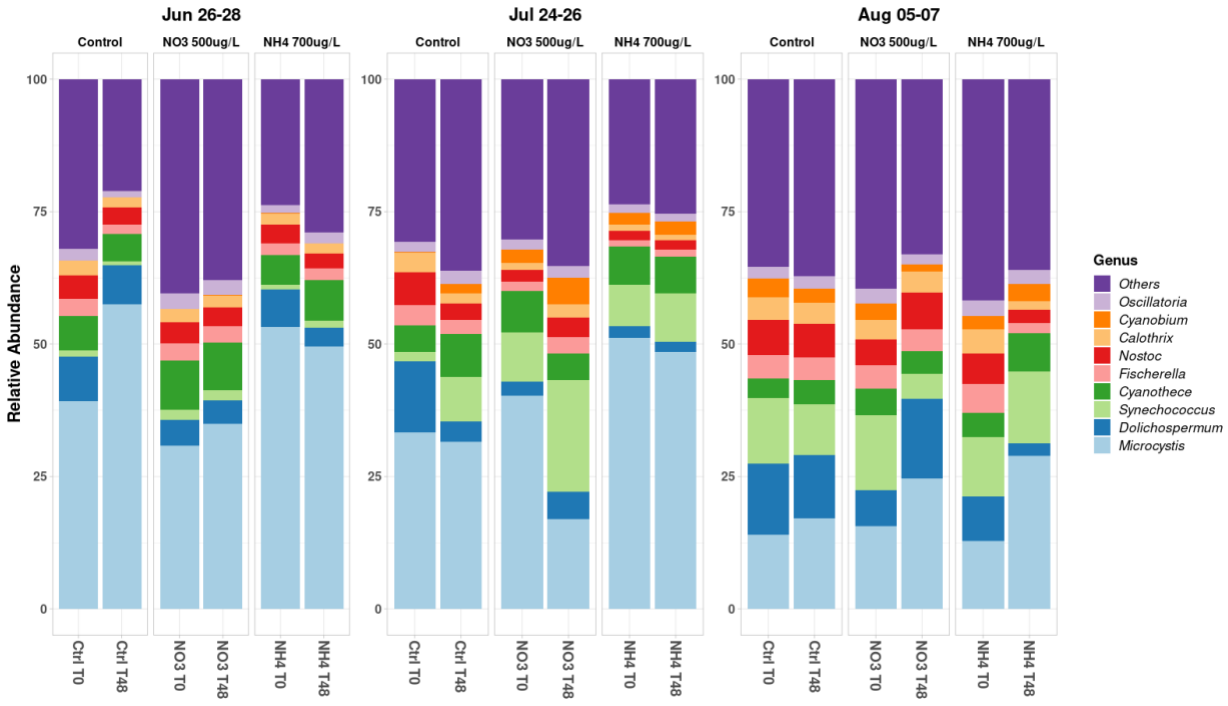

Figure 7.3 Relative abundance cyanobacterial community at genus level in Petit Lac St. François

With sufficient nutrients available, cyanobacterial blooms are expected with a TN:TP ratio between 2 and 20 (N-limited or P in abundance) at both sites (Table C.1 and Table C.2). We also observed

the DIN:SRP ratio changed from less than 13 (N-limited) that favors the growth of N_2-fixing cyanobacteria (*Dolichospermum* and *Nostoc*) at T0 to greater than 50 (P-limited) that enhances non-N_2-cyanobacteria (*Microcystis*) at T48 (Figure 7.2) in the mesocosms containing nutrients in MB [23, 70, 110, 270] (Table C.5 and Table C.7). In contrast, we did not observe the same trend in in PLSF (Figure 7.3) where the nitrogen to phosphorus ratios started at higher values suggesting that nitrogen addition would have less of an effect at PLSF.

The observed genera from microscopic cell counts do not completely match with shotgun metagenomic sequencing results (Figure C.5, Figure C.6, Figure 7.2, and Figure 7.3). *Aphanizomenon*, *Aphanocapsa* and *Aphanothece* were only reported by taxonomic cell counts while *Synechococcus* was only identified by shotgun metagenomics. Several studies showed contrasting results of phytoplankton communities between two approachs of microscopic and high throughput sequencing [34, 55, 238, 257]. The limitations of these methods could be the reason for the difference in community composition structure [34]. For microscopic taxonomic cell counts, low abundance species, or similar morphology among cyanobacterial taxa may lead to the misindentification of cyanobacteria [34-36, 257]. In the case of shotgun metagenomic sequencing, the limitations can include incomplete DNA sequencing libraries or using different libraries [34, 55, 257].

7.6.4 Impact of NH_4^+ and NO_3^- on Cyanotoxins

In the beginning of sampling period, the concentration of total intracellular mirocystins (intracellular ΣMCs) varied around 10^1-10^5 ng/L while total extracellular microcystins (extracellular ΣMCs) was around 10^2-10^4 ng/L in MB and PLSF (Figure 7.4 and Figure 7.5). Twelve microcystins (MCs) including MC-LR, MC-YR, MC-HtyR, MC-RR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF, [Asp^3]MC-RR and [Asp^3]MC-LR, and some cyanopeptides (anabaenopeptin A (AP-A) and anabaenopeptin B (AP-B)) were detected at both sites (Figure C.7 and Figure C.8). In MB, MC-LR, MC-RR and APA were the most abundant intra- and extracellular cyanotoxins observed (Figure C.7 and Figure C.9). MC-LR was the most common in both intra and extracellular cyanotoxins accounting for greater than 75% of total relative abundance in all mesocosms at T0 in PLSF (Figure C.8 and Figure C.10).

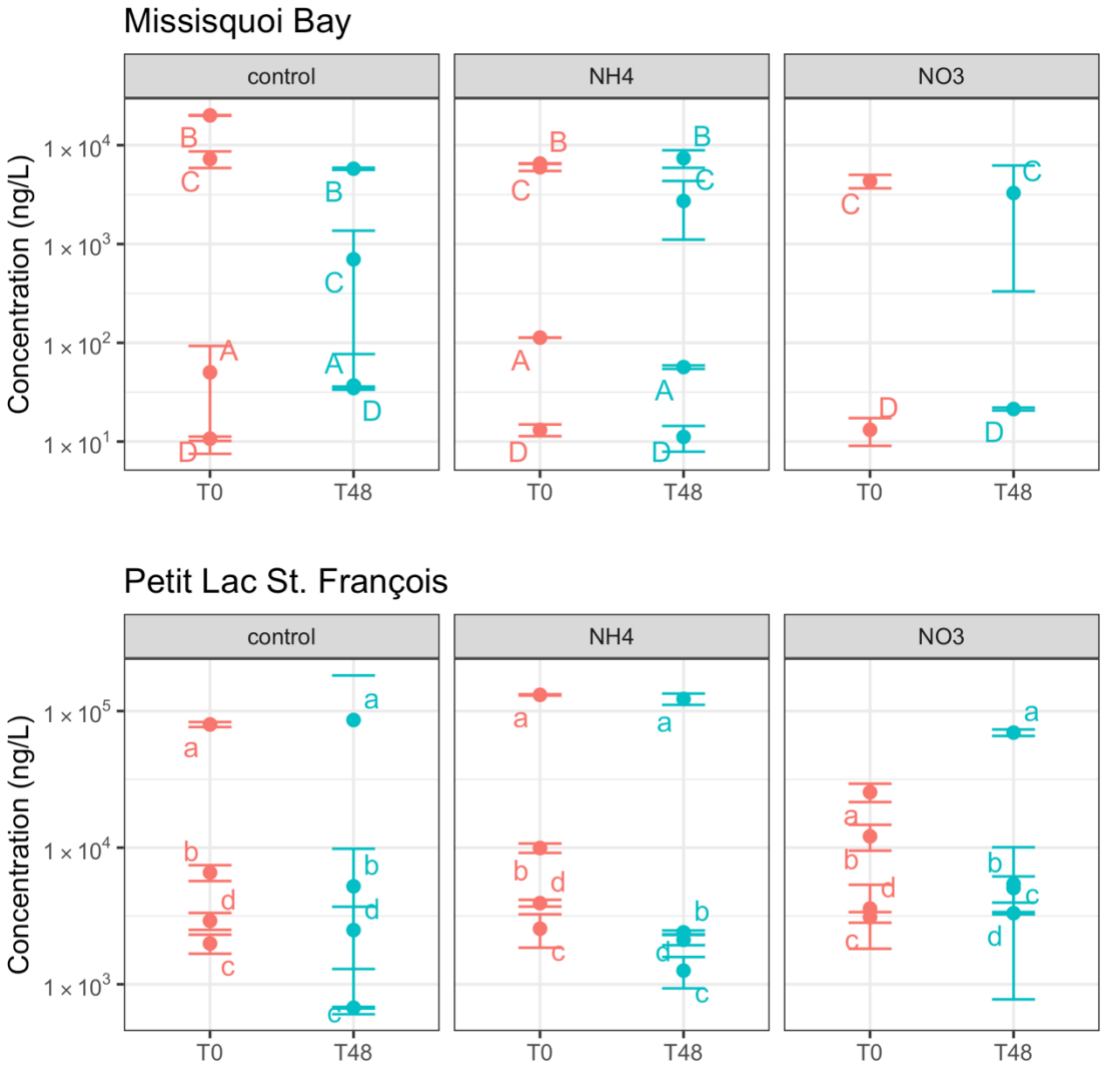

Figure 7.4 Concentration of total intracellular microcystins in the control, NO_3^- (500 µg N/L) and NH_4^+ (700 µg N/L) mesocosms (Mean ± standard deviation). A) 31 July-02 August 2018, B) 24-26 September 2018, C) 13-15 August 2019, D) 20-22 September 2019; a) 26-28 June 2019; b) 24-26 July 2019; c) 05-07 August 2019; d) 09-11 October 2019.

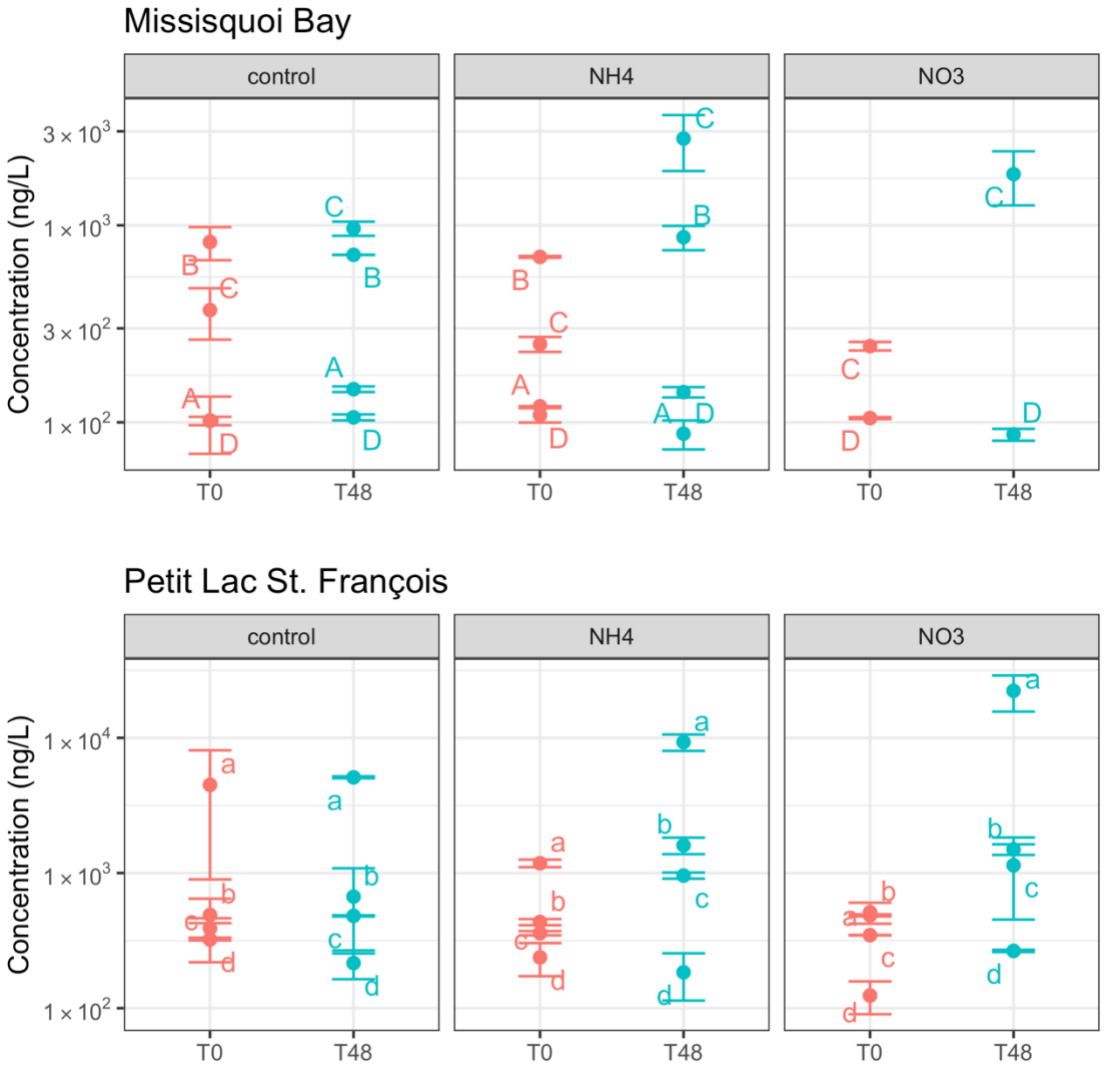

Figure 7.5. Concentration of total extracellular microcystins in the control, NO_3^- (500 µg N/L) and NH_4^+ (700 µg N/L) mesocosms (Mean ± standard deviation). A) 31 July-02 August 2018, B) 24-26 September 2018, C) 13-15 August 2019, D) 20-22 September 2019; a) 26-28 June 2019; b) 24-26 July 2019; c) 05-07 August 2019; d) 09-11 October 2019.

After 48 hours, the concentration of intracellular ΣMCs did not change significantly in the mesocosms with added nutrients as compared to the control mesocosms at both sites. However, there was a significant increase in extracellular ΣMCs and MC-LR in the mesocosms containing NO_3^- 500 µg/L and NH_4^+ 700 µg/L as compared to the control mesocosms (Table C.11). For the individual intracellular toxins, MC-LY and AP-B significantly changed in the mesocosms adding

NO_3^- compared to the control mesocosms and there was no significant differences in individual cyantoxins between the control mesocosms and the mecososm containing NH_4^+. The composition of intracellular cyanotoxins generally remained stable after 48 hours in all mesocosms in almost events in PLSF and MB while the composition of extracellular cyanotoxins varied across at both sites (Figure C.7 and Figure C.8).

The increase of extracellular ΣMCs can be the result of extracellular toxin release following cell death or active toxin production by cyanobacteria. The extracellular ΣMCs did not significantly change in the control mesocosms after two days (Figure 7.5) and *Microcystis* (the main microcystin producing cyanobacteria) cells remained intact even with the harsh conditions of parallel coagulation mesocosms over the 2 days [179, 180]. Therefore, the increase in extracellular ΣMCs was likely enhanced by adding N leading to active toxin production. N-enrichment has been related to the production of cyanotoxins, such as the MCs produced by *Microcystis* spp. and other bloom-forming cyanobacteria [24, 25, 31, 271]. Moreover, Carmichael and Boyer, 2016 [272] demonstrated that N avaibility plays an important role to some extent in the production of MCs in nature though seven amino acids. On a cellular level, N assimilation in cyanobacteria in cyanobacteria is carried out by the transciption factor *ntcA* [118, 273], and *ntc A* bind to the *mcyD* (microcystin biosynthesis gene cluster) promoter region has direct relevance for the regulation of microcystin systhetase of *Microcystis* spp. [274].

7.6.5 Impact of Environmental Conditions on Cyanobacterial Community in the Mesocosms Before and After Nitrogen Addition

Redundancy analysis (RDA) analyses were performed to evaluate the relationships among nitrogen, dominant cyanobacteria's relative abundance and cyanotoxins in Missisquoi Bay and Petit Lac St. François. PC1 and PC2 represented 69.1% and 26% of the variation, respectively at T0. PC1 and PC2 represented 79% and 15.9% of the variation, respectively at T48 (Figure 7.6).

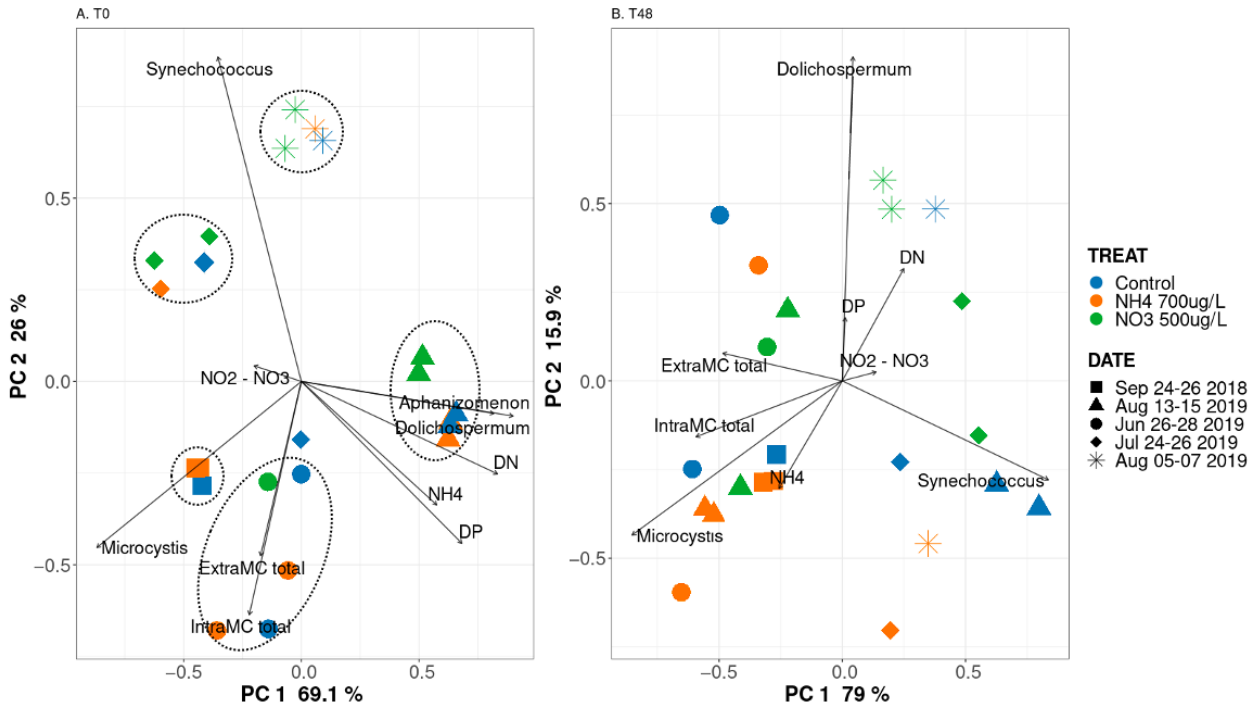

Figure 7.6 Redundancy analysis (RDA) of environmental parameters with respect to cyanobacterial communities in the control, NH_4^+ and NO_3^- mesocosms in Missisquoi Bay (24-26 September, 2018 and 13-15 August, 2019) and Petit Lac St. François (26-28 June, 2019; 24-26 July, 2019 and 05-07 August, 2019). Only significant parameters ($\rho < 0.05$) are shown exception of $NO_2^- + NO_3^-$ parameter.

A relationship was observed between *Dolichospermum* and *Aphanizomenon* with NH_4^+, DN, and DP in the begining of studied period (T0) where *Dolichospermum* and *Aphanizomenon* were the predominant genera in all mesocosms on 13 August, 2019 in MB (Figure 7.6A). In contrast, the association between *Microcystis* and *Synechococcus* and NH_4^+ was not observed at T0 in other events. After two days of exposure (T48), NH_4^+ has a strong impact on the presence of *Microcystis*, which became dominant in the mesocosms adding NH_4^+ on 15 August, 2019. NH_4^+ was associated with *Microcystis* in the mesocosm containing NH_4^+ at T48 on September 26, 2019 in MB, and June 28, 2019, July 26, 2019 and August 07, 2019 in PLSF (Figure 7.6B). In addition, *Microcystis* was associated with intra- and extracellular ΣMCs at both T0 and T48 in this study (Figure 7.6A,B).

These observations are consistent with previous studies reporting that addition of NH_4^+ promoted the presence of cyanobacteria including *Microcystis*. *Microcystis* spp. have high affinity for NH_4^+

[117, 130], and the frequent dominance of *Microcystis* in the middle of summer could be related to NH_4^+ concentrations since NH_3 and NH_4^+ are released from sediments during warmer months through decomposition processes [23, 275, 276]. Previous study of Monchamp *et al*. 2014 [29] assessed the nitrogen form impacted on cyanobacterial community in PLSF and found that NH_4^+ significantly influenced *Microcystis* relative abundance. Similarly, a recent mesocosm study o determine the effect of N on cyanobacterial growth found that NH_4^+ treatment stimulated mostly *Microcystis aeruginosa* [132]. Addition of N could be a result of high N:P that is one of key conditions preferred by non-N_2-fixing species such as *Microcystis* spp. [132, 277]. In addition, *Microcystis* could display a clear optimum growth rate at an high N:P (100:1) [132, 278]. *Microcystis* has been considered as the major producer of MC and *Microcystis* was strongly linked to the microcystin concentrations [13, 279]. It could be a reason for the association between *Microcystis* and both intra- and extracellular ΣMCs at T0 and T48 in this study.

7.7 Conclusions

- *Dolichospermum* and *Microcystis* were the dominant genera throughout the experiments at Petit Lac St. François and Missisquoi Bay. *Aphanothece* spp. and *Aphanizomenon* spp. were also documented as representative genera in Missisquoi Bay;
- After two days of exposure (T48), the total taxonomic cell counts, *Dolichospermum* and *Aphanizomenon*'s cell counts significantly decreased in the mesocosms containing NH_4^+ as compared to the control mesocosms while total taxonomic cell counts and genus's cell counts in NO_3^- mesocosms did not significantly changed when compared to control mesocosms;
- A shift to *Microcystis* in the mesocosms with nitrogen addition was observed two days after the addition in Missisquoi Bay where nitrogen was more limited as compared to Petit Lac Saint François based on nutrient ratios. Cyanobacterial species present in Petit Lac St. François were less sensitive to the addition of nitrogen given the higher ratios of N:P at the begining of study period;
- Intracellular microcystins (MC-RR, MC-YR, MC-HtyR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF, [Asp^3]MC-RR and [Asp^3]MC-LR), anabaenopeptin A (AP-A) and anabaenopeptin B (AP-B) were detected throughout the experiment;

- There was no general significant change in intracellular ΣMCs in the mesocosms following the addition of NH_4^+ or NO_3^- as compared to the control mesocosms after two days of exposure;
- Extracellular ΣMCs and MC-LR increased significantly in mesocosms with NH_4^+ or NO_3^- addition after 48 hours as compared to control mesocosms at both Missisquoi Bay and Petit Lac St. François;
- Intra- and extracellular microcystin concentrations were related to the appearance of *Microcystis*.
- NH_4^+ was associated with *Microcystis* presence 48 hours after the addition of N at both sites.
- Precipitation events leading to increased nitrogen loads in source waters (e.g. stormwater, agricultural runoff, untreated municipal effluents) could result in higher bloom toxicity within a short time frame. Drinking water treatment plants receiving water from surface waters with recurrent blooms should remain vigilant, particularly in the days following such events during cyanobacterial blooms.
- Drinking water sources with recurrent cyanobacterial blooms should identify measures to limit stormwater inputs of nitrogen in addition to phosphorus.
- Results support a reduction of total nitrogen loads in addition to toxic nitrogen concentrations to mitigate the severity to toxic cyanobacterial blooms.

CHAPTER 8 GENERAL DISCUSSION

A summary of this research study is presented according to the following themes:

- Assessment of the efficiency of a coagulant for the removal of cyanobacteria cells and cyanotoxins;
- Impact of coagulation on cyanobacterial community composition;
- Effects of nutrients on cyanobacteria and cyanotoxins;
- Management and treatment implications.

8.1 Assessment of the efficiency of a coagulant on the removal of cyanobacterial cell and cyanotoxins

Fe^{3+} coagulants have been applied for the treatment of cyanobacterial blooms in lakes [46, 173-176]. Fe addition significantly decreased the P concentration in the water column and prevented P release from sediment. In addition, blooms of phytoplankton significantly declined the following summer and microcystins associated with cyanobacterial blooms were reduced [5].

In this thesis (Chapter 5), we studied the effects of ferric sulfate ($Fe_2(SO_4)_3$) on cyanobacterial cells and cyanotoxins from lake water with cyanobacterial blooms on a short time scale. At the beginning of the experiments, *Dolichospermum*, *Microcystis* and *Aphanizomenon* were the most common genera and the total taxonomic cell counts were greater than 10^5 cell/mLs in all mesocosms at both field sites. The total cell counts reduction was more than 96% and 51% with applied doses of 35 mgFe/L and 20 mgFe/L, respectively. *Dolichospermum* spp., *Microcystis*., and *Aphanizomenon*., were well removed with an applied dose of 35 mgFe/L. In addition, several species including *Chroococcus*, *Merismopedia*, and *Coelosphaerium* were removed to below detection limits.

Multiple intracellular microcystins toxins (MC-RR, MC-YR, MC-HtyR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF, [Asp^3]MC-RR and [Asp^3]MC-LR), anatoxin-a (ANA-a), cylindrospermopsin (CYN), and some rarely monitored cyanopeptides (anabaenopeptin A (AP-A) and anabaenopeptin B (AP-B)) were detected and the total intracellular microcystin exceeded 10^3 ng/L at the start of experiment at Missisquoi Bay and Petit Lac St. François. Both applied doses eliminated more than 97% intracellular total microcystins and MC-LR. There was no significant difference in removing intracellular total microcystins and individual intracellular cyanotoxins between the two applied dosages. However, extracellular toxins were not removed significantly after coagulation with both doses. There was no significant difference in removing intra- and extra-cellular cyanotoxins between two applies doses. Therefore, source water treatment will not be highly sensitive to the lack of optimal dose as is required for drinking water treatment within a plant.

8.2 Impact of coagulation on cyanobacterial community composition

High-throughput sequencing has considerable advantages for the analysis of microbial diversity and community structure in drinking water treatment, seawater, and sewage sludge [34, 55, 57, 183, 280]. High-throughput sequencing does not share some of the disadvantages of microscopic approaches such as misidentification of morphologically similar species and the complexity of counting species in aggregates [55, 281, 282]. In Chapter 6, high-throughput sequencing was used to investigate the cyanobacterial community structure using shotgun metagenomics in a short term in situ mesocosm experiment in two lakes following coagulation with ferric sulfate ($Fe_3(SO_4)_3$).

Microscopic taxonomic cell counts and high-throughput sequencing did not completely match although there was considerable overlap. Different biases of microscopic and high-throughput sequencing may provide an explanation for the differences in the structure of community composition [34, 55, 238, 239].

Results from shotgun metagenomics showed that *Dolichospermum* and *Microcystis* were the most dominant genera in Missisquoi Bay and Petit Lac St. François, respectively, at the beginning of all experiments. In Missisquoi Bay, cyanobacterial community composition at the genus level did not change remarkedly in all of the mesocosms after two days on the 10th of August, 2018. In contrast, the most dominant genus shifted from *Dolichospermum* to *Synechococcus* and *Microcystis* in the control mesocosms and the coagulant mesocosms, respectively on the 15th of August, 2019. In Petit Lac St. François, there was no change in the cyanobacterial relative abundance in the control mesocosms while a change of relative abundance in the mesocosms with added 20 and 35 mgFe/L was observed after two days of exposure on the 26th July and 7th August in 2019. That change may be linked to the reduction of the dominant genus in taxonomic cell counts results.

A principal component analysis showed that intracellular microcystin concentration was related to the presence of *Microcystis*. This is in agreement with previous studies [185, 280]. It suggests that conditions favorable for the proliferation of Microcystis in particular should be monitored and barriers should be improved for the removal of *Microcystis* prior to entering the water treatment plant given problems associated with the accumulation of cells and toxins within water treatment plants [55, 160, 181] The Shannon and richness indices did not significantly change in mesocosms

after 48 hours. In Missisquoi Bay and Petit Lac St. François, *Microcystis* was associated with dissolved nitrogen, in accordance with result of previous studies [27, 257].

8.3 Effects of nutrients on cyanobacterial and cyanotoxins

In Chapter 7, we presented the effects of nutrients (NO_3^- 500 µg/L, NH_4^+ 700 µg/L) on cyanobacteria and cyanotoxins in a short time frame in Missisquoi Bay and Petit Lac St. François. Moreover, it also investigate the cyanobacterial community composition using shotgun metagenomic sequencing before and after adding nutrients. In Québec and elsewhere, nitrogen is primarily regulated based on its toxicity. Regulations to limit eutrophication predominantly aim to reduce phosphorus loads. Data are needed in support of future nutrient regulations based on loads and not only toxic concentrations.

Our results showed that the initial avarage taxonomic cell counts were greater than 10^4 cells/mL in all of the mesocosms at both sites. Genera *Dolichospermum* and *Microcystis* were the most bloom forming cyanobacteria in all Petit Lac St. François mesocosms at the beginning of the study period. In Missisquoi Bay, the dominant genus was more diverse than in Petit Lac St. François, including *Microcystis*, *Dolichospermum*, *Aphanothece*, and *Aphanizomenon*. After two days of adding nutrients, the total taxonomic cell counts, *Dolichospermum* and *Aphanizomenon*'s cell counts significantly decreased in the mesocosms adding NH_4^+ while the total taxonomic cell counts and genus's cell counts did not significantly change in the mesocosms containing NO_3^- as compared to the control mesocosms at both sites. Moreover, a shift to *Microcystis* was observed in the mesocosms with nitrogen addition after two days of the addition in Missisquoi Bay where nitrogen was more limited as compared to Petit Lac Saint François based on nutrient ratios. The result of the microscopic and high throughput sequencing methods did not completely match with each other although there were similarities. Each method has biases which can influence results and considering both provides a more complete portrait.

Intracellular microcystins (MC-RR, MC-YR, MC-HtyR, MC-LR, MC-HilR, MC-WR, MC-LA, MC-LY, MC-LW, MC-LF, [Asp^3]MC-RR and [Asp^3]MC-LR), anabaenopeptin A (AP-A) and anabaenopeptin B (AP-B) were detected throughout the experiment. MC-LR was the dominant intra and extracellular cyanotoxin, accounting for more than 75% of total cyanotoxins in all mesocosms at the start of experiment in Missisquoi Bay and at Petit Lac St. François. After two

days following nutrient addition, there was no significant difference in intracellular ΣMCs between control mesocosms and mesocosms with added NH_4^+ or NO_3^-. In contrast, extracellular ΣMCs and MC-LR significantly increased in the mesocosms adding N as compared to control mesocosms at both field sites. Intra- and extracellular microcystin concentrations after two days of nutrient addition was associated with *Microcystis* presence at both sites. Thus, toxin production following sudden nitrogen addition can occur on short time scales relevant to drinking water treatment plant operation.

8.4 Management and treatment implications

Some implications of our findings are presented in the following subsections.

8.4.1 Managing cell damage and release of dissolved toxins during coagulation

Coagulation processes are among the most important steps for the removal of cyanotoxins because most cyanotoxins remain within cells [13]. Cell damage following coagulation-flocculation-sedimentation may be related to the type of coagulant and various operations [50, 181, 189]. Hence, cyanobacterial cells may be lysed and their toxins released into the water during sludge storage process [55, 158]. Several studies have indicated that ferric-based coagulants did not damage the intact cells as compared to aluminium-based coagulants in coagulation treatment [159, 283]. *Microcystis aeruginosa*'s cell remained intact after 10 days of floc storage within $FeCl_3$ sludge [180]. In addition, others have shown that with ferric chloride, treatment, *Microcystis* cells can retain their integrity during coagulation and the intensity of mixing action did not compromise *Microcystis* cells; the extracellular microcystin concentration increased slightly and then decreased below 1 µg/L in the system with the coagulation while their increase exceeded 30 µg/L in the system without coagulation during the storage period [179]. Our results in Chapter 5 showed the concentration of extracellular total microcystin occasionally increased after 48 hours in mesocosms with coagulant addition at both study sites (Figure 5.5). Therefore, it is recommended that treatment plant processes need to be carefully adjusted during algal bloom season. For example, preozonation of raw water before coagulation-flocculation-sediment could also help removal of cells and extracellular cyanotoxins [185].

8.4.2 Impact of coagulants on source waters

Coagulant addition can decrease the pH in water at the time coagulant application. The environmental conditions after 48 hours in mesocosms showed the pH was in the range of 3.5 to 6 in the coagulated mesocosms (Table A.8 and Table A.9). A low pH can be harmful to fish and aquatic organisms [284]. Although cyanobacterial blooms often lead to high pH values in source waters, the application of coagulants must ensure that pH remains within an acceptable range. Besides, the large amount of sludge containing metal salts generated after the treament process may require additional removal techniques, thereby increasing the treatment cost [285, 286]. An alternative solution such as natural coagulants (e.g. seed, starch, shell, gelatin, peel, leaves) can be considered because of their low toxicity and high biodegradability which ensures the safety human health and aquatic life; they also do not generate secondary pollution and are environmentally friendly [286, 287]. Several studies have used natural coagulants to remove cyanobacteria from the water column [288-290]. For example, a previous study applied a natural coagulant (*Moringa oleifera* seeds) to remove *M. aeruginosa* in lab scale and the result showed that *Moringa oleifera* removed almost 80% of *M. aeruginosa* cells [291]. Similarly, Habtemariam *et al*. 2021 [292] evaluated the efficiency of the *Moringa oleifera* coagulant in settling cyanobacterial species present in the reservoir. Results indicated that *Moringa oleifera* of 30 mg/L may eliminate 93.6% cyanobacterial concentration without cell lysis [292].

8.4.3 Cost implication of various coagulant and sludge removal

Costs are an important consideration for coagulation application. Table 8.1 presents cost estimation of several common coagulants.

Table 8.1 Coagulant cost. Adapted from [293]

Coagulant	Typical analysis	Manufacturers	Price (USD/tonne)
Ferric sulfate $Fe_2(SO_4)_3$	12% w/w Fe (III)	Hardman Omega Chemicals	550
Ferric chloride $FeCl_3$	14-15% w/w Fe (III)	Spectrum	1400

Table 8.1 Coagulant cost. Adapted from [293] (cont'd)

Chitosan	Xian Huisun Bio-tech	1500

To reach acceptable removal efficiencies with coagulants in source waters, a large amount of coagulant must be used. The application of coagulant in a water source can lead to high sludge formation at the bottom of the lake or reservoir. Previous study indicated that goethite (iron oxide mineral) was found in Fe-treated sediments and thus, sorption of P onto Fe(III) oxides may have contributed to limiting P diffusion across the sediment-water surface [5]. Besides, P adsorbed to Fe(III) hydroxide may be transformed into form vivianite when anoxia occured [294]. Similarly, Rothe *et al*. 2014 [295] confirmed that formation of vivianite was triggered in sediment after artificial Fe supplementation in a German lake. Interestingly, vivianite P did not retransfer to a redox-sensitive P binding form under oxic condition, and thus Fe addition are promising for long-term P immobilisation in lake sediment [294].

Although this sludge can be considered as a cap on the sediments that prevents phosphorus release, the sludge from the treatment is non-biodegradable [286] and may have an impact on aquatic organisms [296], thus sludge removal might need to be considered. There are several methods of sediment removal: hydraulic dredging, clamshell, and hydro-raking. The chosen method will depend on several factors including sediment structure, the volume of materials removed, budget, and disposal considerations [297]. This adds extra cost and complexity to the process by the inefficient use of metal-based coagulant.

CHAPTER 9 CONCLUSIONS AND RECOMMENDATIONS

9.1 Conclusions

This study provides insight into the effects of a coagulant - ferric sulfate ($Fe_2(SO_4)_3$ and nitrogen (NO_3^- and NH_4^+) on cyanobacteria and their toxins using high throughput sequencing and taxonomic cell counts using microscopy for an *in situ* mesocosm experiment. The following conclusions were drawn along the following themes:

(A) Cyanobacteria and cyanotoxins removal following coagulant addition:

- More than 96% and 51% cyanobacterial removal in the mesocosms with applied dose of 35 mgFe/L and 20 mgFe/L, respectively.
- *Dolichospermum*, *Microcystis*, and *Aphanizomenon* were well removed by both applied doses. In the mesocosms containing 20 mgFe/L, *Aphanothece* and *Coelosphaerium* had a lower removal rate. There were significant differences in removing total cyanobacterial cells among several dominant cyanobacterial genera.
- More than 97% of total intracellular microcystins were eliminated by both applied doses. Ferric sulfate effectively removes intracellular cyanotoxins but not extracellular cyanotoxins.
- Source water treatment may not be highly sensitive to suboptimal dosing since different doses of ferric sulfate have almost the same effectiveness in removing intra- and extracellular cyanotoxins. The effect on surface water body pH will be a more important consideration.

(B) Impact of coagulation on cyanobacterial community composition:

- Cyanobacterial community composition at the genus level changed after two days in the mesocosms with coagulant addition.
- Intracellular microcystin concentrations were related to the appearance of *Microcystis*.
- Diversity and richness indices did not change significantly after coagulation by $Fe_2(SO_4)_3$ in both control and coagulated mesocosms at either site.

- Among nutrient parameters, dissolved nitrogen avaibility was related to *Microcystis* at the two studied sites, whereas *Synechococcus* was influenced by total nitrogen, dissolved nitrogen, dissolved organic carbon, and dissolved phosphorus.
- Coagulation for onsite source treament could be a potential option for removing toxin-producing cyanobacterial species and intracellular toxins from the water column. However, it its impact on cyanobacterial community structure should be considered.

(C) Cyanobacterial community composition and production of cyanotoxins in response to nutrient addition:

- The total taxonomic cell counts, *Dolichospermum* and *Aphanizomenon*'s cell counts significantly decreased in the mesocosms containing NH_4^+ as compared to the control mesocosms while total taxonomic cell counts and genus's cell counts in NO_3^- mesocosms did not significantly change when compared to control mesocosms after two days following addition;
- In the mesocosmss following the addition of NH_4^+ or NO_3^-, a shift to *Microcystis* was observed after two days in Missisquoi Bay where nitrogen was more limited as compared to Petit Lac Saint François based on nutrient ratios.
- There was no general significant change in intracellular ΣMCs in the mesocosms containing NH_4^+ or NO_3^- as compared to the control mesocosms after two days of increased N;
- Extracellular ΣMCs and MC-LR increased significantly in mesocosms with NH_4^+ or NO_3^- addition after 48 hours as compared to control mesocosms;
- Intra- and extracellular microcystin concentrations linked to the appearance of *Microcystis*;
- *Microcystis* presence was influenced by NH_4^+ after 48 hours of the N addition at both sites;
- Higher bloom toxicity could occur quickly as a result of precipitation events that raise the nitrogen loading to source waters (e.g. stormwater, agricultural runoff, untreated

municipal effluents). Drinking water treatment plants receiving water from recurrently bloomed surface waters should be cautious, especially in the days following such incidents during cyanobacterial blooms;

- Drinking water sources with persistent cyanobacterial blooms should investigate strategies to reduce stormwater inputs of nitrogen in addition to phosphorus;
- Results suggest that in order to mitigate the severity of toxic cyanobacterial blooms, total nitrogen loads as well as toxic nitrogen concentrations should be reduced;

(D) Management and treatment implications

- Limitations with regards to extracellular toxin removal following coagulation demonstrated the requirement for processes in treatment plants to remove dissolved toxins (such as pre-ozonation).
- Metal-based coagulants lowered the pH in the water column and produce a sludge containing cyanobacterial cells. Organic coagulants might be suitable alternatives, although their role in phosphorus loading from sediments following microbial degradation would also have to be considered.

9.2 Recommendations

Based on these findings, recommendations for further investigation are to:

- Use SEM (scanning electronic microscopy) on sludge samples to determine the cell integrity after coagulation.
- Study the cyanobacteria in stored sludge, including the potential risk of growth and cyanotoxin release.
- Evaluate the cyanobacterial functional response to coagulation and nutrients.
- Investigate the effect of coagulants on cyanobacterial gene expression using metatranscriptomic analysis.
- Consider the impact different kind of nitrogen (e.g. urea) on cyanobacterial community composition.

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APPENDIX A SUPPLEMENTARY INFORMATION, ARTICLE 1

Journal: Toxins

Title: The Effects of Ferric Sulfate ($Fe_2(SO_4)_3$) on the Removal of Cyanobacteria and Cyanotoxins: A Mesocosm Experiment

Author: Kim Thien Nguyen Le, Eyerusalem Goitom, Hana Trigui, Sébastien Sauvé, Michèle Prévost and Sarah Dorner

Table A.1 List of cyanobacteria species. Associated toxin information adapted from [91]

No	MB		PLSF		Associated toxin group belonging to genera
	Genera	**Species**	**Genera**	**Species**	
1	*Aphanizomenon*	*A. flos-aquae* *A. issatschenkoi* *A. gracile*	*Aphanizomenon*	*A. flos-aquae* *A. issatschenkoi*	Anatoxin-a (ANA-a), Cylindrospermopsin (CYN), Saxitoxin (STX)
2	*Aphanocapsa*	*A. delicatissima* *A. planctonica* *A. holsatica*	*Aphanocapsa*	*A. delicatissima* *A. planctonica*	
3	*Aphanothece*	*A. clathrata brevis* *A. nidulans* *A. smithii*	*Aphanothece*	*A. clathrata brevis*	
4	*Chroococcus*	*C. limneticus* *C. minimus* *C. prescottii*	*Chroococcus*	*C. limneticus* *C. dispersus* *C. prescottii*	
5	*Coelosphaerium*	*C. kuetzinggianum*	*Coelosphaerium*	*C. kuetzinggianum*	
6	*Dolichospermum*	*D. spiroides* *D. circinalis* *D. planctonicum*	*Dolichospermum*	*D. spiroides* *D. circinalis* *D. planctonicum* *D. mendotae*	Microcystis (MC), Anatoxin-a (ANA-a), Anatoxin-a (S), Saxitoxins (STX)
7	*Merismopedia*	*M. tenuissima* *M. punctata* *M. minima*	*Merismopedia*	*M. tenuissima* *M. punctata* *M. minima*	
8	*Microcystis*	*M. aeruginosa* *M. wesenbergii*	*Microcystis*	*M. aeruginosa* *M. wesenbergii*	Microcystin (MC)
9	*Pseudanabaena*	*P. limnetica* *P. mucicola*	*Pseudanabaena*	*P. limnetica*	

Table A.2 Removal effectiveness (%) of taxonomic cell counts of individual cyanobacterial genus after 48 hours (Mean±Standard deviation)

Event	Treat	Total cell counts	*Aphanizomenon*	*Aphanocapsa*	*Aphanothece*	*Chroococcus*	*Coelosphaerium*	*Dolichospermum*	*Merismopedia*	*Microcystis*	*Pseudanabaena*
MB September 10-12, 2018	20 mgFe/L	99.96±0.04	97.84±3.05	100.0±0.00	98.30±0.21	100.0±0.00	NA	99.88±0.04	100.0±0.00	99.97±0.01	99.97±2.81
	35 mgFe/L	99.94±0.04	97.69±1.12	100.0±0.00	97.27±0.11	100.0±0.00	NA	99.81±0.05	100.0±0.00	99.65±0.05	97.75±0.79
MB September 24-26, 2018	20 mgFe/L	71.91±5.38	68.55±1.94	89.27±6.55	64.38±2.83	NA	NA	75.22±7.48	100.0±0.00	86.92±2.14	72.69±3.93
	35 mgFe/L	96.39±1.29	80.58±7.53	76.56±2.31	69.09±1.63	NA	NA	98.51±1.19	100.0±0.00	93.98±2.61	71.59±8.54
MB August 13-15, 2019	20 mgFe/L	94.27±1.67	93.91±1.64	NA	87.73±6.22	NA	100.0±0.00	97.17±3.14	NA	86.55±1.62	NA
	35 mgFe/L	99.35±0.11	99.26±0.15	NA	96.51±1.44	NA	100.0±0.00	99.97±0.01	NA	100.0±0.00	NA
PLSF June 26-28, 2019	20 mgFe/L	85.22±6.43	77.17±8.16	NA	-37.5±1.67	NA	35.11±5.01	84.66±4.07	NA	92.66±8.64	NA
	35 mgFe/L	98.99±1.06	98.66±1.44	NA	88.50±4.58	NA	100.0±0.00	99.03±0.95	NA	95.82±4.36	NA
PLSF July 24-26, 2019	20 mgFe/L	51.98±6.21	76.76±4.51	NA	-13.5±1.24	100.0±0.00	19.36±1.14	66.57±4.71	77.5±0.28	66.99±0.95	NA
	35 mgFe/L	99.11±0.11	97.93±0.28	NA	92.65±0.96	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	NA
PLSF August 05-07, 2019	20 mgFe/L	78.21±6.72	72.22±6.32	NA	79.47±4.98	100.0±0.00	75.86±4.33	77.97±5.04	92.14±3.59	96.51±5.39	NA
	35 mgFe/L	99.72±0.65	98.22±0.65	NA	93.46±2.34	NA	100.0±0.00	100.0±0.00	100.0±0.00	99.97±0.01	NA

Removal effectiveness percentage (%) = $\frac{T0 - T48}{T0} \times 100$.

NA: no value

Table A.3 Pairwise Kruskal-Wallis test, showing differences in removal of total cell counts and individual cyanobacterial genus between control mesocosms and mesocosms with dose of 20 mgFe/L, control mesocosms and mesocosms with dose of 35 mgFe/L, mesocosms with dose of 20 mgFe/L and 35 mgFe/L in Missisquoi Bay and Petit-Lac-St-François (*p*-value< 0.05 is significant)

Species	***p*-value**			***df***	***chi-squared***	***p*-value**
	control-20 mgFe/L	control-35 mgFe/L	20 mgFe/L-35 mgFe/L			
Dolichospermum	0.002	<0.001	0.001	2	24.12	5.7E-06
Aphanothece	0.018	<0.001	0.003	2	17.81	0.0001
Chroococcus	0.047	0.019	0.018	2	7.49	0.0236
Aphanocapsa	0.084	0.281	0.758	2	5.16	0.0754
Microcystis	<0.001	<0.001	0.013	2	23.68	7.1E-06
Merismopedia	0.009	0.019	0.021	2	8.65	0.0132
Aphanizomenon	0.009	<0.001	0.019	2	13.66	0.0010
Coelosphaerium	0.558	0.043	0.011	2	6.43	0.0401
Total cell counts	0.002	<0.001	0.043	2	18.59	9.1E-05

Table A.4 Removal effectiveness (%) of total intracellular microcystins (intracellular ΣMCs) and individual intracellular cyanotoxins in mesocosms with dose of 20 mgFe/L and 35 mgFe/L after 48 hours (Mean±Standard deviation). Dominant and representative cyanotoxins are shown

Event	Treat	ΣMCs	MC-LR	MC-RR	MC-LY	MC-LA	CYN	ANA	APA	APB
MB September 10-12, 2018	20 mgFe/L	99.91±0.15	99.67±0.05	99.70±0.10	60.58±0.51	97.86±0.14	-87.91±0.46	-140±4.34	98.72±2.78	99.09±1.81
	35 mgFe/L	99.92±0.02	99.70±0.09	99.74±0.05	61.57±0.19	98.21±0.41	NA	-150±5.21	98.61±4.56	99.21±1.89
MB September 24-26, 2018	20 mgFe/L	99.65±0.01	99.95±0.02	99.86±1.89	89.43±2.03	99.76±2.92	NA	-23±0.98	80.11±1.23	90.91±1.12
	35 mgFe/L	99.89±0.02	99.89±0.03	99.86±2.11	85.53±1.32	99.71±2.87	NA	-98±2.11	88.31±0.78	90.58±2.78
MB August 13-15, 2019	20 mgFe/L	98.92±1.01	99.15±0.58	99.47±0.04	92.34±1.34	98.06±0.37	43.37±2.35	NA	99.77±1.34	93.21±0.03
	35 mgFe/L	99.70±0.19	99.52±0.01	99.91±0.04	95.39±0.98	96.06±0.24	70.01±1.56	NA	99.29±1.45	99.81±2.01
PLSF June 26-28, 2019	20 mgFe/L	97.97±0.26	98.33±1.50	90.83±0.13	97.81±2.31	97.58±0.05	NA	NA	NA	91.27±1.09
	35 mgFe/L	99.83±1.12	99.96±1.01	89.77±0.05	99.88±1.14	96.42±0.01	NA	NA	42.25±3.45	91.16±0.13
PLSF July 24-26, 2019	20 mgFe/L	99.75±0.07	99.37±0.24	43.92±2.01	98.62±1.07	-44.01±0.03	NA	NA	NA	22.94±2.34
	35 mgFe/L	99.75±0.03	99.90±0.25	58.86±0.19	99.45±0.46	3.65±0.01	NA	NA	NA	33.81±2.23
PLSF August 05-07, 2019	20 mgFe/L	98.96±0.41	99.56±0.32	76.46±0.81	98.57±1.45	-109±0.04	NA	NA	NA	50.86±0.09
	35 mgFe/L	98.05±3.81	99.22±0.69	85.59±0.91	97.26±2.06	-46.98±0.98	-130	NA	NA	72.53±0.81

Removal effectiveness percentage (%) = $\frac{T0 - T48}{T0} \times 100$.

NA: no value

Table A.5 Pairwise Kruskal-Wallis test, showing differences in removal of total intracellular microcystins (intracellular ΣMCs) and individual intracellular cyanotoxins between control mesocosms and mesocosms with dose of 20 mgFe/L, control mesocosms and mesocosms with dose of 35 mgFe/L, mesocosms with dose of 20 mgFe/L and 35 mgFe/L in Missisquoi Bay and Petit-Lac-St-François (*p*-value<0.05 is significant)

Toxins	***p*-value**			***df***	***chi-squared***	***p*-value**
	control-20 mgFe/L	control-35 mgFe/L	20 mgFe/L-35 mgFe/L			
MC-LR	<0.001	<0.001	0.386	2	20.12	4.2E-05
MC-LA	0.057	0.050	0.603	2	4.76	0.0922
MC-LY	<0.001	0.001	0.488	2	14.61	0.0006
MC-RR	0.003	0.001	0.453	2	11.31	0.0034
MC-LF	0.078	0.021	0.475	2	5.96	0.0501
MC-LW	0.356	0.119	0.681	2	2.44	0.2941
MC-HilR	0.005	0.004	0.862	2	11.05	0.0039
MC-YR	0.073	0.098	0.802	2	4.19	0.1235
MC-WR	0.785	0.811	0.335	2	0.56	0.7548
MC-HtyR	0.864	0.547	0.381	2	1.15	0.561
[Asp^3]MC-LR	0.001	0.001	0.876	2	20.05	4.4E-5
CYN	0.328	0.626	0.684	2	0.91	0.6348
ANA-a	0.169	0.054	0.409	2	3.99	0.1354
AP-A	0.324	0.129	0.862	2	2.16	0.3385
AP-B	0.083	0.024	0.184	2	6.69	0.0334
ΣMCs	<0.001	<0.001	0.057	2	24.98	3.7E-06

Table A.6 Pairwise Kruskal-Wallis test, showing differences in removal of total extracellular microcystins (extracellular ΣMCs) and individual extracellular cyanotoxins between control mesocosms and mesocosms with dose of 20 mgFe/L, control mesocosms and mesocosms with dose of 35 mgFe/L, mesocosms with dose of 20 mgFe/L and 35 mgFe/L in Missisquoi Bay and Petit-Lac-St-François (*p*-value <0.05 is significant)

Toxins	***p*-value**			***df***	***chi-squared***	***p*-value**
	control-20 mgFe/L	control-35 mgFe/L	20 mgFe/L-35 mgFe/L			
MC-LR	0.053	0.564	0.113	2	5.10	0.078
MC-LA	0.684	0.172	0.148	2	1.36	0.505
MC-LY	0.965	0.073	0.231	2	6.28	0.043
MC-RR	0.355	0.369	0.488	2	2.51	0.285
MC-LW	0.149	0.616	0.514	2	2.11	0.347
[Asp^3]MC-LR	0.149	0.569	0.076	2	2.95	0.228
[Asp^3]MC-RR	0.088	0.089	0.771	2	4.37	0.112
CYN	0.306	0.151	0.094	2	2.15	0.340
AP-A	0.056	0.099	0.894	2	2.56	0.227
AP-B	0.208	0.089	0.414	2	2.55	0.278
ΣMCs	0.225	0.525	0.106	2	7.04	0.029

Table A.7 Environmental conditions of lake water samples in control mesocosms at T48 (Mean ± standard deviation)

Parameter	Missisquoi Bay			Petit-Lac-St-François		
	Event A September 12 2018	**Event B September 26 2018**	**Event C August 15 2019**	**Event a June 28 2019**	**Event b July 26 2019**	**Event c August 07 2019**
Chlorophyll-*a* (RFU)	No data	No data	64.72 ± 0.59	3.37 ± 0.32	5.63 ± 0.16	7.88 ± 0.17
Phycocyanin (RFU)	No data	No data	169.21 ± 0.34	16.42 ± 0.15	0.87 ± 0.03	6.78 ± 0.16
pH	7.47 ± 0.07	No data	6.33 ± 0.09	9.93 ± 0.06	8.36 ± 0.07	7.37 ± 0.15
TDS (mg/L)	105 ± 0.00	No data	140.0 ± 4.24	151.0 ± 5.65	118.5 ± 0.71	121.0 ± 0.00
Temp (°C)	22.7 ± 0.17	No data	22.09 ± 0.17	27.81 ± 0.41	25.37 ± 0.34	25.25 ± 0.02
TOC (mg C/L)	19.97 ± 0.24	5.46 ± 0.00	700.0 ± 23.19	11.39 ± 0.23	10.57 ± 0.21	10.19 ± 0.44
DOC (mg C/L)	12.17 ± 0.39	5.08 ± 0.05	73.48 ± 1.45	9.83 ± 0.06	10.07 ± 0.83	9.81 ± 0.07
TN (mg N/L)	5.46 ± 0.69	1.68 ± 0.01	6.84 ± 1.78	11.21 ± 1.07	1.01 ± 0.15	1.28 ± 0.05
TP (µg P/L)	320.92 ± 4.48	177.49 ± 21.03	2074.60 ± 20.22	603.01 ± 3.17	72.08 ± 5.32	89.29 ± 4.81
DN (mg N/L)	2.02 ± 0.001	0.48 ± 0.009	1.56 ± 0.11	0.91 ± 0.25	0.58 ± 0.00	0.63 ± 0.03
DP (µg P/L)	40.47 ± 1.17	15.43 ± 0.25	215.01 ± 11.61	108.06 ± 2.22	16.46 ± 1.16	21.81 ± 2.13

Table A.8 Environmental conditions of lake water samples in mesocosms with dose of 20 mgFe/L at T48 (Mean ± standard deviation)

Parameter	Missisquoi Bay			Petit-Lac-St-François		
	Event A September 12 2018	**Event B September 26 2018**	**Event C August 15 2019**	**Event a June 28 2019**	**Event b July 26 2019**	**Event c August 07 2019**
Chlorophyll-*a* (RFU)	No data	No data	1.04 ± 0.002	0.08 ± 0.00	1.18 ± 0.01	0.82 ± 0.07
Phycocyanin (RFU)	No data	No data	33.69 ± 5.21	0.49 ± 0.05	0.54 ± 0.00	1.03 ± 0.05
pH	4.9 ± 0.04	No data	5.08 ± 1.01	6.09 ± 0.01	7.70 ± 0.19	5.66 ± 0.02
TDS (mg/L)	138 ± 0.00	No data	152.00 ± 0.00	130.00 ± 0.00	139.00 ± 0.00	146.00 ± 0.00
Temp (°C)	21.8 ± 0.01	No data	22.95 ± 0.04	23.92 ± 0.02	25.21 ± 0.01	25.03 ± 0.01
TOC (mg C/L)	4.52 ± 0.15	2.22 ± 0.09	23.76 ± 4.52	17.56 ± 2.71	3.81 ± 0.51	3.69 ± 0.11
DOC (mg C/L)	3.21 ± 0.03	2.39 ± 0.03	22.62 ± 1.92	15.10 ± 2.87	3.63 ± 0.24	3.44 ± 0.01
TN (mg N/L)	0.67 ± 0.01	1.29 ± 0.01	4.16 ± 1.01	3.71 ± 0.48	0.55 ± 0.007	0.35 ± 0.009
TP (µg P/L)	8.47 ± 0.35	126.02 ± 14.54	167.73 ± 6.34	130.02 ± 14.49	20.12 ± 1.15	14.73 ± 0.71
DN (mg N/L)	0.64 ± 0.001	0.39 ± 0.01	3.28 ± 0.29	1.28 ± 0.04	0.36 ± 0.02	0.23 ± 0.001
DP (µg P/L)	5.51 ± 0.02	7.14 ± 0.14	60.08 ± 12.79	19.34 ± 1.44	6.72 ± 1.56	3.56 ± 0.21

Table A.9 Environmental conditions of lake water samples in mesocosms with dose of 35 mgFe/L at T48 (Mean ± standard deviation)

Parameter	Missisquoi Bay			Petit-Lac-St-François		
	Event A September 12 2018	**Event B September 26 2018**	**Event C August 15 2019**	**Event a June 28 2019**	**Event b July 26 2019**	**Event c August 07 2019**
Chlorophyll-*a* (RFU)	No data	No data	1.05 ± 0.002	0.01 ± 0.00	0.09 ± 0.007	0.01 ± 0.00
Phycocyanin (RFU)	No data	No data	29.69 ± 1.13	0.05 ± 0.00	0.11 ± 0.00	0.015 ± 0.007
pH	3.95 ± 0.08	No data	4.01 ± 0.13	4.47 ± 0.15	4.1 ± 0.02	4.01 ± 0.05
TDS (mg/L)	198 ± 0.00	No data	221.5 ± 9.81	285.01 ± 7.12	228.00 ± 0.00	296.50 ± 2.12
Temp (°C)	21.05 ± 0.01	No data	22.83 ± 0.12	27.73 ± 0.33	25.26 ± 0.03	24.69 ± 0.33
TOC (mg C/L)	4.01 ± 0.06	2.18 ± 0.09	22.02 ± 0.41	5.68 ± 0.34	2.27 ± 0.31	1.68 ± 0.01
DOC (mg C/L)	3.71 ± 0.01	2.41 ± 0.03	21.07 ± 0.91	7.31 ± 0.10	1.87 ± 0.21	1.87 ± 0.10
TN (mg N/L)	0.67 ± 0.01	1.07 ± 0.02	4.06 ± 0.15	0.95 ± 0.01	0.28 ± 0.007	0.29 ± 0.007
TP (µg P/L)	8.83 ± 0.56	109.97 ± 23.21	146.44 ± 7.81	29.29 ± 0.73	6.58 ± 1.71	4.86 ± 0.82
DN (mg N/L)	0.63 ± 0.001	0.41 ± 0.007	2.93 ± 0.08	0.72 ± 0.04	0.25 ± 0.001	0.27 ± 0.00
DP (µg P/L)	7.11 ± 0.04	6.92 ± 0.04	64.71 ± 4.23	17.99 ± 2.02	4.49 ± 0.11	4.01 ± 0.19

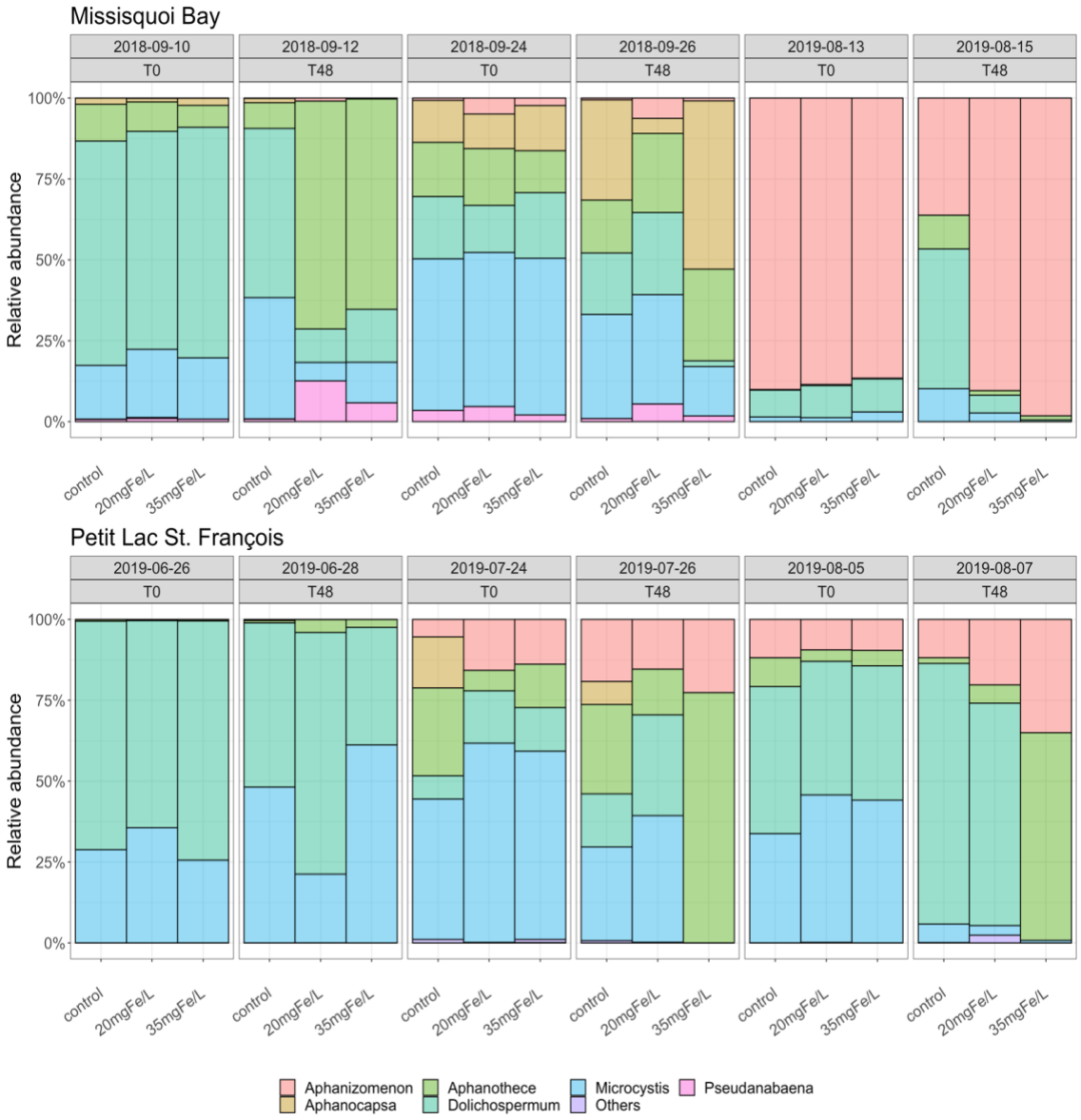

Figure A.1 Relative abundance of cyanobacterial cells at genus level

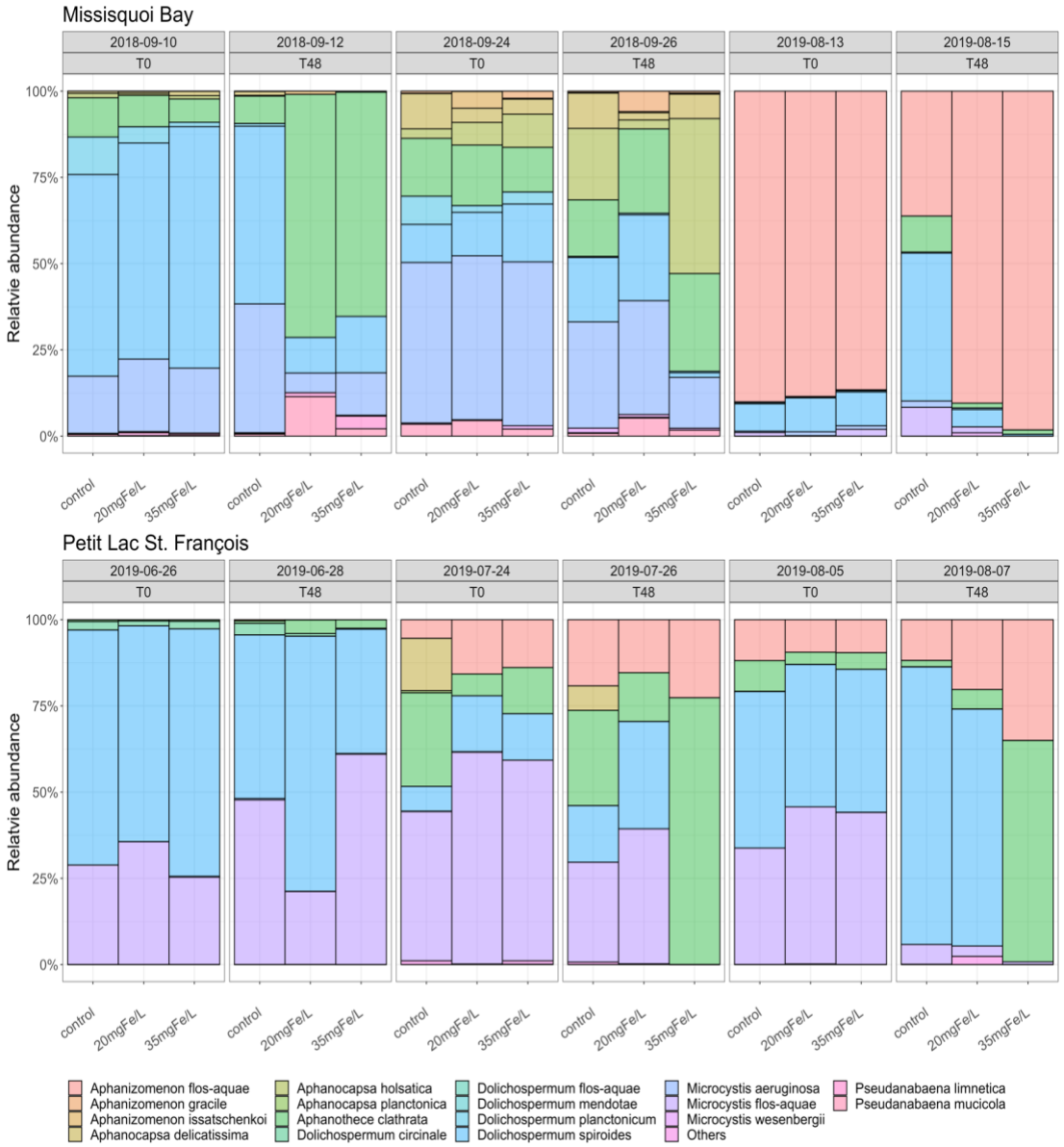

Figure A.2 Relative abundance of cyanobacterial cells at species level

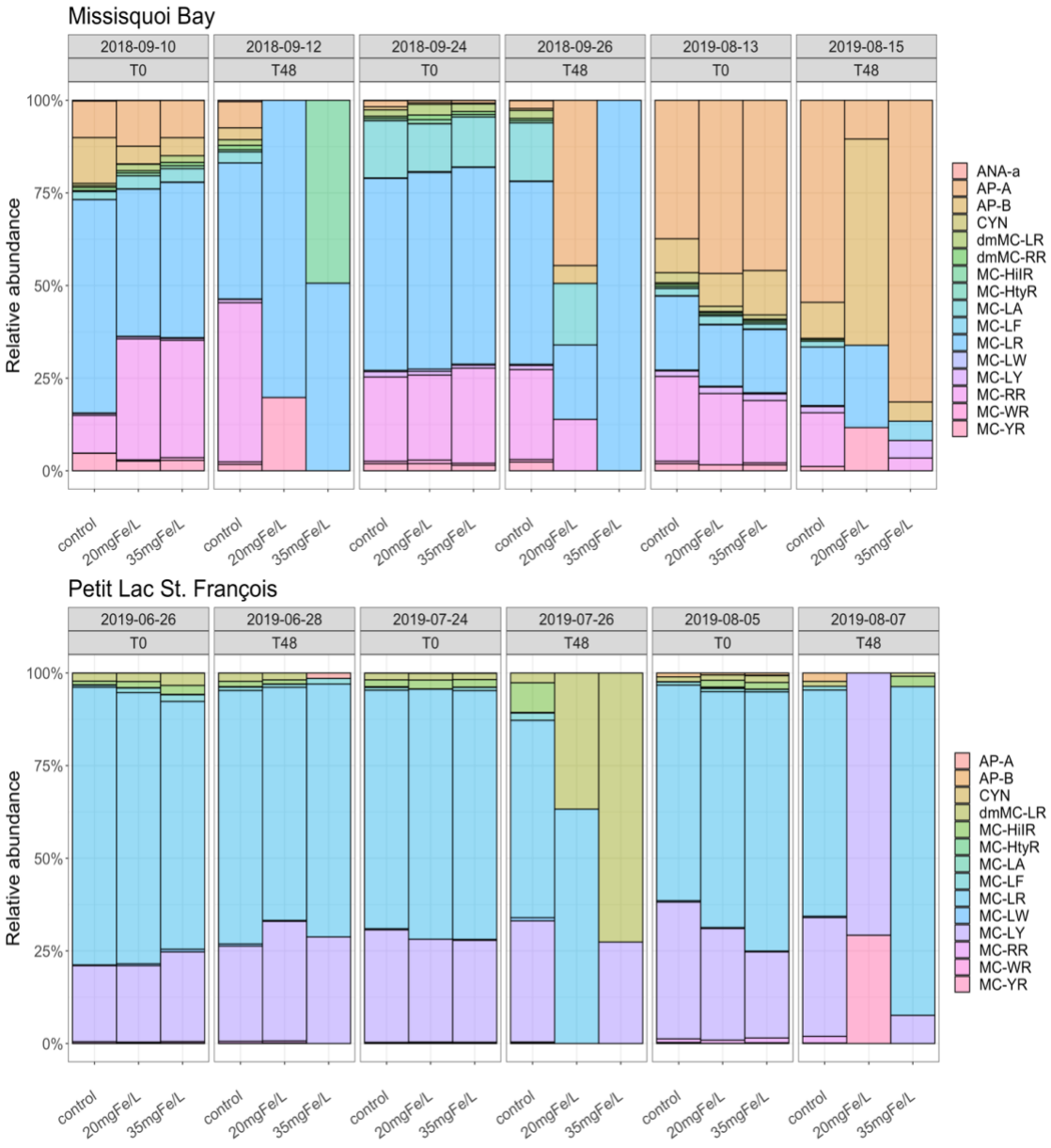

Figure A.3 Relative abundance of intracellular cyanotoxins

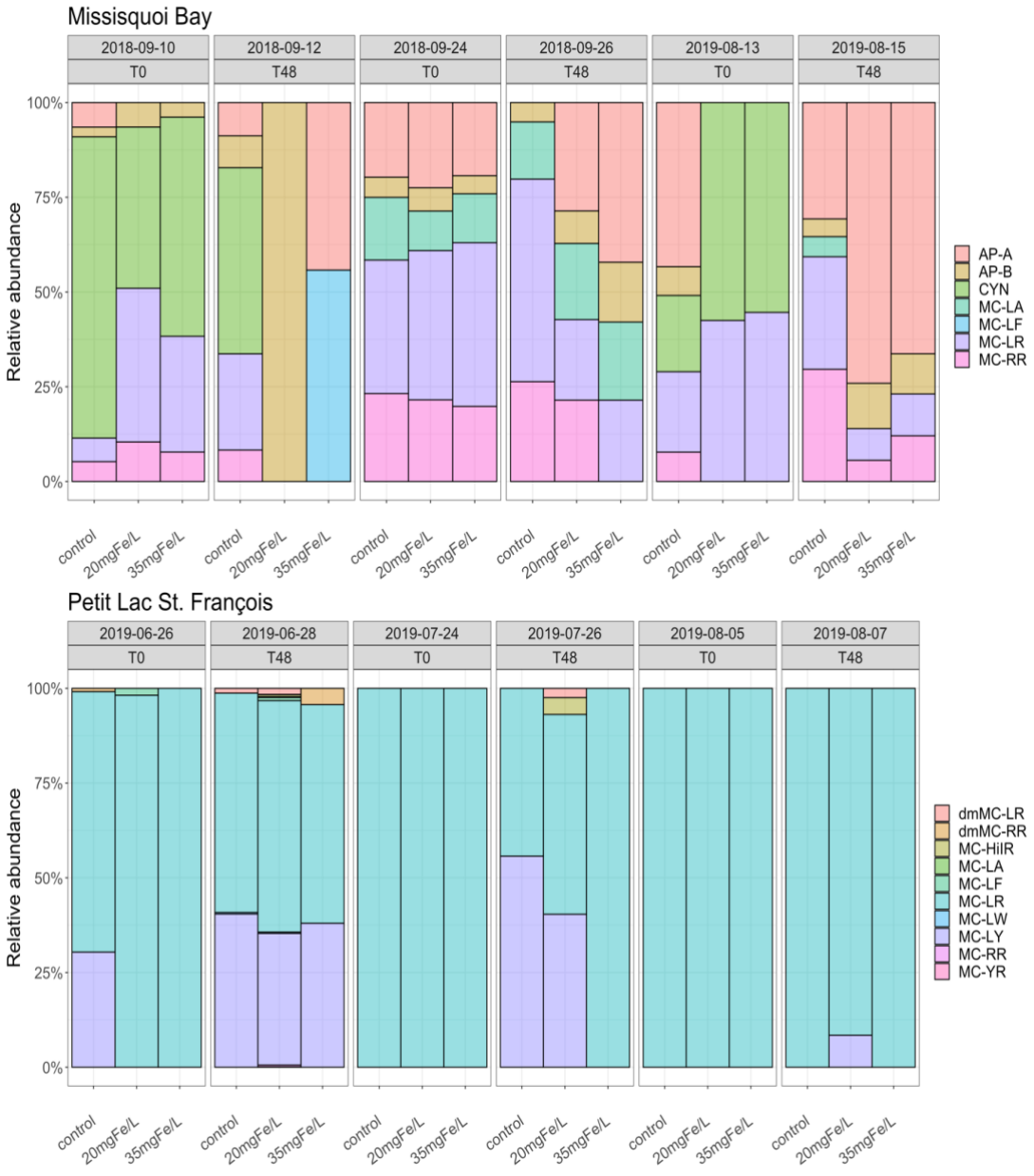

Figure A.4 Relative abundance of extracellular cyanotoxins

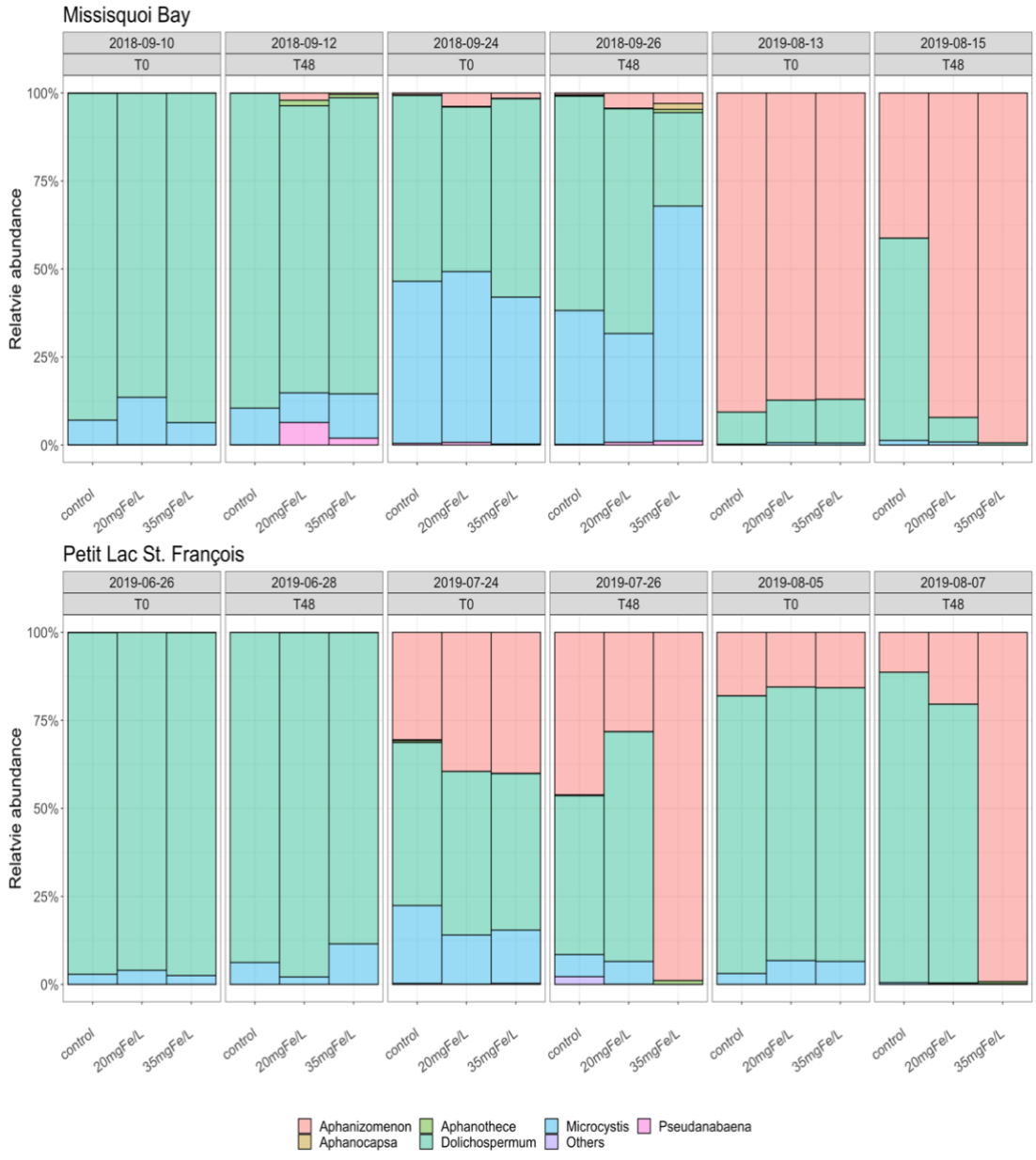

Figure A.5 Relative abundance of cyanobacterial biovolume at genus level

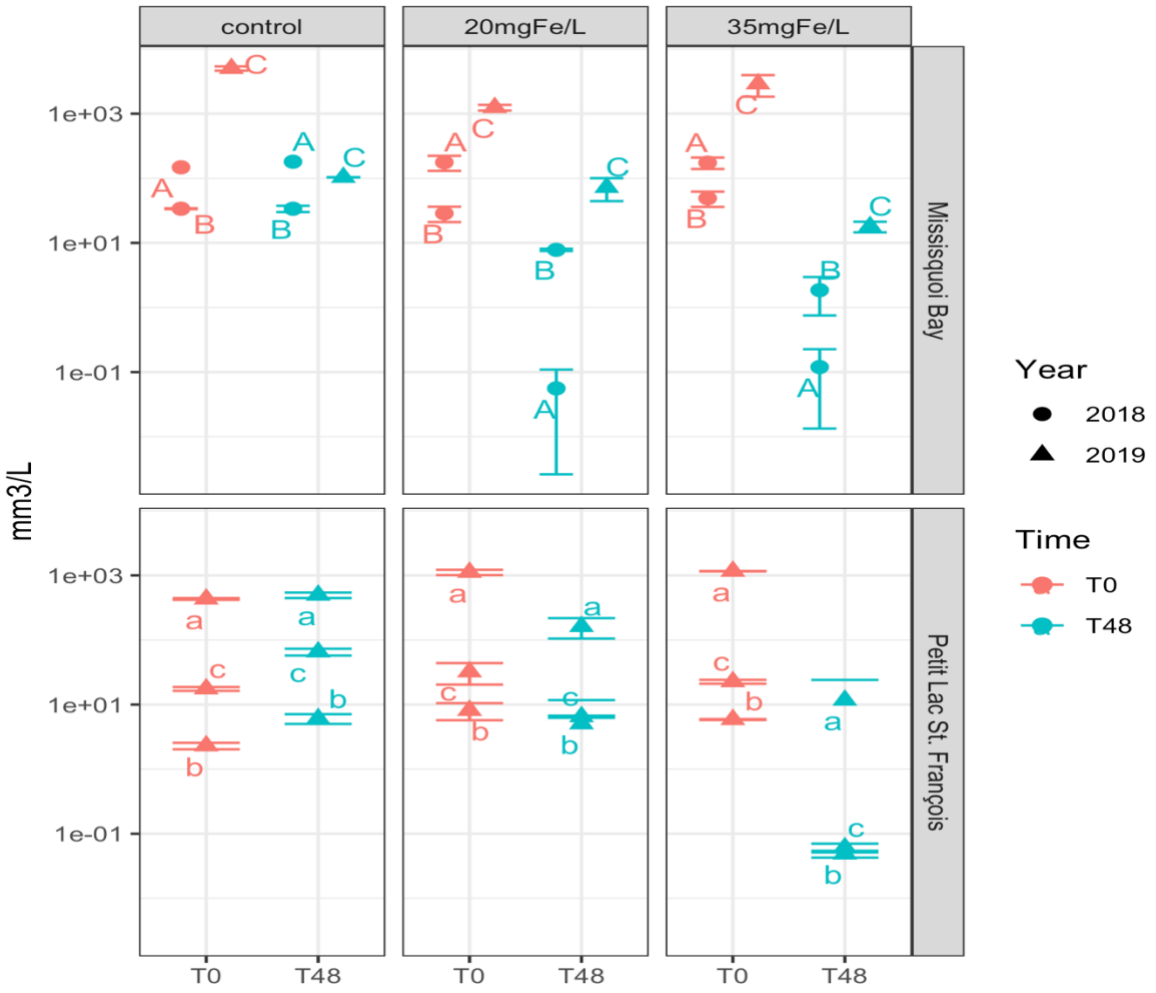

Figure A.6 Total cyanobacterial biovolume (Mean ± standard deviation): A) September 10-12, 2018; B) September 24-26, 2018; C) August 13-15, 2019; a) June 26-28, 2019; b) July 24-26, 2019; c) August 05-07, 2019

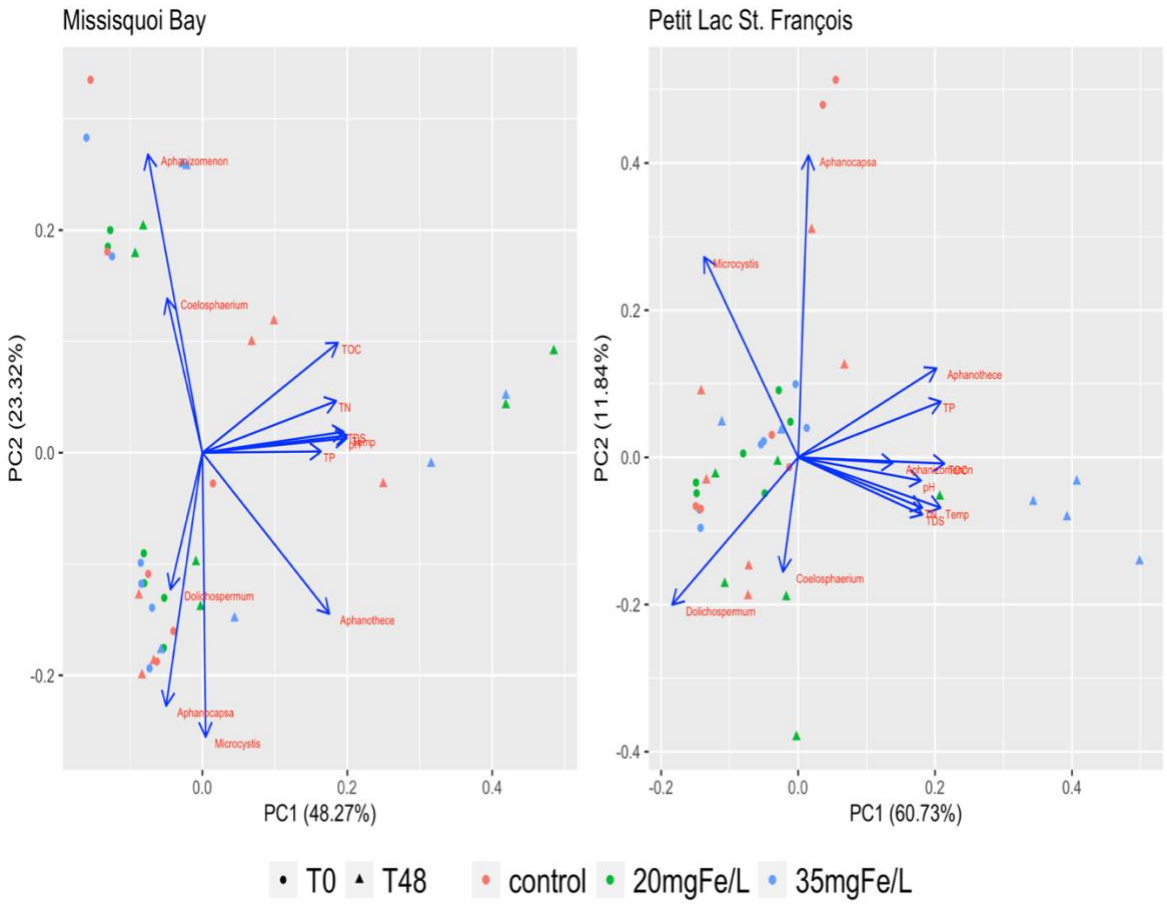

Figure A.7 Principal component analysis (PCA) of cyanobacterial cell counts respect to environmental conditions in control, 20 mgFe/L and 35 mgFe/L mesocosms in Missisquoi Bay and Petit Lac St. François

APPENDIX B SUPPLEMENTARY INFORMATION, ARTICLE 2

Journal: Toxins

Title: Shotgun metagenomic sequencing to assess cyanobacterial community composition following coagulation of cyanobacterial blooms

Author: Kim Thien Nguyen Le, Juan Francisco Guerra Maldonado, Eyerusalem Goitom, Hana Trigui, Yves Terrat, Thanh-Luan Nguyen, Barry Husk, B. Jesse Shapiro, Sébastien Sauvé, Michèle Prévost and Sarah Dorner

Table B.1 Removal effectiveness (%) of taxonomic cell counts of individual cyanobacterial genus after 48 hours (Mean±Standard deviation) in Missisquoi Bay (MB) and Petit Lac St. François (PLSF). Adapted from the author of [185]

Event	Treat	Total cell counts	*Aphanizomenon*	*Aphanocapsa*	*Aphanothece*	*Coelosphaerium*	*Dolichospermum*	*Merismopedia*	*Microcystis*	*Pseudanabaena*
MB September 10-12, 2018	20 mgFe/L	99.96±0.04	97.84±3.05	100.0±0.00	98.30±0.21	NA	99.88±0.04	100.0±0.00	99.97±0.01	99.97±2.8
	35 mgFe/L	99.94±0.04	97.69±1.12	100.0±0.00	97.27±0.11	NA	99.81±0.05	100.0±0.00	99.65±0.05	97.75±0.9
MB September 24-26, 2018	20 mgFe/L	71.91±5.38	68.55±1.94	89.27±6.55	64.38±2.83	NA	75.22±7.48	100.0±0.00	86.92±2.14	72.69±3.3
	35 mgFe/L	96.39±1.29	80.58±7.53	76.56±2.31	69.09±1.63	NA	98.51±1.19	100.0±0.00	93.98±2.61	71.59±8.5
MB August 13-15, 2019	20 mgFe/L	94.27±1.67	93.91±1.64	NA	87.73±6.22	100.0±0.00	97.17±3.14	NA	86.55±1.62	NA
	35 mgFe/L	99.35±0.11	99.26±0.15	NA	96.51±1.44	100.0±0.00	99.97±0.01	NA	100.0±0.00	NA
PLSF June 26-28, 2019	20 mgFe/L	85.22±6.43	77.17±8.16	NA	-37.5±1.67	35.11±5.01	84.66±4.07	NA	92.66±8.64	NA
	35 mgFe/L	98.99±1.06	98.66±1.44	NA	88.50±4.58	100.0±0.00	99.03±0.95	NA	95.82±4.36	NA
PLSF July 24-26, 2019	20 mgFe/L	51.98±6.21	76.76±4.51	NA	-13.5±1.24	19.36±1.14	66.57±4.71	77.5±0.28	66.99±0.95	NA
	35 mgFe/L	99.11±0.11	97.93±0.28	NA	92.65±0.96	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	NA
PLSF August 05-07, 2019	20 mgFe/L	78.21±6.72	72.22±6.32	NA	79.47±4.98	75.86±4.33	77.97±5.04	92.14±3.59	96.51±5.39	NA
	35 mgFe/L	99.72±0.65	98.22±0.65	NA	93.46±2.34	100.0±0.00	100.0±0.00	100.0±0.00	99.97±0.01	NA

Removal effectiveness percentage (%) = $\frac{T0 - T48}{T0} \times 100$.

NA: no value

Table B.2 Removal effectiveness (%) of taxonomic cell counts of individual cyanobacterial genus after 48 hours on August 08-10, 2018, in Missisquoi Bay (Mean ± Standard deviation)

Event	Treat	Total cell counts	*Aphanocapsa*	*Aphanothece*	*Dolichospermum*	*Microcystis*
August 08-10, 2018	20 mgFe/L	50.01±0.04	100.0±0.00	53.36±0.21	77.88±0.04	99.97±0.01
	35 mgFe/L	96.94±0.04	100.0±0.00	67.27±0.11	97.81±0.05	

Removal effectiveness percentage (%) = $\frac{T0 - T48}{T0} \times 100$.

NA: no value

Table B.3 Mean ± standard deviation value for environmental conditions of the sampled water in the control mesocosms on August 08-10, 2018, in Missisquoi Bay (n=2)

Parameters	August 08 2018	August 10 2018
Chlorophyll-*a* (RFU)	20.01 ± 2.45	-
Phycocyanin (RFU)	155.16 ± 2.96	-
pH	8.61 ± 0.07	-
DO (mg/L)	9.11 ± 0.21	-
Temp (°C)	26.70 ± 0.21	-
TOC (mg C/L)	2387.5 ± 45.96	185.51 ± 4.01
DOC (mg C/L)	38.41 ± 0.27	36.10 ± 1.46
TN (mg N/L)	14.44 ± 0.83	14.85 ± 0.69
TP (µg P/L)	3155.01 ± 67.45	1769.02 ± 89.39
DN (mg N/L)	0.10 ± 0.001	5.46 ± 0.57
DP (µg P/L)	328.22 ± 2.07	168.58 ± 5.79

- : No data

Table B.4 Environmental conditions of lake water samples in control mesocosms (n = 6) at T0 (Mean ± standard deviation). Adapted from author of [185]

Parameters	Missisquoi Bay			Petit Lac St. François		
	(A) **10 September 2018**	**(B)** **24 September 2018**	**(C)** **13 August 2019**	**(a)** **26 June 2019**	**(b)** **24 July 2019**	**(c)** **05 August 2019**
Total cell counts (cells/mL)	998,183 ± 35,034	547,325 ± 16,578	17,408,158 ± 138,898	6,033,197 ± 316,425	109,193 ± 3578	235,723 ± 5986
Chlorophyll-*a* (RFU)	No data	No data	62.22 ± 1.03	3.97 ± 0.04	5.14 ± 0.06	6.27 ± 0.08
pH	6.5 ± 0.08	6.4 ± 0.07	8.05 ± 0.24	8.08 ± 0.07	7.84 ± 0.03	8.01 ± 0.01
TDS (mg/L)	101 ± 0.00	100 ± 0.00	98.00 ± 0.00	122.50 ± 0.71	116.00 ± 0.00	115.00 ± 0.00
Temp (°C)	21.8 ± 0.01	18.7 ± 0.12	26.89 ± 0.21	25.96 ± 0.49	25.14 ± 0.12	24.43 ± 0.24
TOC (mg C/L)	15.22 ± 0.25	5.55 ± 0.07	885.00 ± 33.34	175.00 ± 9.50	11.10 ± 0.38	10.76 ± 0.13
DOC (mg C/L)	7.50 ± 0.05	5.00 ± 0.00	19.34 ± 1.67	9.80 ± 0.11	9.83 ± 0.13	11.34 ± 1.58
TN (mg N/L)	5.55 ± 0.65	2.75 ± 0.08	7.84 ± 2.95	12.85 ± 0.57	1.11 ± 0.05	1.29 ± 0.008
TP (µg P/L)	360.51 ± 0.01	292.11 ± 13.28	4336.92 ± 74.65	723.55 ± 24.97	110.38 ± 1.89	131.78 ± 7.71
DN (mg N/L)	0.45 ± 0.004	0.52 ± 0.009	2.66 ± 0.12	0.73 ± 0.08	0.60 ± 0.02	0.50 ± 0.02
DP (µg P/L)	17.05 ± 0.07	17.99 ± 0.45	302.17 ± 12.98	139.06 ± 3.35	23.92 ± 4.91	42.86 ± 1.13

Table B.5 Environmental conditions of lake water samples in control mesocosms at T48 (Mean ± standard deviation). Adapted from author of [185]

Parameters	Missisquoi Bay			Petit-Lac-St-François		
	Event A **September 12 2018**	**Event B** **September 26 2018**	**Event C** **August 15 2019**	**Event a** **June 28 2019**	**Event b** **July 26 2019**	**Event c** **August 07 2019**
Chlorophyll-*a* (RFU)	No data	No data	64.72 ± 0.59	3.37 ± 0.32	5.63 ± 0.16	7.88 ± 0.17
pH	7.47 ± 0.07	No data	6.33 ± 0.09	9.93 ± 0.06	8.36 ± 0.07	7.37 ± 0.15
TDS (mg/L)	105 ± 0.00	No data	140.0 ± 4.24	151.0 ± 5.65	118.5 ± 0.71	121.0 ± 0.00
Temp (°C)	22.7 ± 0.17	No data	22.09 ± 0.17	27.81 ± 0.41	25.37 ± 0.34	25.25 ± 0.02
TOC (mg C/L)	19.97 ± 0.24	5.46 ± 0.00	700.0 ± 23.19	11.39 ± 0.23	10.57 ± 0.21	10.19 ± 0.44
DOC (mg C/L)	12.17 ± 0.39	5.08 ± 0.05	73.48 ± 1.45	9.83 ± 0.06	10.07 ± 0.83	9.81 ± 0.07
TN (mg N/L)	5.46 ± 0.69	1.68 ± 0.01	6.84 ± 1.78	11.21 ± 1.07	1.01 ± 0.15	1.28 ± 0.05
TP (µg P/L)	320.92 ± 40	177.49 ± 21.	2074.60 ± 20	603.01 ± 31	72.08 ± 5.32	89.29 ± 4.81
DN (mg N/L)	2.02 ± 0.001	0.48 ± 0.009	1.56 ± 0.11	0.91 ± 0.25	0.58 ± 0.00	0.63 ± 0.03
DP (µg P/L)	40.47 ± 1.17	15.43 ± 0.25	215.01 ± 11.61	108.06 ± 2.22	16.46 ± 1.16	21.81 ± 2.13

Table B.6 Environmental conditions of lake water samples in mesocosms with dose of 20 mgFe/L at T48 (Mean ± standard deviation)

Parameters	Missisquoi Bay			Petit-Lac-St-François		
	Event A **September 12 2018**	**Event B** **September 26 2018**	**Event C** **August 15 2019**	**Event a** **June 28 2019**	**Event b** **July 26 2019**	**Event c** **August 07 2019**
Chlorophyll-*a* (RFU)	No data	No data	1.04 ± 0.002	0.08 ± 0.00	1.18 ± 0.01	0.82 ± 0.07
pH	4.9 ± 0.04	No data	5.08 ± 1.01	6.09 ± 0.01	7.70 ± 0.19	5.66 ± 0.02
TDS (mg/L)	138 ± 0.00	No data	152.00 ± 0.00	130.00 ± 0.00	139.00 ± 0.00	146.00 ± 0.00
Temp (°C)	21.8 ± 0.01	No data	22.95 ± 0.04	23.92 ± 0.02	25.21 ± 0.01	25.03 ± 0.01
TOC (mg C/L)	4.52 ± 0.15	2.22 ± 0.09	23.76 ± 4.52	17.56 ± 2.71	3.81 ± 0.51	3.69 ± 0.11
DOC (mg C/L)	3.21 ± 0.03	2.39 ± 0.03	22.62 ± 1.92	15.10 ± 2.87	3.63 ± 0.24	3.44 ± 0.01
TN (mg N/L)	0.67 ± 0.01	1.29 ± 0.01	4.16 ± 1.01	3.71 ± 0.48	0.55 ± 0.007	0.35 ± 0.009
TP (µg P/L)	8.47 ± 0.35	126.02 ± 14.54	167.73 ± 6.34	130.02 ± 14.49	20.12 ± 1.15	14.73 ± 0.71
DN (mg N/L)	0.64 ± 0.001	0.39 ± 0.01	3.28 ± 0.29	1.28 ± 0.04	0.36 ± 0.02	0.23 ± 0.001
DP (µg P/L)	5.51 ± 0.02	7.14 ± 0.14	60.08 ± 12.79	19.34 ± 1.44	6.72 ± 1.56	3.56 ± 0.21

Table B.7 Environmental conditions of lake water samples in mesocosms with dose of 35 mgFe/L at T48 (Mean ± standard deviation)

Parameters	Missisquoi Bay			Petit-Lac-St-François		
	Event A **September 12** **2018**	**Event B** **September 26** **2018**	**Event C** **August 15** **2019**	**Event a** **June 28** **2019**	**Event b** **July 26** **2019**	**Event c** **August 07** **2019**
Chlorophyll-*a* (RFU)	No data	No data	1.05 ± 0.002	0.01 ± 0.00	0.09 ± 0.007	0.01 ± 0.00
pH	3.95 ± 0.08	No data	4.01 ± 0.13	4.47 ± 0.15	4.1 ± 0.02	4.01 ± 0.05
TDS (mg/L)	198 ± 0.00	No data	221.5 ± 9.81	285.01 ± 7.12	228.00 ± 0.00	296.50 ± 2.12
Temp (°C)	21.05 ± 0.01	No data	22.83 ± 0.12	27.73 ± 0.33	25.26 ± 0.03	24.69 ± 0.33
TOC (mg C/L)	4.01 ± 0.06	2.18 ± 0.09	22.02 ± 0.41	5.68 ± 0.34	2.27 ± 0.31	1.68 ± 0.01
DOC (mg C/L)	3.71 ± 0.01	2.41 ± 0.03	21.07 ± 0.91	7.31 ± 0.10	1.87 ± 0.21	1.87 ± 0.10
TN (mg N/L)	0.67 ± 0.01	1.07 ± 0.02	4.06 ± 0.15	0.95 ± 0.01	0.28 ± 0.007	0.29 ± 0.007
TP (µg P/L)	8.83 ± 0.56	109.97 ± 23.1	146.44 ± 7.81	29.29 ± 0.73	6.58 ± 1.71	4.86 ± 0.82
DN (mg N/L)	0.63 ± 0.001	0.41 ± 0.007	2.93 ± 0.08	0.72 ± 0.04	0.25 ± 0.001	0.27 ± 0.00
DP (µg P/L)	7.11 ± 0.04	6.92 ± 0.04	64.71 ± 4.23	17.99 ± 2.02	4.49 ± 0.11	4.01 ± 0.19

Table B.8 Pairwise Kruskal-Wallis test, showing differences in changing of the species richness and Shannon indices at genus level between control mesocosms and mesocosms with dose of 20 mgFe/L, control mesocosms and mesocosms with dose of 35 mgFe/L, mesocosms with dose of 20 mgFe/L and 35 mgFe/L after 48 hours in Missisquoi Bay and Petit Lac St. François (p-value< 0.05)

		df	***chi-squared***	***p*-value**
Missisquoi Bay	***Richness***	1	1.218	0.264
	Shannon	1	0.561	0.452
Petit Lac St. François	***Richness***	1	1.334	0.248
	Shannon	1	0.044	0.833

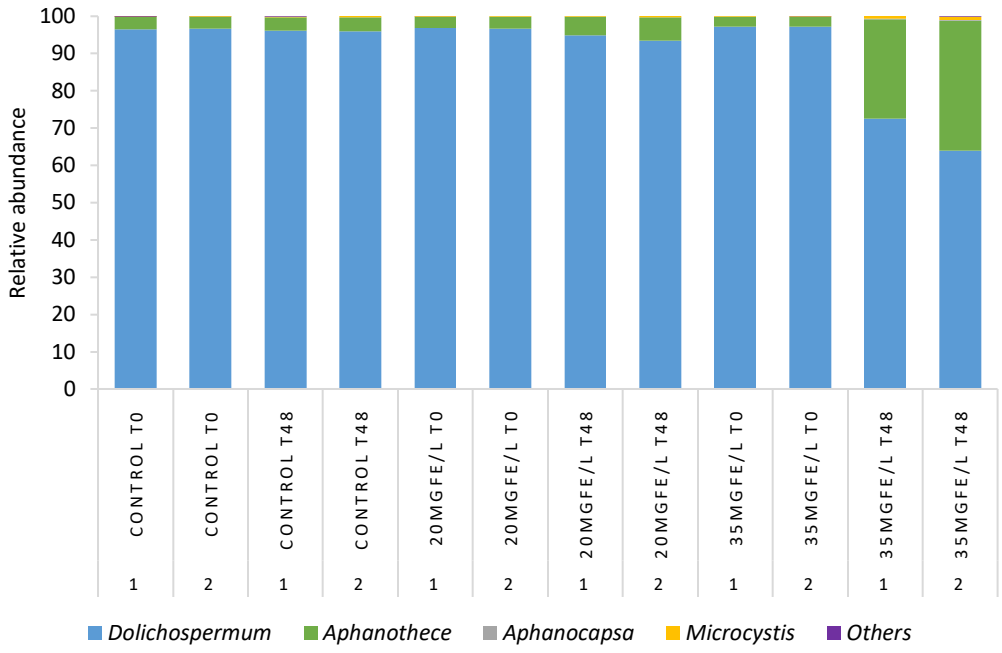

Figure B.1 Cyanobacterial relative abundance at genus level in the control, 20 mgFe/L and 35 mgFe/L mesocosms in Missisquoi Bay in August 08 (T0) and August 10 (T48), 2018

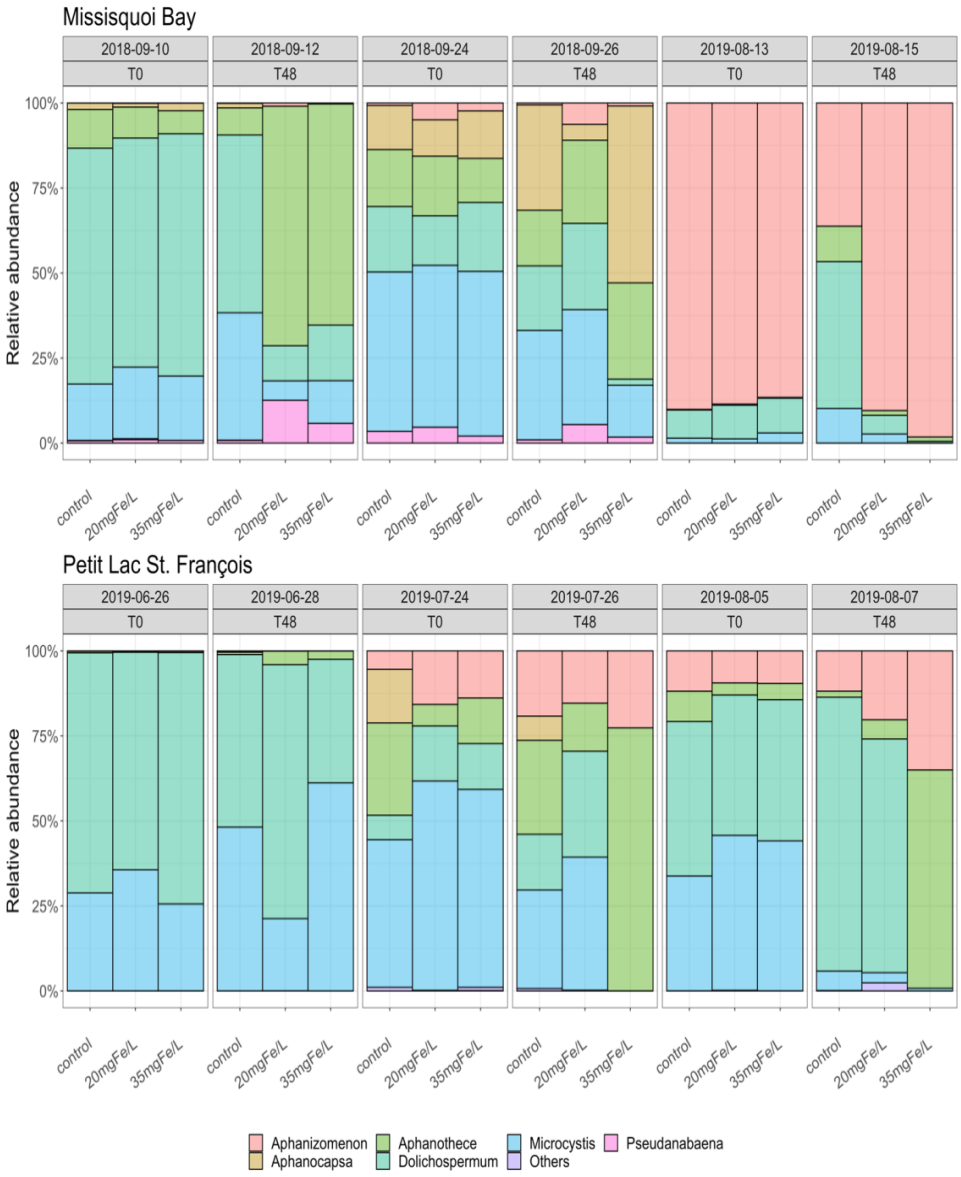

Figure B.2 Cyanobacterial relative abundance at genus level in the control, 20 mgFe/L and 35 mgFe/L mesocosms in Missisquoi Bay and Petit Lac St. François. Adapted from [185]

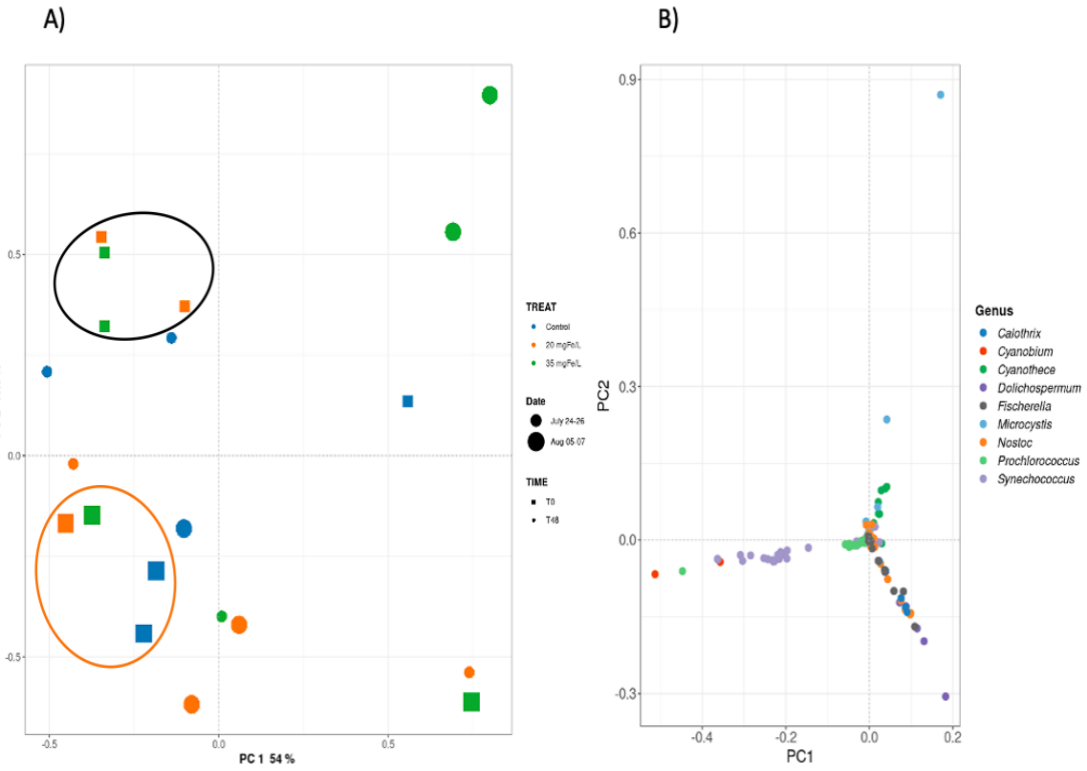

Figure B.3 Principal components analysis (PCA) of the normalized relative abundance of cyanobacteria community composition in control, 20 mgFe/L and 35 mgFe/L mesocosms with respect to genus abundance in Petit Lac St. François. (a) PCA analysis of cyanobacterial community following coagulation; (b) Data are plotted following the genus-level classification

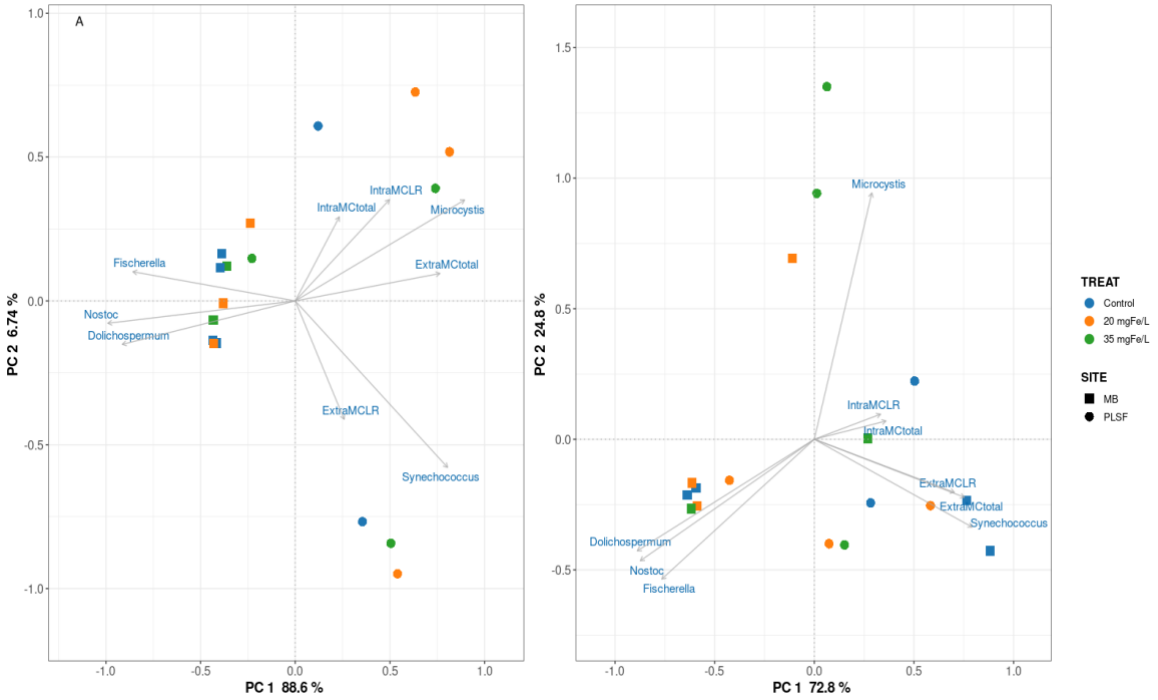

Figure B.4 Principal components analysis (PCA) of the normalized relative abundance of cyanobacteria community composition in control, 20 mgFe/L and 35 mgFe/L mesocosms with respect to intra- and extracellular total microcystins, MC-LR in Missisquoi Bay and Petit Lac St. François at T0 (left panel) and T48 (right panel)

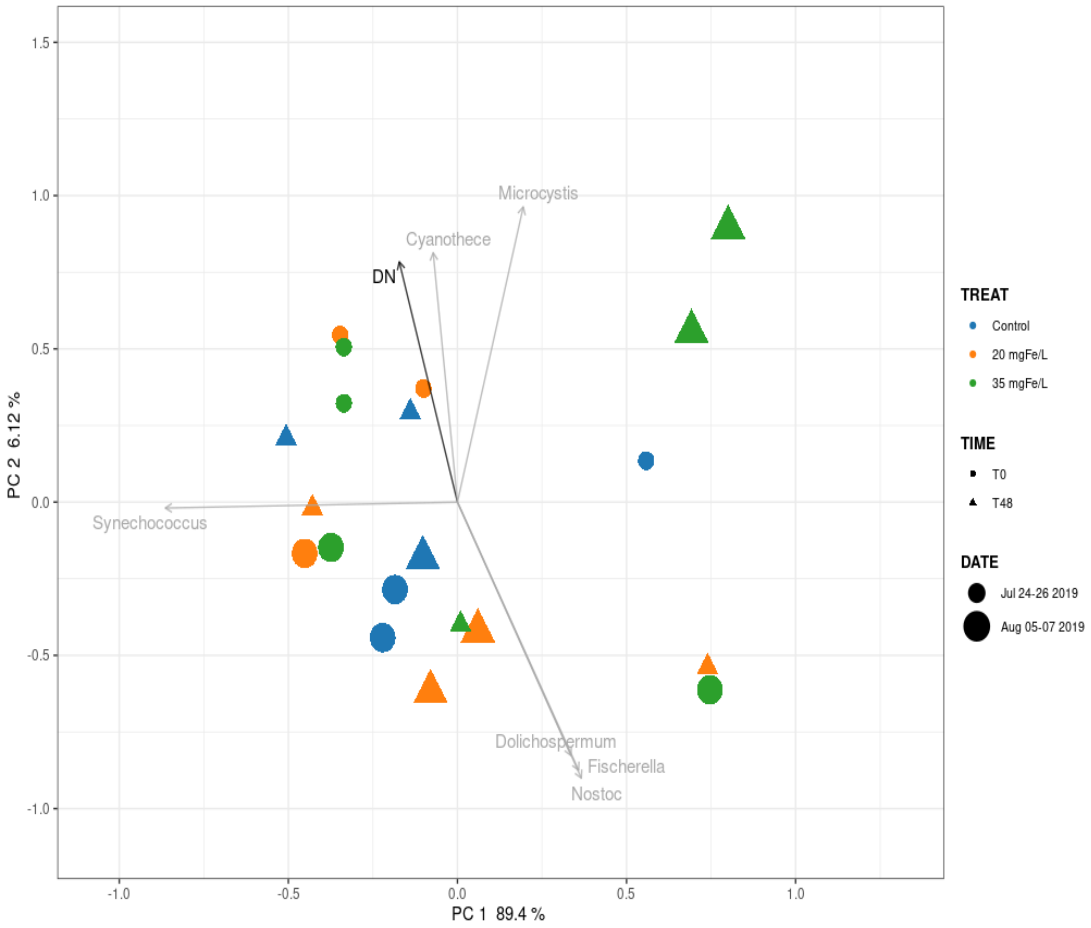

Figure B.5 Principal components analysis (PCA) of the normalized relative abundance of cyanobacteria community composition in control, 20 mgFe/L and 35 mgFe/L mesocosms with respect to genus abundance in Petit Lac St. François (PLSF). (a) PCA analysis of cyanobacterial community following coagulation; (b) Data are plotted following the genus-level classification

APPENDIX C SUPPLEMENTARY INFORMATION, ARTICLE 3

Submitted on 31st 2023 to Science of The Total Environment Journal

Table C.1 Average concentration of N and P fraction and temperature in all mesocosms in the beginning of sampling period in Missisquoi Bay

	31 July 2018	**24 September 2018**	**13 August 2019**	**20 September 2019**
Total nitrogen (TN) mg N/L	5.83	2.82	8.89	0.59
Dissolved nitrogen (DN) mg N/L	1.25	0.51	2.22	0.36
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.032	0.051	0.01	0.01
Ammonium (NH_3 + NH_4^+) mg N/L	0.174	0.011	0.085	0.0056
Dissolved organic nitrogen (DON) mg N/L	2.78	0.45	2.15	0.48
Kjeldahl nitrogen (TKN) mg N/L	5.78	2.76	8.88	0.58
Total phosphors (TP) µg P/L	1311	337	3101	780
Dissolved phosphorus (DP) µg P/L	101	17.7	210.92	704.4
Particulate phosphorus (PP) µg P/L	1292	235	2589	18.08
Dissolved organic phosphorus (DOP) µg P/L	19.01	13.7	174.1	320
Soluble reactive phosphorus (SRP) µg P/L	45.2	2.5	51.09	5.62
Temperature (°C)	27.05	17.50	26.06	18.18
pH	8.81	8.90	9.02	9.22
TN:TP	4.45	8.37	2.87	0.76
DIN:SRP	4.56	24.8	1.86	2.79

Table C.2 Average concentration of N and P fraction and temperature in all mesocosms in the beginning of sampling period in Petit Lac St. François

	26 June 2019	**24 July 2019**	**05 August 2019**	**09 October 2019**
Total nitrogen (TN) mg N/L	11.45	1.21	1.12	30.0
Dissolved nitrogen (DN) mg N/L	0.750	0.65	0.55	0.51
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.015	0.015	0.01	0.01
Ammonium (NH_3 + NH_4^+) mg N/L	0.00755	0.0055	0.0095	0.0589
Dissolved organic nitrogen (DON) mg N/L	0.36	0.12	0.12	2.23
Kjeldahl nitrogen (TKN) mg N/L	11.43	1.19	1.11	29.99
Total phosphorus (TP) μg P/L	670.01	111.12	137.1	1550.10
Dissolved phosphorus (DP) μg P/L	132.11	25.12	42.35	465.90
Particulate phosphorus (PP) μg P/L	350.89	42.44	53.1	760.56
Dissolved organic phosphorus (DOP) μg P/L	20.01	6.50	7.89	100.01
Soluble reactive phosphorus (SRP) μg P/L	6.68	7.12	8.31	6.99
Temperature (°C)	22.0	25.0	26.5	13.2
pH	9.45	7.82	8.82	8.45
TN:TP	17.09	10.89	8.17	19.35
DIN:SRP	13.56	2.88	2.35	9.86

Table C.3 Average concentration of N and P fraction and temperature in the control mesocosms after 48 hours in Missisquoi Bay

	02 August 2018	**26 September 2018**	**15 August 2019**	**22 September 2019**
Total nitrogen (TN) mg N/L	5.35	1.69	8.38	0.74
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.012	0.000	0.007	0.01
Ammonium (NH_3 + NH_4^+) mg N/L	0.104	0.011	0.055	0.064
Kjeldahl nitrogen (TKN) mg N/L	5.33	1.69	8.37	0.73
Total phosphorus (TP) μg P/L	408.10	192.80	1931	199.12
Dissolved phosphorus (DP) μg P/L	32.70	17.2	707.01	72.08
Particulate phosphorus (PP) μg P/L	395.6	175.6	611.12	112.01
Dissolved organic phosphorus (DOP) μg P/L	12.7	15.4	256.11	50.61
Soluble reactive phosphorus (SRP) μg P/L	32.21	2.50	195.21	11.98
Temperature (°C)	26.78	16.78	25.45	18.92
pH	No data	No data	6.44	8.01
TN:TP	13.11	8.80	4.38	3.71
DIN:SRP	3.61	4.40	0.31	6.37

Table C.4 Average concentration of N and P fraction and temperature in the control mesocosms after 48 hours in Petit Lac St. François

	28 June 2019	**26 July 2019**	**07 August 2019**	**11 October 2019**
Total nitrogen (TN) mg N/L	10.72	1.13	1.68	22.01
Dissolved nitrogen (DN) mg N/L	1.23	0.78	0.65	4.51
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	<0.001	<0.001	<0.001	<0.001
Ammonia and ammonium (NH_3 + NH_4^+) mg N/L	0.0019	0.0078	0.0091	0.021
Kjeldahl nitrogen (TKN) mg N/L	10.71	1.13	1.68	22.00
Total phosphorus (TP) µg P/L	550.23	102.67	92.76	986.51
Dissolved phosphorus (DP) µg P/L	99.89	18.89	27.02	210.11
Particulate phosphorus (PP) µg P/L	214.09	41.31	58.31	654.09
Dissolved organic phosphorus (DOP) µg P/L	50.09	8.91	13.78	121.90
Soluble reactive phosphorus (SRP) µg P/L	5.43	2.48	3.45	4.56
Temperature (°C)	25.51	25.60	25.22	11.91
pH	9.89	8.31	7.48	7.34
TN:TP	19.49	11.07	18.26	21.69
DIN:SRP	0.34	3.14	2.63	4.61

Table C.5 Average concentration of N and P fraction and temperature in the NH_4^+ 700 μg N/L mesocosms after 48 hours in Missisquoi Bay

	02 August 2018	**26 September 2018**	**15 August 2019**	**22 September 2019**
Total nitrogen (TN) mg N/L	7.83	4.82	7.89	3.59
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.045	0.067	0.11	1.37
Ammonium (NH_3 + NH_4^+) mg N/L	1.41	0.96	1.79	1.85
Kjeldahl nitrogen (TKN) mg N/L	7.78	4.75	7.78	2.22
Total phosphorus (TP) μg P/L	566.34	476.67	3048.4	743.8
Dissolved phosphorus (DP) μg P/L	15.56	15.78	1373.60	697.82
Particulate phosphorus (PP) μg P/L	312.12	243.87	1495.34	92.96
Dissolved organic phosphorus (DOP) μg P/L	13.87	12.89	563.48	321.09
Soluble reactive phosphorus (SRP) μg P/L	12.31	10.67	220.33	33.76
Temperature (°C)	26.71	16.78	24.31	18.91
pH	No data	No data	6.01	8.11
TN:TP	13.83	10.12	2.58	4.83
DIN:SRP	118.19	96.25	8.13	95.37

Table C.6 Average concentration of N and P fraction and temperature in the NH_4^+ 700 μg N/L mesocosms after 48 hours in Petit Lac St. François

	28 June 2019	**26 July 2019**	**07 August 2019**	**11 October 2019**
Total nitrogen (TN) mg N/L	2.84	0.65	0.72	0.74
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.65	0.012	0.032	0.33
Ammonium (NH_3 + NH_4^+) mg N/L	1.41	0.96	1.79	0.85
Kjeldahl nitrogen (TKN) mg N/L	2.19	0.638	0.688	0.41
Total phosphorus (TP) μg P/L	496.77	99.09	127.2	3234
Dissolved phosphorus (DP) μg P/L	22.5	27.76	28.85	225.1
Particulate phosphorus (PP) μg P/L	237.14	60.90	98.3	1054.1
Dissolved organic phosphorus (DOP) μg P/L	7.81	20.11	13.11	121.01
Soluble reactive phosphorus (SRP) μg P/L	16.88	3.21	12.63	41.94
Temperature (°C)	25.26	24.67	25.12	11.88
pH	6.73	6.93	6.31	6.59
TN:TP	5.71	6.56	6.77	0.22
DIN:SRP	122.03	302.28	151.83	28.71

Table C.7 Average concentration of N and P fraction and temperature in the NO_3^- 500 μg N/L mesocosms after 48 hours in Missisquoi Bay

	15 August 2019	**22 September 2019**
Total nitrogen (TN) mg N/L	7.35	1.74
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.92	0.77
Ammonia and ammonium (NH_3 + NH_4^+) mg N/L	0.055	0.164
Kjeldahl nitrogen (TKN) mg N/L	6.43	0.97
Total phosphorus (TP) μg P/L	2896.71	748.60
Dissolved phosphorus (DP) μg P/L	1786.53	705.20
Particulate phosphorus (PP) μg P/L	555.10	27.90
Dissolved organic phosphorus (DOP) μg P/L	875.73	456.78
Soluble reactive phosphorus (SRP) μg P/L	35.04	3.75
Temperature (°C)	25.34	18.92
pH	6.27	8.11
TN:TP	2.53	2.32
DIN:SRP	27.74	49.32

Table C.8 Average concentration of N and P fraction and temperature in the NO_3^- 500 μg N/L mesocosms after 48 hours in Petit Lac St. François

	28 June 2019	**26 July 2019**	**07 August 2019**	**11 October 2019**
Total nitrogen (TN) mg N/L	2.37	1.54	1.28	5.58
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.85	0.71	0.92	0.77
Ammonia and ammonium (NH_3 + NH_4^+) mg N/L	0.061	0.033	0.035	0.056
Kjeldahl nitrogen (TKN) mg N/L	1.52	0.83	0.36	4.81
Total phosphorus (TP) μg P/L	339.09	100.99	102.55	398.11
Dissolved phosphorus (DP) μg P/L	32.31	21.34	17.06	153.69
Particulate phosphorus (PP) μg P/L	122.21	79.87	85.5	122.01
Dissolved organic phosphorus (DOP) μg P/L	9.53	5.56	6.67	32.01
Soluble reactive phosphorus (SRP) μg P/L	13.25	3.45	3.72	68.71
Temperature (°C)	24.62	25.12	25.11	42.54
pH	6.95	8.24	8.69	7.01
TN:TP	6.99	15.40	12.54	14.02
DIN:SRP	68.75	215.36	256.72	121.67

Table C.9 Pairwise Kruskal-Wallis test, showing differences in changing of nitrogen and phosphorus fractions in control mesocosms, mesocosms with NO_3^- (500 μg N/L), and mesocosms with NH_4^+ (700 μg N/L) after 48 hours in Petit-Lac-St-François and Missisquoi Bay (*p*-value< 0.05 is significant)

Missisquoi Bay			
	Control	**NH_4^+ 700 μg/L**	**NO_3^- 500 μg/L**
Total nitrogen (TN) mg N/L	0.342	0.200	0.221
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.101	1.000	0.211
Ammonia and ammonium (NH_3 + NH_4^+) mg N/L	0.661	**0.028**	0.226
Kjeldahl nitrogen (TKN) mg N/L	0.303	1.000	0.193
Total phosphorus (TP) μg P/L	0.342	0.885	0.667
Dissolved phosphorus (DP) μg P/L	1.000	1.000	0.333
Particulate phosphorus (PP) μg P/L	0.306	1.000	0.220
Dissolved organic phosphorus (DOP) μg P/L	0.303	1.000	0.193
Soluble reactive phosphorus (SRP) μg P/L	1.000	1.000	0.333
Petit-Lac-St-François			
	Control	**NH_4^+ 700 μg/L**	**NO_3^- 500 μg/L**
Total nitrogen (TN) mg N/L	0.936	0.132	0.809
Oxidized nitrogen (NO_3^- + NO_2^-) mg N/L	0.002	0.062	**0.004**
Ammonia and ammonium (NH_3 + NH_4^+) mg N/L	0.172	**0.004**	0.890
Kjeldahl nitrogen (TKN) mg N/L	0.808	0.125	0.124

Table C.9 Pairwise Kruskal-Wallis test, showing differences in changing of nitrogen and phosphorus fractions in control mesocosms, mesocosms with NO_3^- (500 μg N/L), and mesocosms with NH_4^+ (700 μg N/L) after 48 hours in Petit-Lac-St-François and Missisquoi Bay (*p*-value< 0.05 is significant) (cont'd)

Total phosphorus (TP) μg P/L	**0.126**	**0.377**	**0.809**
Dissolved phosphorus (DP) μg P/L	0.228	0.574	0.221
Particulate phosphorus (PP) μg P/L	0.808	0.374	0.275
Dissolved organic phosphorus (DOP) μg P/L	0.518	0.373	0.347
Soluble reactive phosphorus (SRP) μg P/L	0.004	0.678	0.377

Table C.10 Pairwise Kruskal-Wallis test, showing differences in changing of total cell counts and several dominant genus's cell counts between control mesocosms and mesocosms with NO_3^- (500 μg N/L), control mesocosms and mesocosms with (NH_4^+ 700 μg N/L) after 48 hours in Petit-Lac-St-François and Missisquoi Bay (*p*-value< 0.05 is significant)

	***p*-value**	
	Control – NH_4^+ 700 μg N/L	**Control – NO_3^- 500 μg/L**
Total cell counts	**0.003**	0.075
Dolichospermum	**0.008**	0.546
Microcystis	0.172	0.683
Aphanothece	0.080	0.353
Aphanizomenon	**0.002**	0.681
Aphanocapsa	0.902	0.052
Chroococcus	0.775	0.629
Pseudanabaena	0.327	0.203

Table C.11 Pairwise Kruskal-Wallis test, showing differences in changing of total intra- and extracellular microcystins (ΣMCs), microcystins, APA and APB between control mesocosms and mesocosms with NO_3^- (500 μg N/L), control mesocosms and mesocosms with NH_4^+ (700 μg N/L) after 48 hours in Petit-Lac-St-François and Missisquoi Bay (*p*-value< 0.05 is significant)

	***p*-value**	
	Control – NH_4^+ 700 μg N/L	**Control – NO_3^- 500 μg/L**
Intracellular Cyanotoxins		
ΣMCs	0.471	0.150
MC-LR	0.260	0.093
MC-LY	0.722	**0.010**
MC-LA	0.518	0.022
MC-LW	0.514	0.147
MC-LF	0.328	0.124
MC-WR	0.221	0.623
MC-RR	0.360	0.016
MC-YR	0.909	0.181
APA	0.552	0.286
APB	0.947	**0.018**
Extracellular Cyanotoxins		
ΣMCs	**0.010**	**0.012**

Table C.11 Pairwise Kruskal-Wallis test, showing differences in changing of total intra- and extracellular microcystins (ΣMCs), microcystins, APA and APB between control mesocosms and mesocosms with NO_3^- (500 μg N/L), control mesocosms and mesocosms with NH_4^+ (700 μg N/L) after 48 hours in Petit-Lac-St-François and Missisquoi Bay (*p*-value< 0.05 is significant) (cont'd)

MC-LR	**0.067**	**0.054**
MC-LY	-	-
MC-LA	0.197	0.121
MC-LW	-	-
MC-LF	-	-
MC-WR	-	-
MC-RR	0.107	**0.047**
MC-YR	-	-
APA	-	-
APB	-	-

-: no data

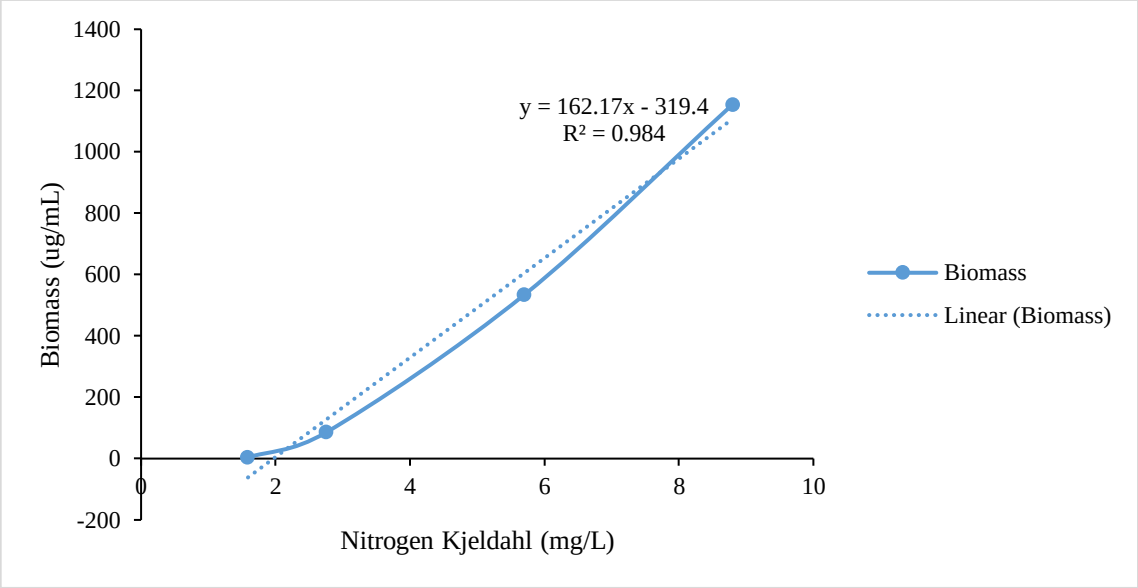

Figure C.1 Linear correlation between total cyanobacterial biomass and nitrogen Kjeldahl in all mesososms in the beginning of sampling period in Missisquoi Bay

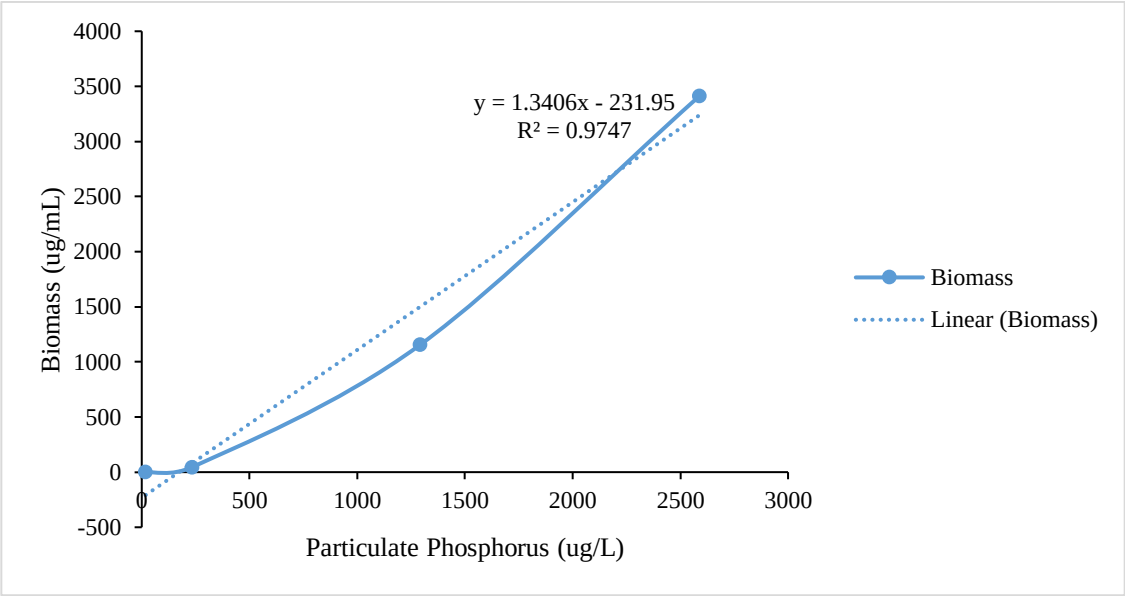

Figure C.2 Linear correlation between total cyanobacterial biomass and particulate phosphorus in all mesososms in the beginning of sampling period in Missisquoi Bay

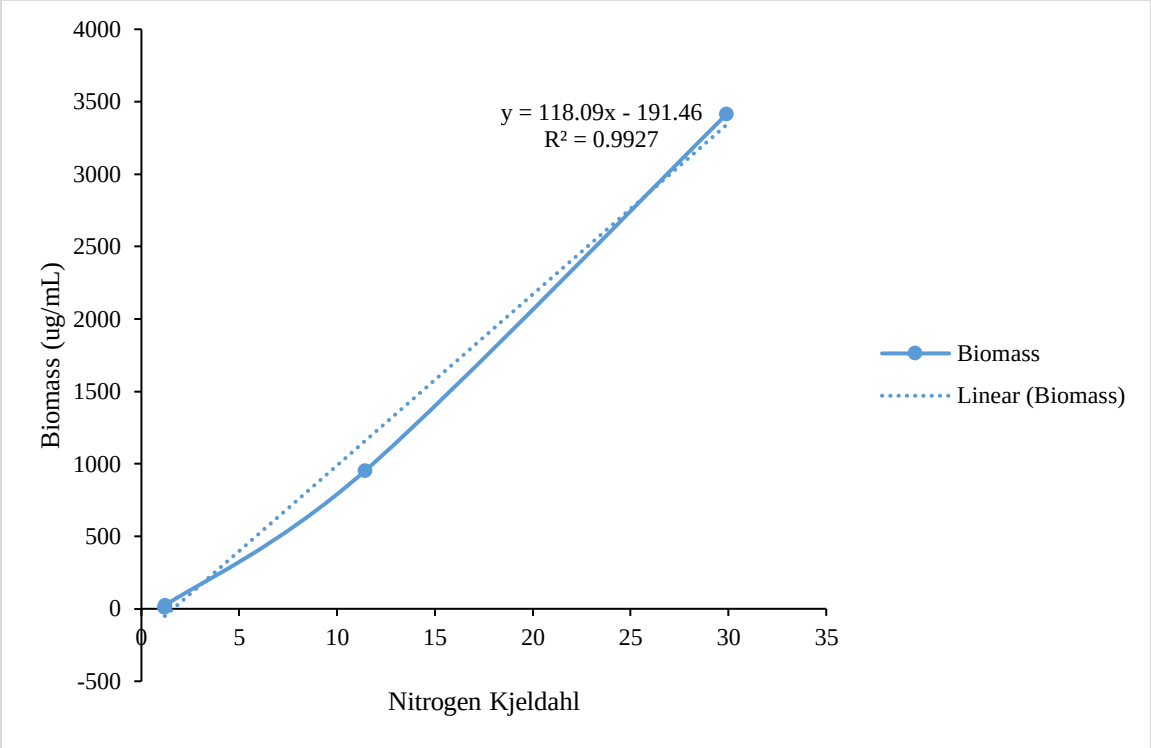

Figure C.3 Linear correlation between total cyanobacterial biomass and nitrogen Kjeldahl in all mesososms in the beginning of sampling period in Petit-Lac-St-François

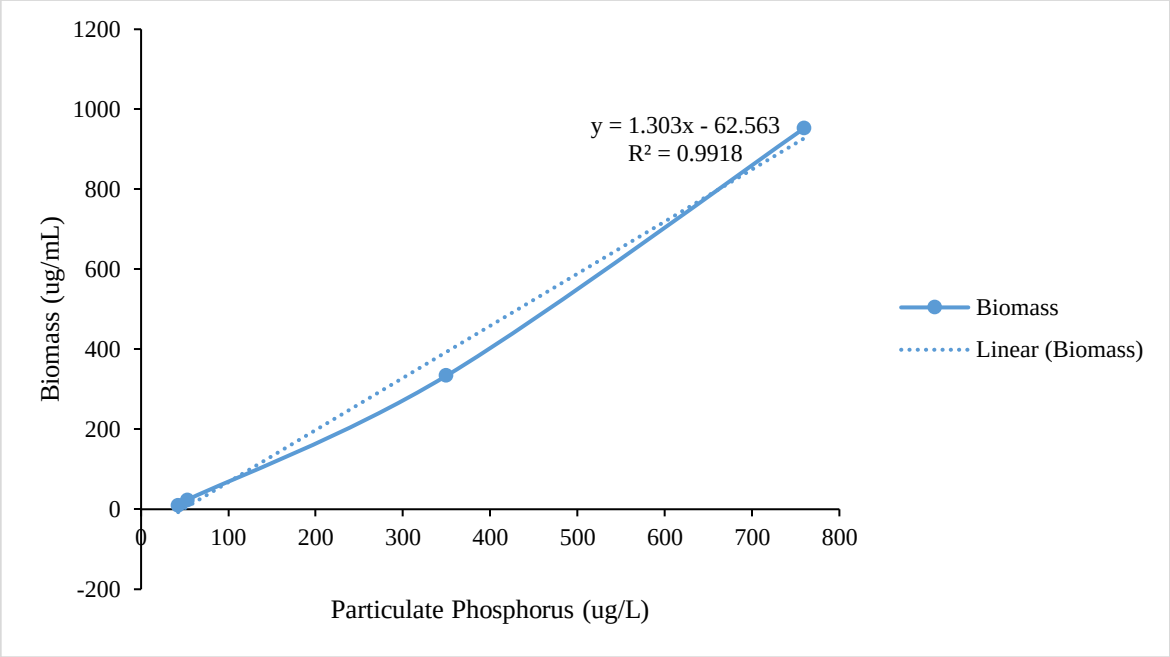

Figure C.4 Linear correlation between total cyanobacterial biomass and particulate phosphorus in all mesososms in the beginning of sampling period in Petit-Lac-St-François

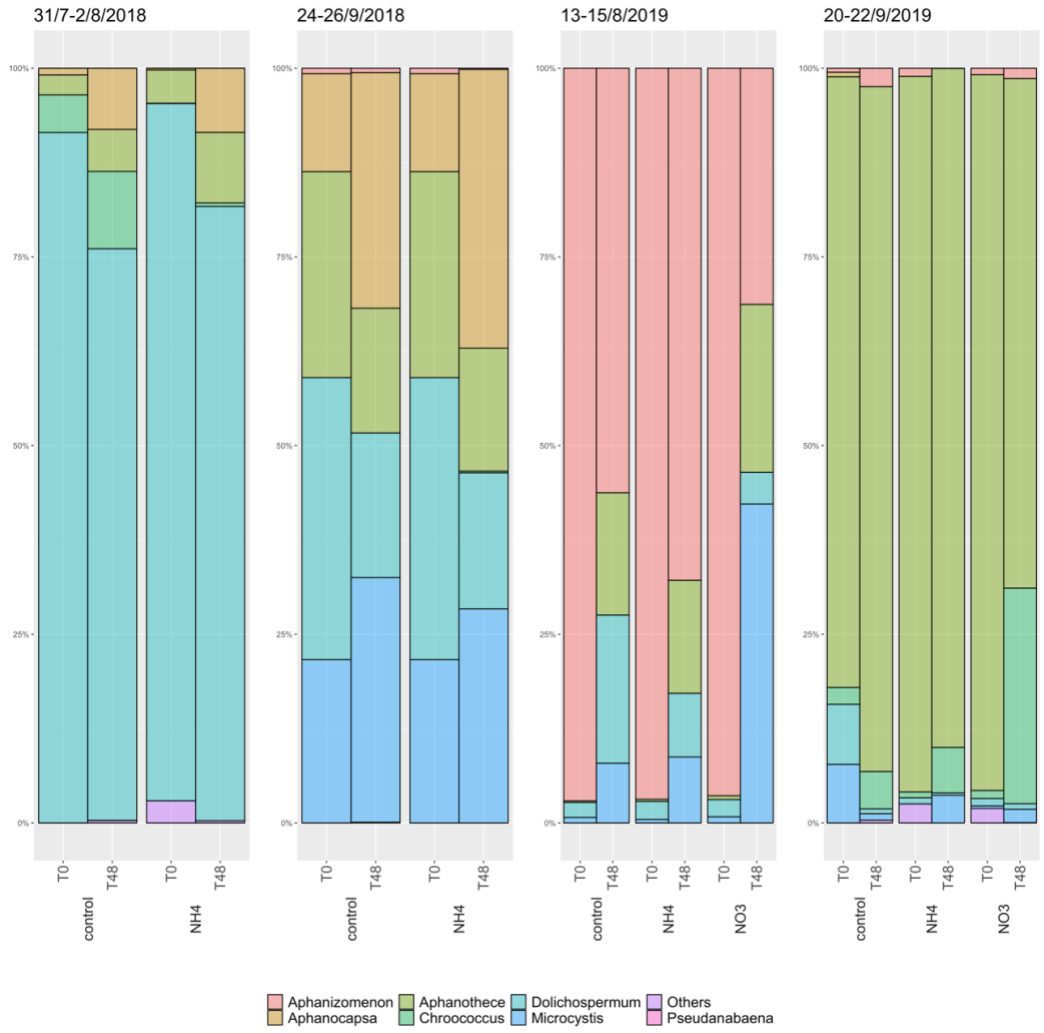

Figure C.5 Relative cell abundance in the mesocosms in Missisquoi Bay

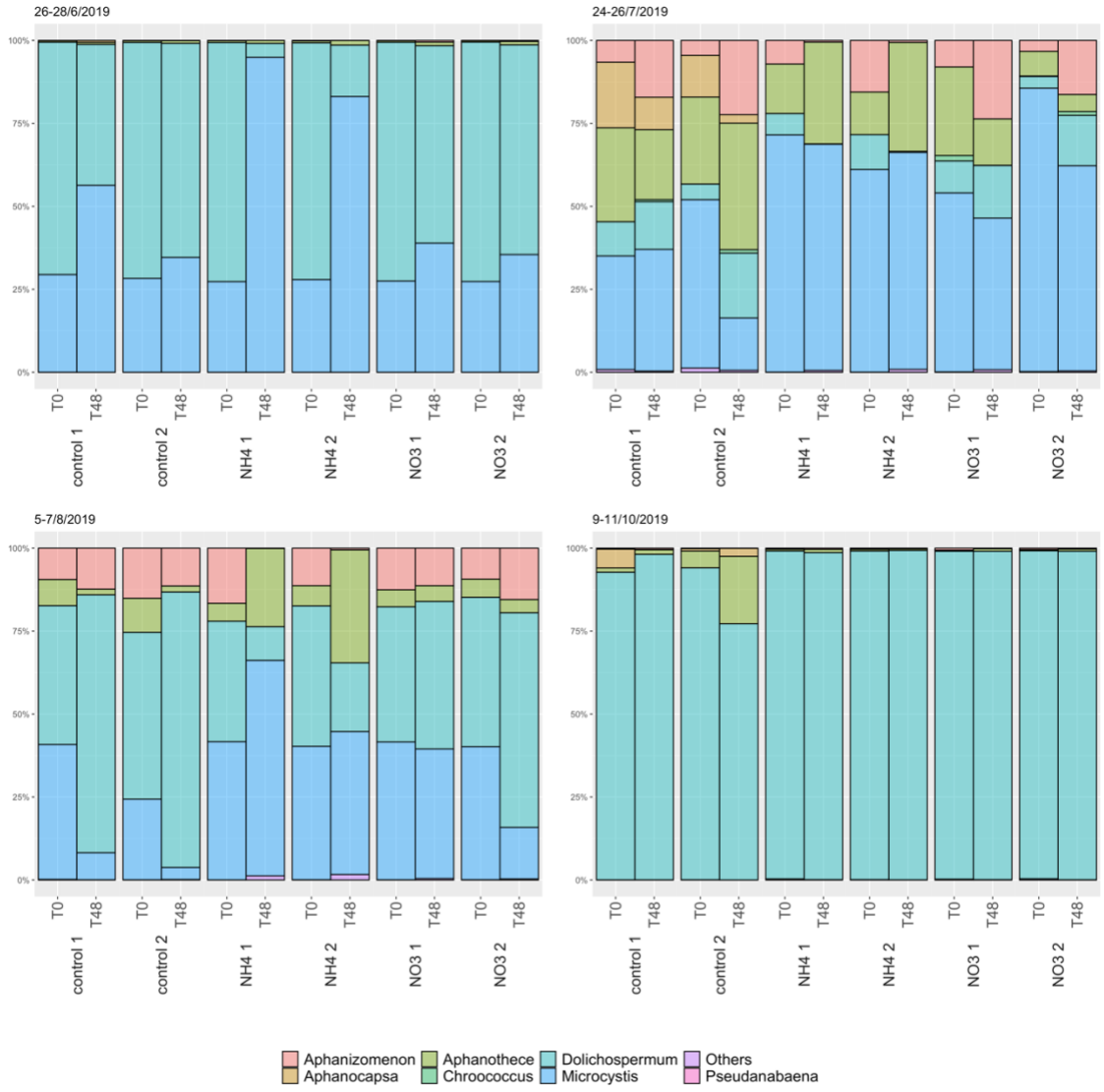

Figure C.6 Relative cell abundance in the mesocosms in Petit-Lac-St-François

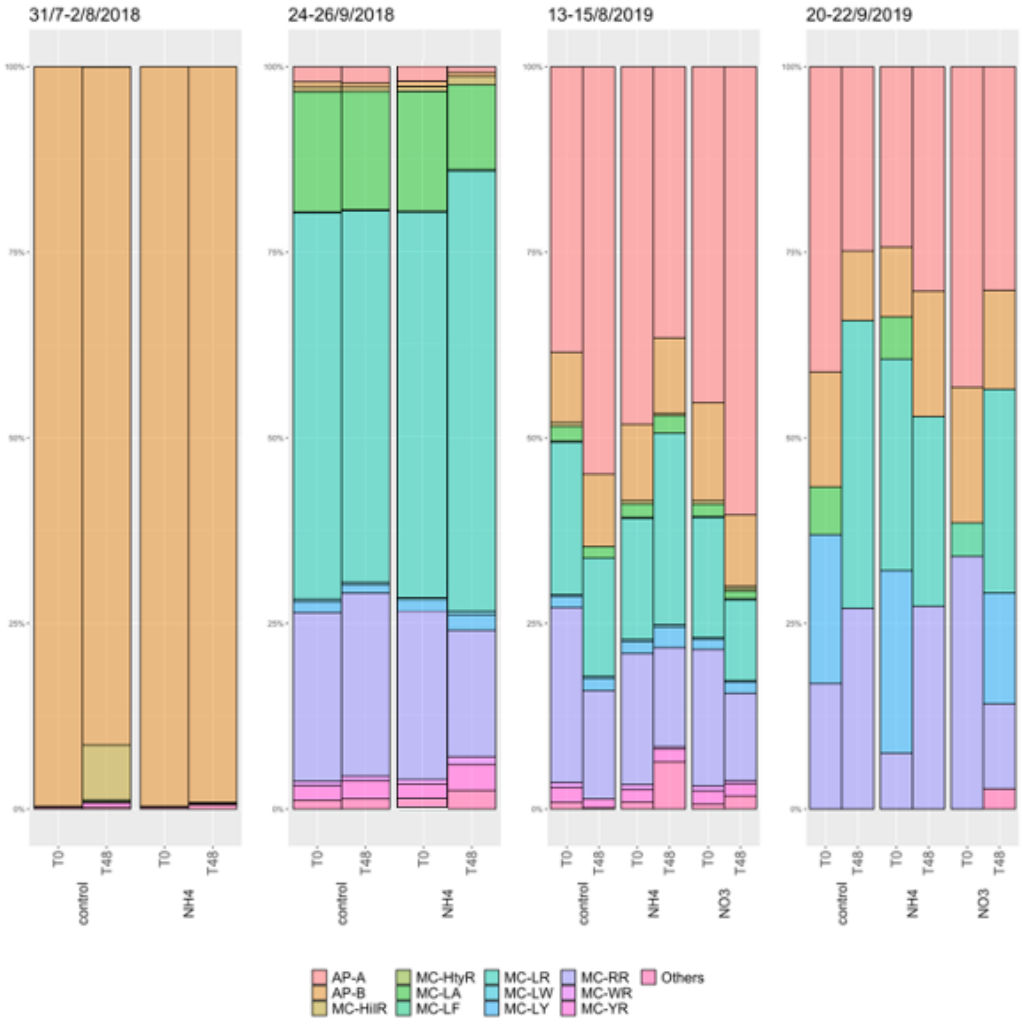

Figure C.7 Relative intracellular cyanotoxin abundance in the mesocosms in Missisquoi Bay

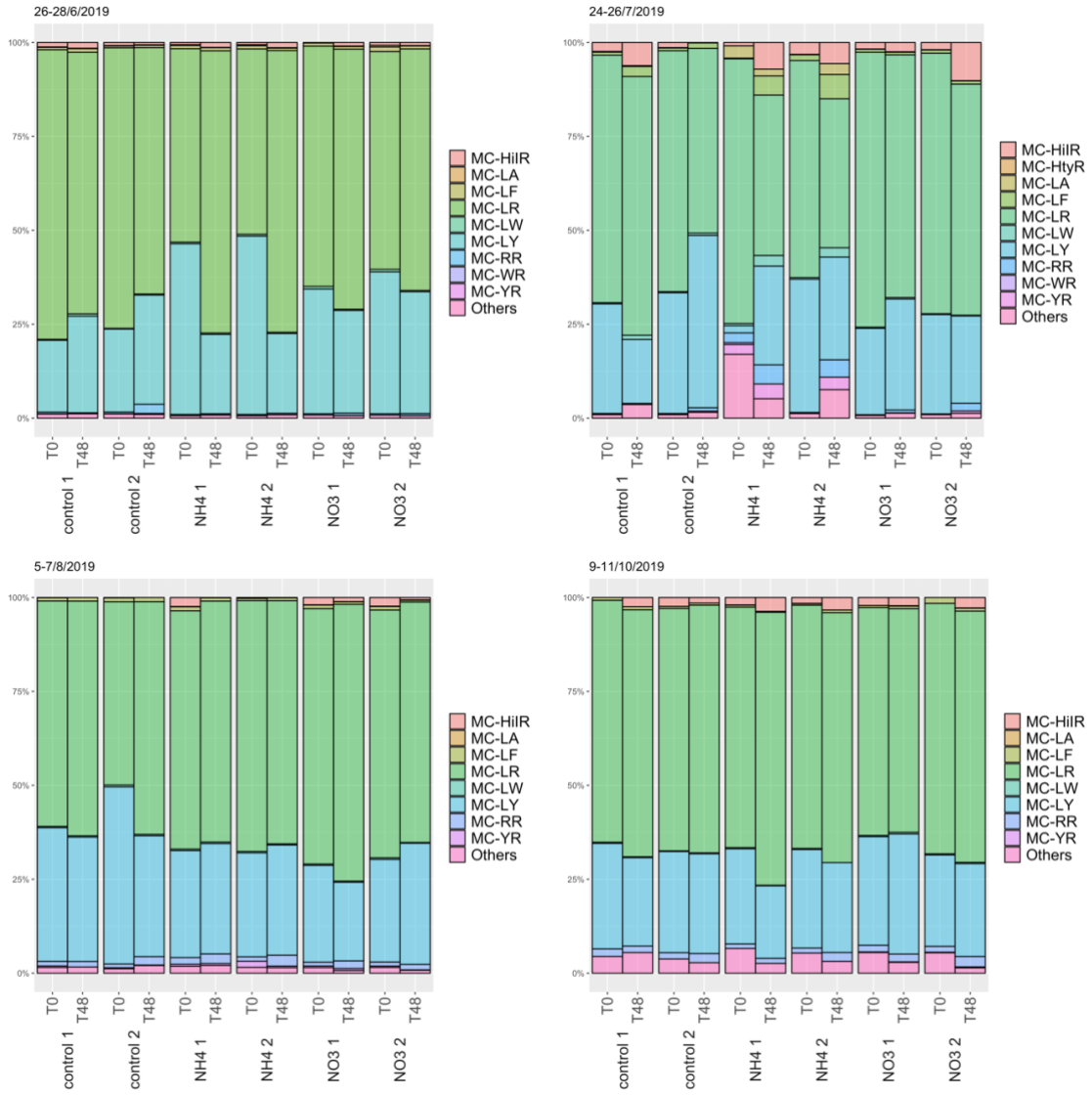

Figure C.8 Relative abundance of total intracellular cyanotoxins in the mesocosms in Petit-Lac-St-François

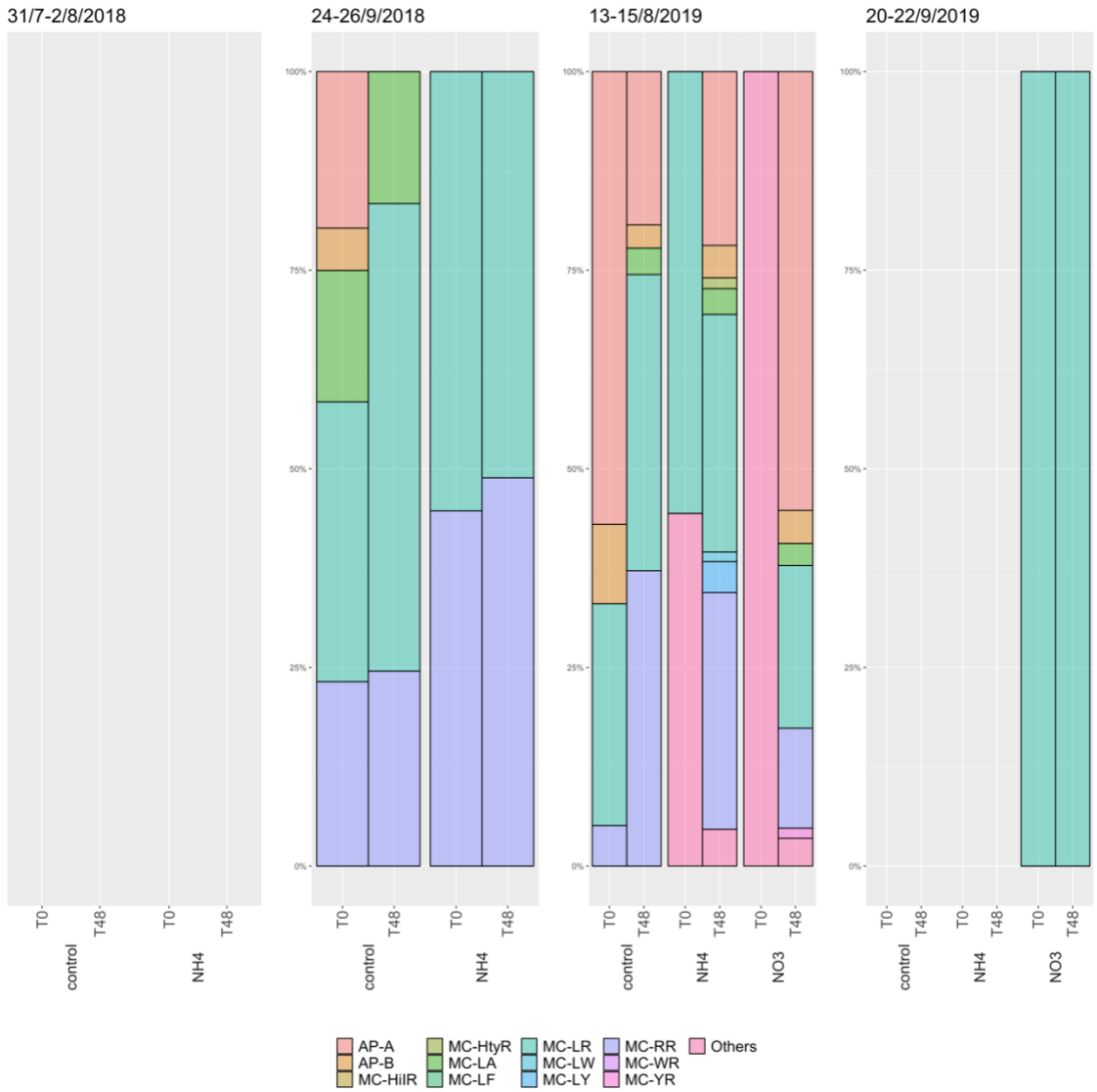

Figure C.9 Relative extracellular cyanotoxin abundance in the mesocosms in Missisquoi Bay

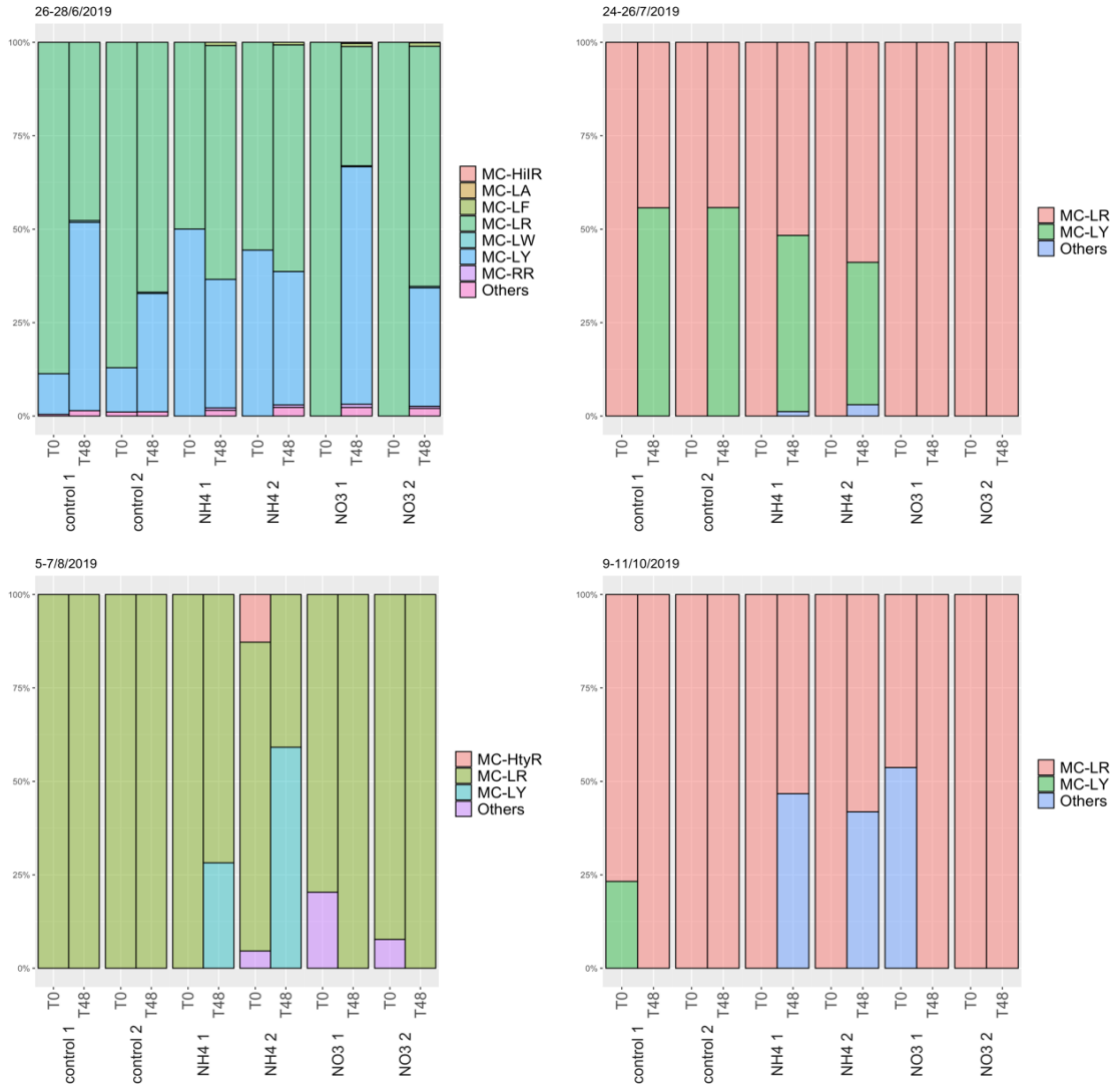

Figure C.10 Relative abundance of total extracellular cyanotoxins in the mesocosms in Petit-Lac St. Francois

END