UNIVERSITÉ DE MONTRÉAL

HISTORICAL TRENDS OF CYANOBACTERIA AND THEIR TOXINS IN FOUR EASTERN CANADIAN SOURCE WATERS

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DEDICATION

I lovingly dedicate this thesis to my parents and my fiancé, who supported me each step of the way.

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Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

RÉSUMÉ

La mesure in situ des concentrations de cyanotoxines est laborieuse. L'étude de la dynamique des cyanobactéries et cyanotoxines est un complément important dans le domaine de l'eau. L'objectif de cette étude était de déterminer les cyanobactéries dominantes et la relation entre la biomasse et la concentration de cyanotoxines dans quatre lacs du Québec sur la base des données historiques du Ministère du Développement Durable de l'Environnement et des Parc (MDDEP). La dynamique étudiée peut être utilisée comme une base pour établir une méthode efficace pour mesurer rapidement les cyanotoxines et d'assurer une meilleure élimination des cyanotoxines dans l'eau potable.

Des campagnes d'échantillonnage ont été effectuées par le MDDEP de 2000 à 2008 dans quatre lacs, Baie Missisquoi, lac Nairne, lac Brome et lac William. Cyanotoxines ont été surveillés et mesurés par des méthodes de laboratoire. Les résultats de ces observations ont permis d'effectuer une vaste surveillance des variations spatio-temporelles de l'abondance de cyanobactéries et des espèces de cyanobactéries et cyanotoxines dans les quatre lacs. L'analyse des données démontre que les concentrations de mcirocystine LR équivalent (MC-LR éq) détectées dans l'écume étaient beaucoup plus élevées que les seuils d'alerte établis par l'organisation mondiale de la santé (OMS).

Il'est difficile de déterminer quelles étaient les espèces dominantes les plus abondantes dans l'eau. Cependant, les espèces dominantes ont été facilement identifiées dans l'écume. La concentration d'anatoxine détectée a toujours été faible, même inférieure à la limite de détection (LOD) (Annexe 2). Bien que l'anatoxine soit potentiellement produite par les cyanobactéries, Aphanizomenon flos-aquae et Anabaena flos-aquae étaient les espèces les plus fréquemment présentes dans les échantillons. L'abondance de cyanobactéries potentiellement MC produites dans l'écume était toujours accompagnée de forte concentration de MC-LR éq. L'analyse des données montre que la relation entre la biomasse des cyanobactéries et les concentrations de MC-LR éq n'est pas claire, cependant, quand l'eau etait dominée par des espèces spécifiques, les relations étaient beaucoup plus apparentes.

ABSTRACT

The concentration of cyanotoxins is hard to be measured in situ. The study of the dynamics of cyanobacteria and cyanotoxins is a strong complement to the drinking water scientific knowledge. The goal of this study was to identify the dominant cyanobacteria and the relationship between the biomass and the concentration of cyanotoxins in four lakes in Quebec based on the historical data obtained from the Quebec Ministry of Durable Development of Environment and Parks (MDDEP). The dynamic studied can be used as a base to establish an effective method for rapid measurement of cyanotoxins and to ensure better removal of cyanotoxins in drinking water.

Sampling was conducted from 2000 to 2008 in four lakes, Missisquoi Bay, Lake Nairne, Lake Brome and Lake William. Cyanotoxins were monitored and measured by laboratory methods. The results of these monitoring showed large spatial-temporal variations of cyanobacterial abundance, cyanobacteria species, and cyanotoxins in these four lakes. The concentrations of Microcystin-LR equivalent (MC-LR eq) detected in the scums were much higher than the alert threshold established by World Health Organization (WHO).

It was difficult to determine the dominant cyanobacterial species as well as the most abundant species in these waters. However, in the scum, the dominant species were easily identified. The concentration of anatoxin detected was always low even lower than the Limit of Detection (LOD) (Appendix 2), although the potentially anatoxin producing cyanobacteria, *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* were the more frequent present species in samples. The abundance of potentially MC producing cyanobacteria in water always accompanied with high concentration of MC-LR eq. Data analysis demonstrates that the relationship between the biomass of cyanobacteria and the concentrations of MC-LR eq is not clear, however when water dominated by specific species, the relationships were much clearer.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BM	Bay Missisquoi
LN	Lake Nairne
LB	Lake Brome
LW	Lake William
CB	Cyanobacteria
MC	Microcystin
MC-LR eq	Microcystin-LR equivalent

LIST OF APPENDICES

INTRODUCTION

An increasing occurrence of cyanobacterial blooms, caused by eutrophication, has been observed in freshwater sources all over the world over the past decade. The primary consequence of this bloom occurrence has been the reduction of ecological quality of water and a sustained increase in public health risks (Codd, 2000). Many water systems, including drinking water treatment plants, suffer from extensive cyanobacterial blooms (Lahti et al., 2001). Most of the relevant literature indicates that cyanobacteria is responsible for producing of a variety of toxins harboring a variety of chemical and toxicological properties. These toxins can be responsible for widespread poisoning of domestic animal, fish, and recently humans (Carmichael et al., 2001).

The World Health Organization (WHO) established guidelines in 1999 that require drinking water treatment plants to monitor the concentration of cyanobacteria and cyanotoxins in treated water (I. Chorus & J. Bartram, 1999). In order to effectively control the health impacts due to cyanotoxins, several countries such as Australia, Canada and New Zealand have established recommendations of maximum concentrations of cyanotoxins contained in drinking water.

The identification and quantification of conventional cyanobacteria and cyanotoxins require laboratory analysis. Such analysis might include species identification; pigment extraction; high performance liquid chromatography (HPLC); and enzyme-linked immunosorbent assays (ELISA). These analyses are precise, they are however costly and time-consuming. Cyanobacterial densities can rapidly increase in favorable water conditions. Conventional cyanobacteria and cyanotoxin monitoring are invalid in real-time monitoring for assessing the alert level of potential risk of cyanotoxins present in water source (N. McQuaid, Zamyadi, Prévost, Bird, & Dorner, 2011).

Innovative online cyanobacterial monitoring systems have been recently proposed such as *in vivo* fluorescence probe which allows *in situ* estimation of cyanobacterial abundance quickly and accurately (Beutler et al., 2002). However, the concentration of cyanotoxin is difficult to measure *in situ*; the results of measurement are affected by environmental factors such as turbidity. So, the study of the dynamics of cyanobacteria and cyanotoxins is a strong complement to this important issue.

Most research about cyanobacteria and cyanotoxins has focused on one lake over the course of one or two years. However, the relationship between cyanobacteria and its correspondent cyanotoxins has high spatial-temporal variability. It lacks sufficient historical data to support the

difference in relationship between the cyanobacteria and cyanotoxins caused by geographical and temporal distributions.

This study aims to investigate the dynamic between cyanobacteria and its cyanotoxins produced by means of analyzing a large historical database of four lakes in Quebec from 2000 to 2008. Data was provided by the Ministère du Développement Durable de l'Environnement et des Parc (MDDEP). The dynamic study can be used as a base to establish an effective method for quickly measuring the cyanotoxins and to ensure better elimination of cyanotoxins in drinking water.

This research is part of a study funded by the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT).

The specific objectives of this study are:

1) To determine the species and toxins that dominate blooms and scum of cyanobacteria in lakes in Quebec in order to understand whether the proliferations were dominated by one or a few species.
2) To analyze the correlation between the abundance and/or the total biomass of cyanobacteria with cyanotoxins produced.
3) To determine if the presence/abundance of species is an indicator for detecting the presence of microcystin or anatoxin and to propose potential values for toxic species most often dominant.

CHAPTER 1 LITERATURE REVIEW

Cyanobacterial blooms have become more widespread, especially in some regions; the blooms have caused deterioration in some aquatic environments and serious problems for water use, particularly in drinking-water treatment (I. Chorus & J. Bartram, 1999). Temperature, light and nutrients probably play a very important role in proliferation of algae or cyanobacteria (Ingrid Chorus & Jamie Bartram, 1999). The occurrence of cyanobacteria and its toxins can provoke a potential risk to humans and animals through exposure to contaminated water.

1.1 Eutrophication and occurrence of cyanobacteria blooms

Eutrophication is a widespread pollution problem in many lakes, rivers, and reservoirs worldwide. Human activities and agricultural practices can lead to increased nitrogen and phosphorous accumulation in water bodies. This excessive accumulation of phosphorous, nitrogen, and other nutrient compounds accelerates eutrophication, which in turn provides favorable conditions for the proliferation of phytoplankton, especially in slow-flowing water sources.

Eutrophication in the presence of advantageous temperature and light conditions favors the growth of algae or cyanobacteria. When a significant proliferation of algae or cyanobacteria is dominated by one or few species, the phenomenon is identified as blooms. A very dense accumulation of cyanobacteria at the surface of a lake, river, or reservoir is identified as cyanobacterial scum (Blais, 2007). Cyanobacterial blooms occur without warning and last only a few days or weeks. Worth mentioning: toxic cyanobacterial blooms have been reported in over 45 countries (Blais, 2007), especially microcystin producing cyanobacteria-dominated blooms. According to some research, the seasonal variation of algal and cyanobacterial communities can be observed: diatoms with small flagellates dominate the water resources in winter and spring, followed by green algae in late spring and early summer, then in eutrophic waters, cyanobacteria dominate the summer phytoplankton (Ingrid Chorus & Jamie Bartram, 1999).

1.1.1 The situation in Quebec

The occurrence of cyanobacteria has become a great concern in the province of Quebec over the past decade, because the reported number of lakes dominated by cyanobacteria (over 20,000

cells/ml) has increased from 34 in 2004 to 108 in 2008 (Ministère du Développement Durable de l'Environnement et des Parcs (MDDEP), 2008). Over 356 cyanobacterial cases were documented (Ministère du Développement Durable de l'Environnement et des Parcs (MDDEP), 2007).

From 2001 to 2003, the Ministry of Sustainable Development, Environment and Parks (MDDEP) began to conduct regular monitoring of cyanobacteria and cyanotoxins in raw and treated water from three water supply stations: Bedford (Baie Missisquoi), Daveluyville, et Plessisville (Rivière Bécancour), all of which were affected by blooms of cyanobacteria (Institut National de Santé Publique du Québec (INSPQ), 2004a). In raw water, 42 species of cyanobacteria were identified; 13 of which were known to produce different cyanotoxins.

In the following years, reports of water bodies affected by cyanobacteria have been increasing. In March 2005, the MDDEP published the results of the monitoring for the presence of cyanobacteria and their toxins in six drinking water stations. The results showed that the abundance of cyanobacteria and the concentration of cyanotoxins exceeded the recommended maximum acceptable concentration (Robert, Tremblay, & DeBlois, 2005).

1.2 Properties of cyanobacteria

Cyanobacteria are primitive organisms and have existed on earth for over 2.5 billion years (Lau, Sapienza, & Doolittle, 1980). Cyanobacteria have strong competitive advantages over other phytoplankton by changing their environment (Lavoie, Laurion, Warren, & Vincent, 2007). Cyanobacteria have a remarkable combination of properties found both in algae and bacteria (Ingrid Chorus & Jamie Bartram, 1999). Their cellular structure is similar to bacteria but they can conduct photosynthesis like other types of algae. Cyanobacteria have an excellent ability to accumulate and store essential nutrients, such as phosphorous, and to grow in a low-nutrient condition. Dinitrogen fixation from the atmosphere is another function for some species of cyanobacteria, giving them the simplest nutritional requirements of all living organisms (Ingrid Chorus & Jamie Bartram, 1999). Many species of cyanobacteria possess gas vesicles which can help them adjust their position in water, and thus find a positive condition for growth. However, in extreme cold and lack of nutrition conditions, cyanobacteria cannot survive.

1.2.1 Cyanobacterial counts and cell volumes

Biovolume (mm^3/L) can be obtained from cell counts by determining the average cell volume for each species or unit counted and then multiplying this value by the cell number present in the sample. The result is the total volume of each species. Different cyanobacterial species have diverse biovolumes. Table 1.1 indicates the average measured biovolume of Cyanobacteria detected in samples collected in untreated water.

Table 1.1: Average measured biovolume of cyanobacteria (adapted from (N. McQuaid, et al., 2011))

Cyanobacterial genus	Cyanobacterial species	Average measured biovolume (μm^3)
Microcystis sp.	*M. flos-aquae*	14.1
	M. aeruginosa	65.5
	M. wesenbergii	87
Anabaena sp.	*A. flos-aquae*	179.6
	A. spiroides crassa	1022.6
Planktothrix sp.	N.A	157
Oscillatoria sp.	*O. tenuis*	98.2
Pseudanabaena sp.	*P. mucicola*	5.3
Aphanizomenon sp.	*A. flos-aquae,*	89.1
Aphanothece sp.	*A. minutissima,*	0.5
Chroococcus sp.	*Chroococcus dispersus,*	65.4
Merismopedia sp.	*M. tenuissima*	2.1
	M. punctata	8.2
Cuspidothrix sp.	*C. isaatschenkoi,*	75.4
Aphanocapsa sp.	*A. parasitica*,	1.8
Snowella sp.	*S. lacustris,*	12.7
Planktolyngbya sp.	N.A	3.1

1.2.2 Factors affecting cyanobacteria growth and bloom formation

The properties of cyanobacteria determine the numerous factors that can affect the development of bloom. The formation of the cyanobacterial bloom can be caused by several typical changes of environmental conditions, such as increased nutrient inputs and increased light intensity.

Chlorophyll a and phycobiliproteins contained in cyanobacteria are the pigments that absorb light and are responsible for photosynthesis. Cyanobacteria can harvest light energy more efficiently than other phytoplankton species by using these pigments. Some cyanobacteria are sensitive to the long period high-light exposure. However, intermittent exposure to high light intensity enhances the growth of cyanobacteria to a maximal rate (Loogman, 1982). Cyanobacteria require low energy to maintain its function and structure (Gons, 1977), which means that, even in the proliferation of phytoplankton, cyanobacteria have a competitive advantage to ensure growth and the formation of bloom.

As mentioned earlier, cyanobacterial blooms usually occur in eutrophic water resources, in which the concentrations of phosphorus and nitrogen are much higher. The ability of fixing nitrogen (N) and accumulating phosphorous (P) enable cyanobacteria to survive in the lowest nutrients concentration. Some cyanobacterial species can survive in low nitrogen water by a particular ability to fix N_2 from the atmosphere and store it for later use (Oliver & Ganf, 2000). However, cyanobacterial blooms can't last for long in cases of low-nutrient, cold, and rapidly flowing water (Lavoie, et al., 2007).

Another factor is climate change. For example the greenhouse effect, increases temperatures worldwide. Temperatures over 25 °C provide suitable conditions for the development of cyanobacterial bloom (Robarts & Zohary, 1987). This can explain why cyanobacteria dominance has been observed primarily in summer time. Long retention of water favors the cyanobacteria to form bloom due to a slow growth rate. High pH and low dissolved oxygen have caused the growth of cyanobacteria (Pick & Lean, 1987).

1.3 Cyanotoxins

1.3.1 The classification of toxins

Cyanotoxins can be classified by different properties. The cyanobacterial toxins composed by a number of chemical compounds, predominantly identified as alkaloids, peptides, and lipopolysaccharides (LPS) (I. R. Falconer, 2005). Depending on the target organs in humans, the main toxins identified are hepatotoxic cyclic peptides (microcystins and nodularins); neurotoxic alkaloids (anatoxins and saxitoxins); cytotoxic alkaloids; dermatotoxic alkaloids; and irritant toxins (lipopolysaccharides) (Ingrid Chorus & Jamie Bartram, 1999)(Table 1.2).

Over 46 cyanobacterial species have been recognized as toxins producers (Ernst, Dietz, Hoeger, & Dietrich, 2005). Among the many toxins, microcystins, anatoxin-a, cylindrospermopsin and saxitoxins have received widespread attention and research (Duy, Lam, Shaw, & Connell, 2000; Shaw, Seawright, Moore, & Lam, 2000). Microcystins are the most reported toxins worldwide.

Table 1.2: List of cyanotoxin and producer organisms (Svrcek & Smith, 2004).

Toxin group*(Type)*	**Producer cyanobacteria genera**	**Toxic**	**Drinking water quality and public health significance** *(irritant effect)*
Cyclic peptides			
Microcystins: Microcystin-LR Microcystin-RR Microcystin-YR Microcystin-LA Microcystin-LW	*Anabaena, Anabaenopsis,* *Aphanocapsa, Hapalosiphon,* *Microcystis, Microcystis aeruginosa,* *Nostoc, Oscillatoria*	Hepatotoxic	• Hepatoenteritis, • Acute toxicity unlikely in large water supplies, • Chronic liver damage with chronic exposure, • Tumor growth promotion, The relationship between the tumor growth promotion properties of these toxins and carcinogenicity needs to be determined
Nodularins	*Nodularia spumigena* (mainly brackish water)	Hepatotoxic	• As for Microcystins, • Nodularia is not found in reservoirs ; only blooms in estuarine lakes
Alkaloids			
Neurotoxic alkaloids Anatoxin-a, Anatoxin-a(S) Saxitoxins*	*Anabaena, Aphanizomenon, Oscillatoria* *Anabaena, Oscillatoria* *Anabaena, Anabaena circinalis, Aphanizomenon,* *Cylindrospermopsis, Lyngbya*	Neurotoxic Neurotoxic Neurotoxic	• Acute poisoning results in death by paralysis and respiratory failure • Acute toxicity only at very high cell densities • No known effects from chronic
Cytotoxic alkaloids Cylindrospermopsin	*Anabaena, Aphanizomenon,* *Cylindrospermopsis, Umezakia,* *Cylindrospermopsis raciborskii*	Cytotoxic, Hepatotoxic, Neurotoxic, Genotoxic	• Liver damage • Gastrointestinal tract damage
Dermatotoxic alkaloids Aplysiatoxin Debromoaplysiatoxin Lyngbyatoxin-a	Marine cyanobacteria *Lyngbya, Schizothrix, Oscillatoria* *Lyngbya, Schizothrix, Oscillatoria* *Lyngbya*	Dermatotoxic Dermatotoxic Dermatotoxic	• Oral and gastrointestinal inflammation
Lipopolysaccharides (LPS)	All (Most cyanobacteria)	Endotoxic	• Potentially irritates any exposed tissue (Skin, eye irritation; Skin rashes) • Respiratory allergy • Gastrointestinal disorders • Possible significant for water supply in relation to bathing

1.3.2 Effects on the formation of cyanotoxins and their release

Cyanotoxins, produced by some species of cyanobacteria, have demonstrated a significant risk to human health. The ability to produce toxins makes cyanobacteria the dominant organism in any water body. The production of cyanotoxins is a complex process of biosynthesis that is not discussed in this study. The toxins are formed as secondary metabolites of cyanobacteria (I. R. Falconer, 2005). The majority of studies indicate that cyanobacteria produce most toxins under conditions which are most favorable for their growth (Ingrid Chorus & Jamie Bartram, 1999).

Based on the study, caynotoxins are produced and contained within the actively growing cyanobacterial cells (Sivonen, 1990). Studies have also shown that less than 10 – 20 percent of toxins in cultures of cyanobacteria are typically extracellular (Negri, Jones, Blackburn, Oshima, & Onodera, 1997; Sivonen, 1990). The release of the toxins from the cells generally occurs during the senescence, death, and lysis of the cyanobacterial cells (Negri, et al., 1997; Rapala, Sivonen, Lyra, & Niemelä, 1997).

Laboratory studies have shown that particular environmental factors on cyanobacteria can induce changes in toxicity or toxin concentration (Ingrid Chorus & Jamie Bartram, 1999). Culture age and temperature are the two most important elements in the formation of toxins. Moreover, the effect of these two factors is common on the majority of toxin-producing. For example, an investigation showed that each year *Microcystis aeruginosa* was non-toxic at the beginning of the growing season, and it became highly toxic during the first bloom (Benndorf & Henning, 1989). Temperatures between 18°C and 25°C favor the toxic content in cyanobacterial cells; in contrast, too low or too high temperatures will limit the quantity of toxins. The effects of N and P on the toxin production by cyanobacteria are highly variable (Orr & Jones, 1998).

1.4 Toxicity of cyanotoxins

The symptoms of poisoning or injury caused by the presence of cyanotoxins in drinking water or other sources of water have been demonstrated by epidemiological evidence reported in several countries, including Brazil, Australia, North and South America, Africa, and Europe. Research results about the toxicity and the health effect associated with the cyanotoxins caused great concern. Although the toxicity tests of cyanotoxins are usually conducted on animals under controlled laboratory conditions, the information provided about the toxicity is useful (Codd,

2000). However, it cannot be directly extrapolated to human populations (Ingrid Chorus & Jamie Bartram, 1999). The lethal dosage of the main cyanotoxins is listed in Table 1.3. The toxicity can vary according to the type of toxins. In this research, we focus on the toxicity of two cyanotoxins related to this study: Microcystins and Anatoxins.

Table 1.3: Acute toxicity of various cyanotoxins (adapted from: (Hitzfeld, Höger, & Dietrich, 2000) (Svrcek & Smith, 2004)).

Name	**LD_{50}*** (i.p. mouse µg/kg body weight)
Hepatotoxins	
Microcystins:	
Microcystin-LR	50
Microcystin-LA	50
Microcystin-YR	70
Microcystin-RR	600
Nodularins	30 to 50
Cylindrospermopsin (hepatotoxic in pure form)	200 to 2100
Neurotoxins	
Anatoxin-a and homoanatoxin-a	200-250
Anatoxin-a(S)	20-40
Saxitoxins (PST)	10-30

* LD_{50} : lethal dose resulting in 50 per cent deaths

1.4.1 Microcystins

Microcystins are produced mainly by *Microcystis spp*., *Anabaena spp.,* and other species. Microcystins are classified as hepatoxins, which is the unique group of compounds that can cause acute liver damage (World Health Organization (WHO), 1998)). Microcystins, being cyclic peptides, are extremely stable and resistant to chemical hydrolysis or oxidation at near-neutral pH levels (Ingrid Chorus & Jamie Bartram, 1999). The exposure routes of this toxin vary, including oral ingestion from contaminated water and food, inhalation, or dermal contact (Dietrich, Fischer, Michle, & Hoeger, 2008; World Health Organization (WHO), 1998, 2003). The symptoms of human exposure to this toxin are gastroenteritis and allergic or irritation reactions, but the

primary target is the liver. The animal studies showed that 50-70 percent of microcystins rapidly accumulate in the liver. Death can occur in one to three hours. Significant evidence exists to show that over 70 human deaths were caused by the exposure to microcystins from dialysis water in 1996 in Caruaru, Brazil (Carmichael, 2001).

The commonly accepted i.p LD_{50} for microcystin-LR in mice is between 50 and 158 μg/kg. The total oral LD_{50} is 5,000 μg/kg for mice. The i.p. LD_{50} for microcystin-RR is about tenfold higher (Ingrid Chorus & Jamie Bartram, 1999).

1.4.2 Anatoxin-a

The potent neurotoxin, anatoxin-a, from *Anabaena flos-aquae* has frequently been involved in animal and wildfowl poisoning (Ressom et al., 1994). Most neurotoxins have shown acute effects in mammals, even with a very low dose of this toxin. The symptoms of exposure to anatoxin-a are drastic, including muscle fasciculations, gasping, convulsions, and opisthotonus (Brookes et al., 2008).

For mice, the i.p. LD_{10} (lowest dose causing death) of anatoxin-a is 250 μg/ kg bw (Stevens & Krieger, 1991) and the i.p. LD_{50} of anatoxin-a is 375 μg kg^{-1} bw (Fitzgeorge, Clark, & Keevil, 1994). The oral LD_{50} for anatoxin-a is greater than 5,000 μg kg^{-1} bw (Fitzgeorge, et al., 1994). No news of human health effects caused by anatoxin-a has been reported.

1.5 Standards and recommendations for cyanobacteria monitoring

In 199, to control the health problems provoked by cyanobacteria and to ensure the safety of drinking water, the WHO (World Health Organization) published a monitoring framework. The Alert Level threshold (ALT) is based on the measure of three criteria: cyanobacterial concentrations; cyanobacterial biovolumes; and chlorophyll A concentrations (Table 1.4).

WHO proposed the maximum concentrations of cyanotoxins in drinking water. A maximum of one μg/L of the hepatoxin microcystins is recommended by an expert group under the auspices of WHO (Ingrid Chorus & Jamie Bartram, 1999). The guideline value is calculated using the following equation:

Guideline value= TDI*bw*P/L

Where:

TDI: Total daily intake µg/kg (0.04 was used)

bw: An average adult body weight (60 kg used)

P: Proportion of total daily intake of the contaminant which is ingested from the drinking water needs (assumed to be 0.8)

L: Typical daily water intake in liters (2 liters used)

Table 1.4: Alert level monitoring framework for DWTPs (adapted from (Ingrid Chorus & Jamie Bartram, 1999)).

Alert level	Criteria	Actions for Drinking Water Treatment Plants
Vigilance	>2,000 cyanobacterial/ml, or >1µg/L Chla, or >0.2mm^3/L	No Bloom
1	<2,000 cyanobacterial/ml, or Between 1µg/L-50µg/L Chla, or Between 1µg/L-50µg/L Chla, or	Weekly counts cells Weekly monitoring of cyanotoxin Public warning
2	>100,000 cyanobacteria/ml, or >50 µg/L Chla, or <10 mm^3/L	Weekly counts cells Weekly monitoring of cyanotoxin Increase information to public warning Alternative water source to be considered

Although many regions globally have adopted the recommendations established by the World Health Organization, some of them have developed complementary recommendations of their own. For example, Quebec's MDDEP proposed an intermediate guideline of 20,000 cyanobacteria cells/ml; the maximum recommended concentration in Quebec is 1.5 µg/L for Microcystins-LR; and the provisional value for anatoxin-a is 3.7 µg/L (Institut National de Santé Publique du Québec (INSPQ), 2004a). Worldwide guidelines and standards for cyanotoxins in treated drinking water are shown in Table 1.5.

Table 1.5: Worldwide guidelines and standards for cyanotoxins in treated drinking water (adapted from (Case, 2006)).

Country/Region/continent	Criteria	Actions for Drinking Water Treatment Plants
World Health Organization	Microcystin 1.0µg/L	Published in "WHO Guidelines for Drinking Water".1996
Canada	1.5µg/L toxins as microcystin LR MAC	Maximum acceptable concentration (MAC) is derived from the tolerable daily intake (TDI). Which is in tern derived from the No-observed adverse effect level (NOAEL)
Quebec	1.5µg/L anatoxin	
Australia	1.3µg/L toxins as microcystin LR	
Africa	None found	
Asia	None found	
European Union and United Kingdom	Assumed to be the same as WHO recommendations. No specific values found	Guidelines indicated that "water should not contain algae" and that were measured in terms of MACs.
New Zealand	≤ 1 potentially toxic cyanobacteria in 10ml sample MAC for toxins Anatoxin (as STX-eq) 3.0µg/L; Anatoxin-a(S): 1.0µg/L; Cylindrospermopsin: 1.0µg/L; Microcystin: 1.0µg/L; Saxitoxins: 1.0µg/L; Nodularin: 1.0µg/L; LPS endotoxin:3.0µg/L	MACs are based on WHO guidelines. Standards provide compliance criteria and compliance is monitored.
Brazil	Microcystin 1.0µg/L Saxitoxin: 3.0µg/L Cylindrospermopsin: 15µg/L	Guidelines for microcystin are mandatory, and guidelines for eq-saxitoxin and ea-cylindrospermopsin are recommended.
United States of America	None currently known	Cyanotoxins are on the Contaminant Candidate List (CCL) and the Environmental Protection Agency is pushing for their inclusion in official legislation.

1.6 Advantages and disadvantages of monitoring methods

Conventional laboratory methods, such as taxonomic analysis (cell counts and biomass measurements), phytoplanktonic pigment extractions, and cyanotoxin analysis (Zamyadi, McQuaid, Prévost, & Dorner, 2012) are accurate but costly, time consuming, unable to respond rapidly to sudden changes in cyanobacterial biovolume. Therefore, an online probe using *in vivo*

fluorescence has been recommended to monitor potentially toxic cyanobacteria. This is a helpful complement to conventional methods (Richardson et al., 2010). The probe can quickly reflect the cyanobacterial biovolume by measuring the light emissions of phycocyanim (PC), which are the fluorescent pigments present in cyanobacteria (Beutler, et al., 2002). Although the online probe is effective for quick water quality assessment, its precision has to be proven. This is due to interference from water environmental factors such as turbidity and chlorophyll-a of Chlorophyta present in water bodies (Zamyadi, et al., 2012).

The study of the dynamic between cyanobacteria and its toxins is a combination of conventional methods and online probes. It can be used in real-time monitoring based on huge laboratory data analyses. It is a useful complement both to conventional methods and online probe monitoring.

CHAPTER 2 MATERIALS AND METHODS

2.1 Description of database

In 2008, École Polytechnique de Montréal (ÉPM) and Université de Montréal (U de M) jointly carried out research on the subject of cyanobacteria under the partnership program coordinated by the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT). The research includes the validation of the fluorometric probes *in vivo*, the development of a rapid measurement of cyanotoxins, and the dynamic of microbiological contamination of drinking water sources.

École Polytechnique obtained access to cyanobacteria data authorized by MDDEP. Two groups of data were analyzed in this study.

1. "Plan de gestion" (since 2004) with abundant classes of dominant genera of cyanobacteria and cyanotoxins detected.
2. "Étude DSÉE" with monitoring in lakes including sampling sites, enumeration, and biomass of cyanobacteria species and cyanotoxins detected.

The "Étude DSÉE" is the primary data used in this study, which is more detailed and complete. Four lakes -- Missisquoi Bay, Lake Nairne, Lake Brome, and Lake William -- were selected to be the basis of this study. . Table 2.1 lists a summary of four lakes monitored.

Table 2.1: Summary of 4 lakes included in the data of Étude DSÉE.

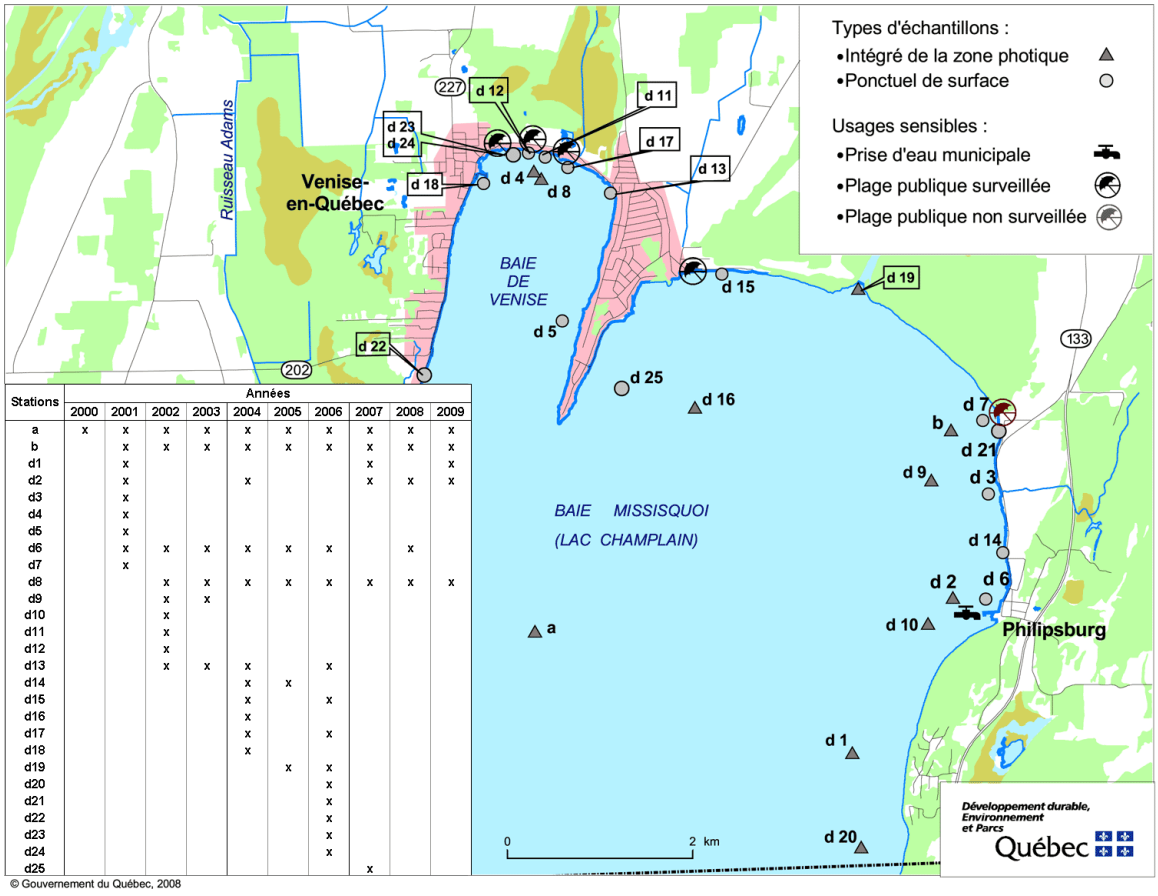
lake	Years monitored	Numbers of stations of sampling*	Toxic cyanobacteria detected (Y/N)	Scums (Y/N)	MC producer detected (Y/N)	Anatoxin producer detected (Y/N)
Missisquoi Bay	2000-2008	27	Y	Y	Y	Y
Nairne	2002-2008	8	Y	Y	Y	Y
Brome	2001 2003	3	Y	N	Y	Y
William	2000-2003	23	Y	Y	Y	Y

***The total number of sampling stations listed includes all the points being monitored, but not all stations were monitored in each year.**

2.2 Lake description and sampling locations

2.2.1 Missisquoi Bay

The surface area of Missisquoi Bay is 77.5 km^2, and its average depth is 2.8 m. Because it is eutrophic, the Missisquoi Bay in Quebec became one of the most important lakes for research on cyanobacteria in recent years. Almost every year, the scum of cyanobacteria has appeared on this lake in the summertime. The inputs injected into the Bay are groundwater, a tributary of Lake Champlain, and runoff from agricultural lands (Galvez & Levine, 2003). The Bay supplies the drinking water for over 4,100 residents (Statistics Canada, 2006) and also serves as a recreational site.



Stations	Années									
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
a	x	x	x	x	x	x	x	x	x	x
b		x	x	x	x	x	x	x	x	x
d1		x						x		x
d2		x			x			x	x	x
d3		x								
d4		x								
d5		x								
d6		x	x	x	x	x	x		x	
d7		x								
d8			x	x	x	x	x	x	x	x
d9			x	x						
d10			x							
d11			x							
d12			x							
d13			x	x	x		x			
d14					x	x				
d15					x		x			
d16					x					
d17					x		x			
d18					x					
d19						x	x			
d20							x			
d21							x			
d22							x			
d23							x			
d24							x			
d25								x		

Figure 2.1: Map of the distributions of monitoring stations in Missisquoi Bay (adapted from MDDEP)

A total of 27 sampling stations were distributed in the center and along the lake's shore. The sites of municipal water intake, a public monitored beach and a public non-monitored beach and their

nearby water areas have received intensive monitoring. Not all of the stations were monitored each year (form in the Figure 2.1), except station d6, which was located in the water intake.

2.2.2 Nairne Lake

Nairne Lake is located in the hinterland of Charlevoix, about 20 km west of La Malbaie and equidistant from the rivers and the Gulf Malbaie, which covers an area of 240 hectares. It is the heart of a drainage basin. By itself, it drains a watershed of 25 km^2 of which the discharge is a tributary of the Malbaie River (http://lacnairne.org/lac.html). Lake Nairne serves as the only water-sports site for the north shore of St-Laurent and east of Quebec City.

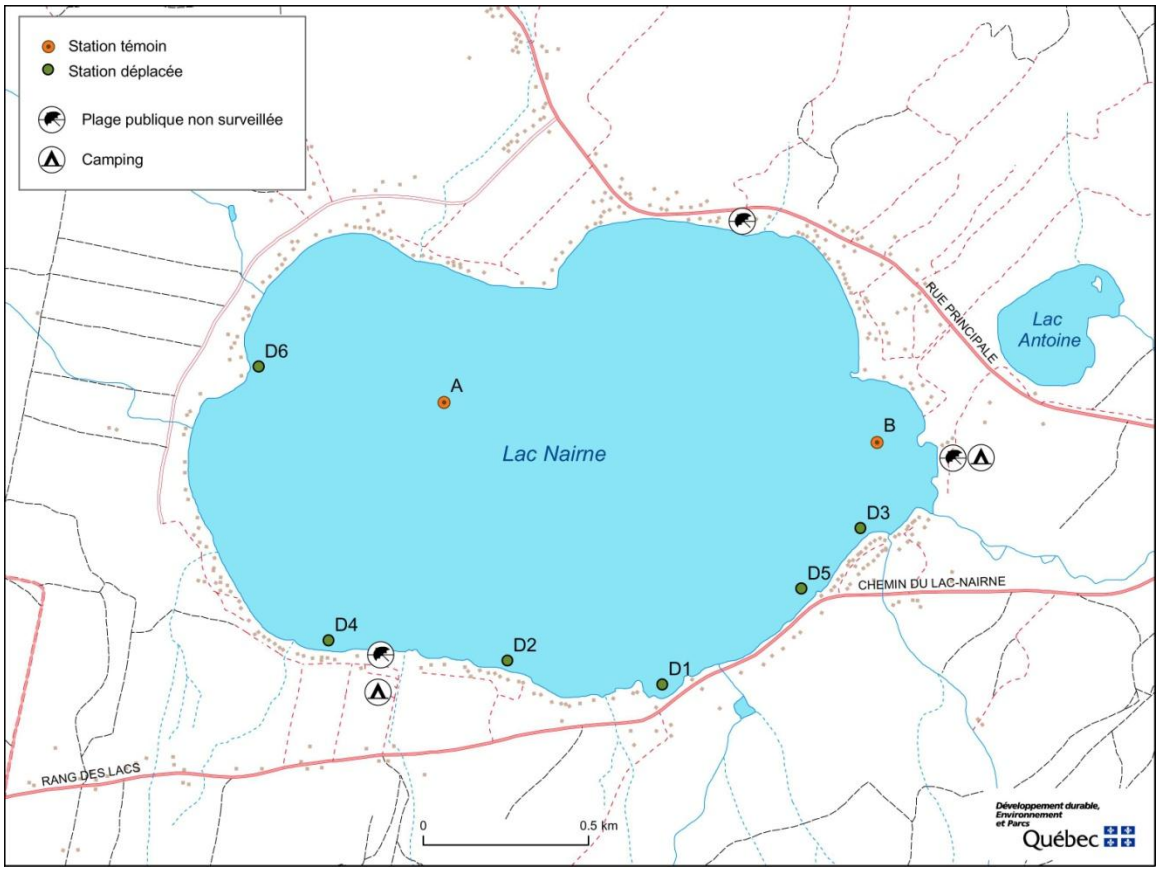


Figure 2.2: Map of the distributions of monitoring stations in Lake Nairne (adapted from MDDEP).

The sampling sites are located along the coastal lake, concentrated in the southeast of Nairne Lake. Stations A and B are the two fixed sampling stations; the other sites are variable year by year. The areas of non-monitoring public beach are also considered in the range of monitoring.

2.2.3 Lake Brome

Brome Lake, (French: *Lac Brome*), is located in the Brome-Missisquoi Regional County Municipality of the Montérégie administrative region of Quebec, Canada (Wikipedia http://en.wikipedia.org/wiki/Brome_Lake,_Quebec). The population in this area is over 5,200 (Statistics Canada, 2006). Like the other recreational sites, Lake Brome is used as a beach for swimming and fishing.

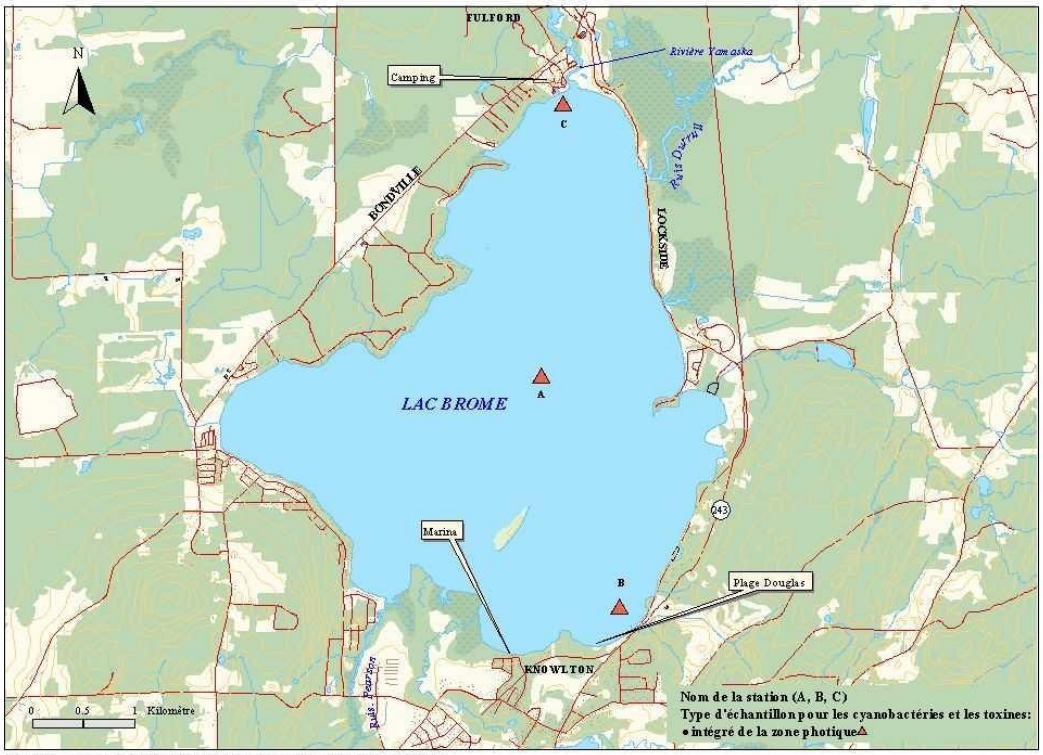


Figure : Localisation des stations au lac Brome en 2001

Figure 2.3: Map of the distributions of monitoring stations in Lake Brome (adapted from MDDEP).

Compared with the other three lakes, Brome Lake had the fewest points of sampling -- only three stations -- and the monitoring period was the shortest (from 2000 to 2003). During the monitoring

time, the cyanobacteria scum never appeared, probably due to the locations of sampling being too few to cover the whole lake.

2.2.4 Lake William

Williams Lake has a length of 586 m, with a nearby population of 12,500 in the center of the Cariboo region. The inputs of the lake are affected by the timber industry, cattle-rearing, and mining of copper and molybdenum. It is also a recreational location for fishing, swimming, and camping.

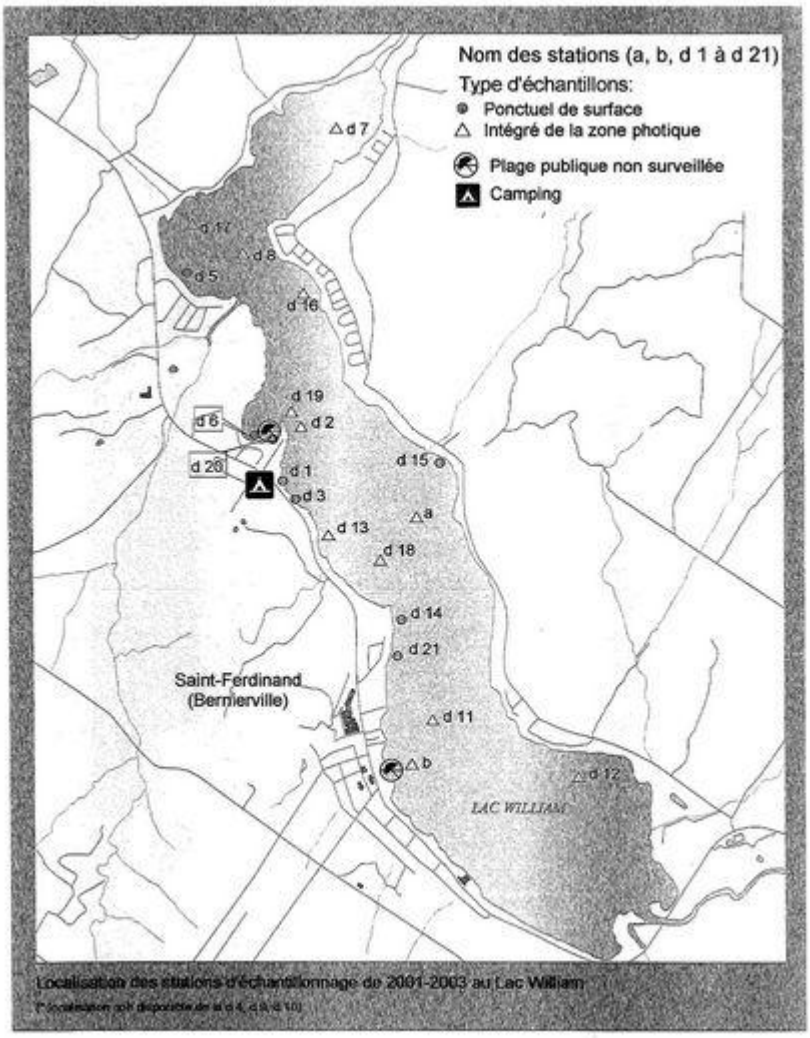


Figure 2.4: Map of the distributions of monitoring stations in Lake William (adapted from MDDEP).

The intensive monitoring stations were scattered in the middle of the lake. There are several points that provided complete data from 2000 to 2003, but for some of the stations, samplings were conducted in just one or two years.

2.3 Methods of analysis

2.3.1 Taxonomic enumeration

Taxonomic counts with species identification were performed using inverse microscopy (Lund, Kipling, & Le Cren, 1958; Wetzel & Likens, 2000) by the Centre d'Expertise en Analyse Environnementale du Québec (CEAEQ) at MDDEP. pH, turbidity, temperature, initial and residual chlorine dosage values were collected from the records of the DWTP for the time period of concern.

2.3.2 Toxins tests and calculation

The total cyanotoxin (µg/L) provided by MDDEP combines the extracellular cyanotoxin and intercellular cyanotoxin. Concentrations of microcystins are then reported in microcystin-LR concentration by multiplying their concentration by their toxicity equivalent factor. If the analytical result in microcystin (MC) is smaller than the limit of detection (LOD) of the method, whichever concentration was measured is given by default half the LOD. The overall concentration is equal to the sum of the concentrations of microcystins toxic equivalent for each microcystin identified. Preliminary calculations were adapted from (Institut National de Santé Publique du Québec (INSPQ), 2004b). Saxitoxin, neosaxitoxin, and cylindrospermopsin were analyzed only in Missisquoi Bay in 2008, and all results were under the detection limit.

Table 2.2: Example of equivalent toxicity calculation using the concentrations of toxins in a sample.

Toxins	Concentration (µg/L)	TEF*	MC Equivalent toxicity (µg/L)
Microcystin-LR	**60**	**1.0**	**60**
Microcystin-LA		**1.0**	
Microcystin-YR	**65**	**1.0**	**65**
Microcystin-YM		**1.0**	
Microcystin-RR	**235**	**0.1**	**23.5**
Sum	**360**		**136.75**

***TEF= toxicity equivalent factor.**

2.4 Methods of field sampling

Regular sampling campaigns were conducted early in the season, before the appearance of bloom as a portrait "precursor" of the lake. The samples collected were at 0-1 m deep from the surface of the water but they were previously collected from the photic zone. If there was one station, it was positioned where the water column was the deepest. The additional station was placed where there was a suitable location for a possible development of bloom, according to the prevailing winds and historical knowledge of the water. The sampling procedure had undergone several changes over the years for bloom:

* In 2002, samples collected in bloom contained the entire thickness of the photic zone (transparency X 2.7). The integrated photic zone came from a sample using a Kemmerer bottle at several intervals to cover the entire photic zone except a certain thickness above the sediment (about 0.3 to 0.5 m).

* In 2003, tubes were developed to sample the photic zone in a single sample. These tubes had a maximum length of 6 m. When the photic zone exceeded that depth, only the first six meters were sampled (Note: for the Studies DSEE, the tubes were all six meters). However, the management plan required for a tube to be provided to each of the regional (county) MDDEP. The tube provide

could be four, five, or six meters depending on the needs expressed by the county in terms of its waters.

* From 2007, the integrated sample of the photic zone was replaced by an integrated sample 0-1 m with a tube.

For scum, the sample was a surface sample. Only cyanobacteria and cyanotoxins were analyzed for these samples. For rural surface water level, a surface sample was also taken. These samples were collected when the bloom was only along shore and the column of water was not deep enough to collect at 0-1 m.

The sample collected was different depending on the type of sampling and different sampling methods (when the sample was embedded in the photic zone, the volume sampled depended directly on the thickness of the photic zone). The sample was mixed and separated into different bottles to analyze each parameter. Surface samples are collected with one-liter wide-mouth glass jars. Samples were stored in a cooler during transport to the lab and then at 4 ºC in the refrigerator until analysis.

2.5Statistical analysis

All historical data was collected by MDDEP and analysis was processed on Statistica 8 (Statsoft, Tulsa, Oklaholma, USA).

CHAPTER 3 RESULTS AND DISCUSSION

3.1 Determination of the dominant species

The number of algal species appearing in water sources is abundant, including toxic cyanobacterial species, non-toxic cyanobacterial species, and other blue-green algae. Cyanobacteria cannot produce cyanotoxin. “No cyanotoxin is identified” is classified as non-toxic cyanobacteria. The goal of section 3.1 is to identify which species dominated the water sources across different years and lakes. Historical data of the four lakes over the past nine years allows us to clearly identify the most important species of toxic dominant cyanobacteria, and it is beneficial for further research on the relationship between cyanobacteria and the toxins produced.

3.1.1 Dominance of toxic cyanobacteria, non-toxic cyanobacteria, and other blue-green algae in Missisquoi Bay

In these four lakes, the Missisquoi Bay data included the entire nine years site measurements. The number of samples and sampling stations shown in Table 3.1 varied throughout the measuring period. The table illustrates that non-toxic cyanobacteria were never the species that dominated the lake. In almost all samples, the percentage of biomass of non-toxic cyanobacteria as a proportion of total phytoplankton is less than 50 percent, often frequently even less than 5 percent.

In contrast, among a majority of samples, the proportion of cyanobacterial biomass was over 50 percent, especially in 2001, 2002, 2004 and 2006. Obviously, in 2007 and 2008, algae other than toxic cyanobacteria dominated Missisquoi Bay. This result is similar to those detected by a probe in (Natasha McQuaid, 2009).

This study contained further research on spatial-temporal distribution of toxic cyanobacteria; non-toxic cyanobacteria; and other algae. A large volume of toxic cyanobacteria normally appeared in mid-July and decreased at the beginning of September in Missisquoi Bay. However, in the first three years (2000-2002), station A was almost dominated by other algae, even in mid-July. Toxic cyanobacteria represented a very small proportion of the phytoplankton, and biomass was close to zero. Compared to station A, toxic cyanobacteria dominated in mid-August in station B.

Table 3.1: The number of sampling stations and samples of Missisquoi Bay from 2000 to 2008.

	2000	2001	2002	2003	2004	2005	2006	2007	2008
No. of sampling stations	1	9	9	6	11	6	13	6	5
No. of samples	5	31	28	24	27	21	29	19	13
No. of samples that %biomass of toxic CB >50%	0	20	13	8	16	8	22	0	3
No. of samples that %biomass of non-toxic CB >50%	0	0	7	5	1	0	0	1	0
No. of samples that %biomass of other algae>50%	5	11	1	11	9	12	7	18	9

According to the sampling map in Missisquoi Bay (Figure 2.1), most sampling stations were located along the lakeshore, except station A which is in the center of the lake. The dominant situation also varied from station to station. Biomass of toxic cyanobacteria measured in station A was always lower than that of other stations. The factors for spatial difference in phytoplankton distribution could be explained by the waves and wind, which can homogenize the water column and accumulate high concentrations of toxic cyanobacteria along the shore.

3.1.2 Dominance of toxic cyanobacteria, non-toxic cyanobacteria, and other blue-green algae in Lake Nairne

The number of sampling stations in Lake Nairne is apparently fewer than that of Missisquoi Bay. Analysis in Table 3.2 provides a clear picture of differences of cyanobacterial dominance among different water sources. Toxic cyanobacteria were the most important species that dominated Lake Nairne in 2002 and 2006, the same as Missisquoi Bay. However, the frequency of cyanobacterial dominance in 2004 dropped to less than 50 percent. Toxic cyanobacteria detected dominated almost all samples of measured in 2007, in contrast to Missisquoi Bay where it was dominated by algae other than the toxic cyanobacteria that year. The difference between these

two lakes may be caused by different water conditions, such as pH, quantity of nutrients, temperature, etc.

Table 3.2: The number of sampling stations and samples of Lake Nairne from 2002 to 2008.

	2002	2003	2004	2005	2006	2007	2008
No. of sampling station	**4**	**3**	**7**	**3**	**4**	**3**	**3**
No. of samples	**6**	**14**	**15**	**13**	**10**	**13**	**5**
No. of samples that %biomass of toxic CB >50%	**4**	**9**	**6**	**1**	**8**	**13**	**1**
No. of samples that %biomass of non-toxic CB >50%	**0**	**2**	**0**	**0**	**0**	**0**	**1**
No. of samples that %biomass of other algae >50%	**2**	**3**	**8**	**12**	**2**	**0**	**1**

When compared to Missisquoi Bay, dominance distribution of cyanobacteria in Lake Nairne has both similarities and differences. First of all, the proportion of non-toxic cyanobacteria was always lower than toxic cyanobacteria and other algae in any time and any year, but with one exception. On June 16, 2003, the non-toxic cyanobacteria represented 30 percent of total phytoplankton, and on September 15, 2003, it increased to 60-80 percent of phytoplankton and then became the dominant species in Lake Nairne. The same situation was found in Missisquoi Bay on August 27, 2002. This illustrates that in certain circumstances, reproduction of non-toxic cyanobacteria could overgrow the other two species. Due to lack of additional information about water quality, the effects of environmental condition to the proliferation cannot be determined.

In the beginning of each year's seasons, other algae dominated the lake, and the proportions of other algae were even over 95 percent. This phenomenon was also found in Missisquoi Bay. The discrepancy between the two lakes is the time when the toxic cyanobacteria reproduce greatly. In Missisquoi Bay, the abundance of toxic cyanobacteria normally appeared between July and August and began to reduce in September. However, the period of toxic cyanobacterial dominance appeared in late August and September even lasted to October in Lake Nairne.

Same as Missisquoi, station A located is at the center of the lake and station B is near the shore. The trend of cyanobacterial dominance was similar when comparing the two stations, but the cyanobacterial biomass accumulated in station B was always more than that measured in station A. This could prove again that the waves and wind have the ability to homogenize the water column and accumulate high concentration of toxic cyanobacteria along the shore.

3.1.3 Dominance of toxic cyanobacteria, non-toxic cyanobacteria, and other blue-green algae in Lake Brome

In Lake Brome, data was collected only in 2001, 2002, and 2003. The measurements were concentrated in three stations. The scum never occurred in Lake Brome as shown in Table 3.3. Other algae were the dominant phytoplankton in this lake.

Table 3.3: The number of sampling stations and samples of Lake Brome from 2001 to 2003.

	2001	2002	2003
No. of sampling stations	**3**	**3**	**3**
No. of samples	**14**	**12**	**15**
No. of samples that %biomass of toxic CB >50%	**6**	**6**	**2**
No. of samples that %biomass of non-toxic CB >50%	**0**	**0**	**0**
No. of samples that %biomass of other algae >50%	**8**	**6**	**13**

In 2001, the cyanobacteria were detected in early August, but no longer detected at all on August 28th in all three stations. Then, the sample taken on September 24 showed that the toxic cyanobacteria appeared again at a proportion of biomass over 50 percent.

The comparison among three stations shows that the distribution of cyanobacterial proportion in station A is very similar to station B. However, the distribution in station C was totally different, except in 2002.

3.1.4 Dominance of toxic cyanobacteria, non-toxic cyanobacteria, and the other blue-green algae in Lake William

Sampling stations are located along the entire shore of Lake William. Although there are sufficient sampling stations, the measurements were mainly taken in stations A and B. Other stations were measured only one day when a large bloom of green algae was observed. Toxic cyanobacteria dominated more frequently than other blue-green algae, and the percentage of non-toxic cyanobacterial biomass of total phytoplankton was still the lowest.

Table 3.4: The number of sampling stations and samples of Lake William from 2000 to 2003.

	2000	2001	2002	2003
No. of sampling stations	**1**	**6**	**7**	**11**
No. of samples	**6**	**23**	**22**	**23**
No. of samples that %biomass of toxic CB >50%	**4**	**15**	**13**	**18**
No. of samples that %biomass of non-toxic CB >50%	**0**	**0**	**0**	**0**
No. of samples that %biomass of other algae >50%	**2**	**8**	**9**	**5**

Due to the shape of Lake William, although station A is in the center of the lake, it is very close to the shore. This is unlike the other three lakes. Consequently, the biomass measured in stations A and station B was different to those of Missisquoi and Lake Nairne. In this lake, the biomass measured in station A is slightly higher than that measured in station B. As Table 3.4 shows, toxic cyanobacteria were still the dominant species in that lake.

3.2 Determination of dominance of specific species of cyanobacteria in water

This section is a further study on spatial-temporal variation of specific cyanobacterial species in each lake from 2000 to 2008. Cyanobacterial blooms are monitored by using biomass (mg/m^3) measurements coupled with the examination of the species present. Usually, the water resources were dominated by one or multiple cyanobacterial species when they are exhibiting bloom or scum. The biological diversity of cyanobacteria determines its occurrence in different water bodies and conditions. It is difficult to demonstrate definitely which species will reproduce in what kind of water quality. However, based on the study of the vast historical monitoring data, the four lakes seem to be dominated by certain species in the past years, although there were differences among stations monitored throughout the years.

Due to the significant variation of cyanobacterial cell volumes in size, taxonomic results derived from monitoring sample are reported by cyanobacterial biomass (mg/m^3) rather than cyanobacterial density (cell/ml). The results using biomass will be clearer and more accurate.

3.2.1 Cyanobacterial analysis at the Missisquoi Bay (2000-2008)

In 2000, only station A was monitored due the lack of bloom development in the Bay. In the beginning of the season (May 24), *Coelosphaerium kuetzingianum* dominated the center of the lake, the biomass achieved over 50 percent, but the amount was reduced gradually in the following three months. That is, until September 17 when it was replaced by *Anabaena flos-aquae* which potentially produce microcystins and anatoxin-a.

Microcystis sp., Anabaena flos-aquae, Aphanizomenon flos-aquae, Microcystis viridis and *Coelosphaerium kuetzingianum* are five main species in 2001 in the Bay. *Coelosphaerium kuetzingianum* can be ignored because of its small biomass. Although we cannot find a clear distribution sequence of these species, they dominated the Bay in 2001. Total biomass measurement determined that *Microcystis sp., Anabaena flos-aquae,* and *Aphanizomenon flos-aquae* were the most abundant cyanobacterial genera during the season.

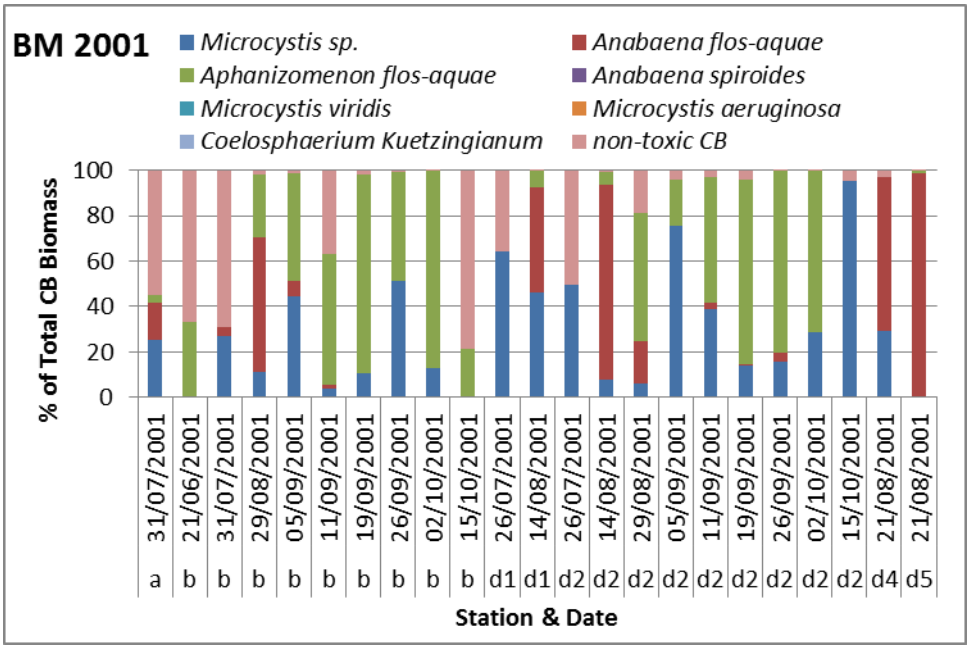


Figure 3.1: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Missisquoi Bay in 2001.

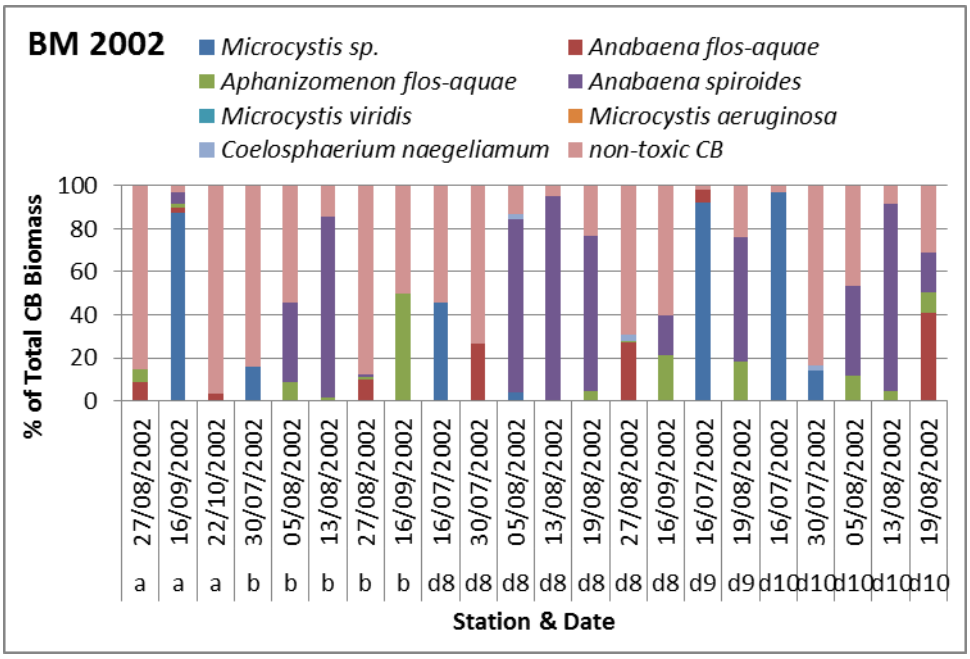


Figure 3.2: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Missisquoi Bay in 2002.

In June and July, 2001 (Figure 3.1), biomass of non-toxic cyanobacteria surpassed other species and became the dominant species. Then, *Anabaena flos-aquae* increased in August and soon was

replaced by *Aphanizomenon flos-aquae* and *Microcystis sp.* The competition started between *Aphanizomenon flos-aquae* and *Microcystis flos-aquae* in September. The monitoring results show that *Aphanizomenon flos-aquae* were always in a dominant position during the entire month. The three cyanobacterial species respectively dominated Missisquoi Bay in different months.

The distribution of cyanobacterial species in Missisquoi Bay in 2002 is very different from that in 2001. *Aphanizomenon flos-aquae* dominated the Bay only on September 16. In contrast, *Anabaena spiroides* became the new dominant species during the seasonal period and reproduced rapidly in August (Figure 3.2). The peak of its biomass was over 80,000 mg/m^3. Non-toxic cyanobacteria dominated almost all stations monitored at Missisqoi Bay in July, same as 2001. It is not yet understood why Missisquoi Bay has been dominated by different species in 2001 and 2002. The same situation was also found in the other years.

Compared to 2001 and 2002, it is difficult to identify dominant cyanobacterial species in 2003 because of the diversity presented. Although we cannot find a special discipline in distribution by time, there should be a spatial consistency.

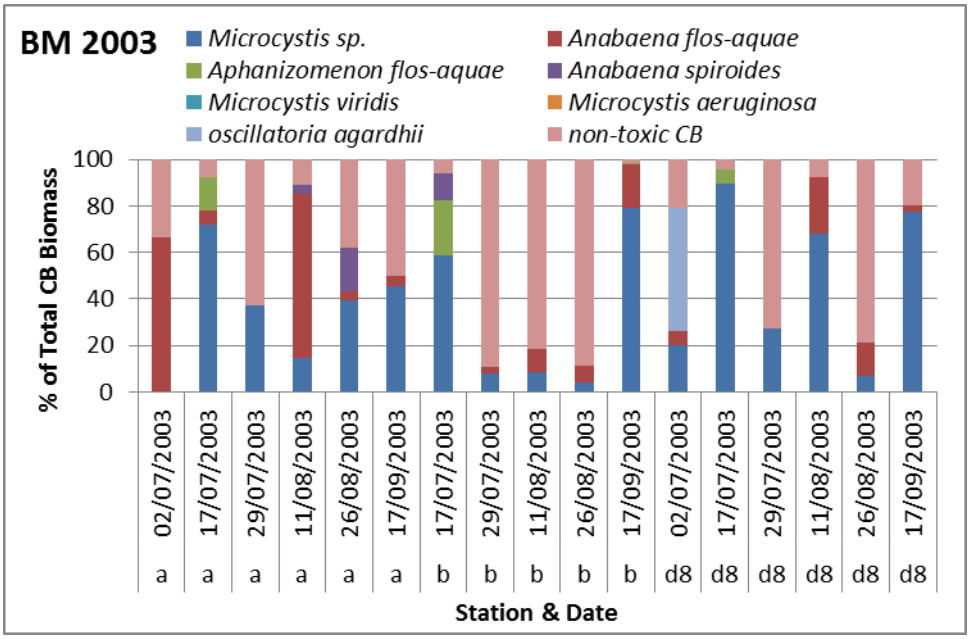


Figure 3.3: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Missisquoi Bay in 2003.

In 2003 and 2004, all four kinds of genera, *Microcystis sp., Anabaena flos-aquae, Aphanizomenon flos-aquae* and *Anabaena spiroides* were identified in almost all stations in the Bay. In 2004, the

fraction of *Microcystis sp.* achieved 94 percent, and 94 percent on July 12 in Stations A and B. Then, *Microcystis viridis* took the place of *Microcystis sp.* with the proportion varied from 27 percent to 80 percent. *Microcystis sp.* potentially produces microcystins which are very toxic to human and animals. In 2005, the cyanobacterial genera with the highest biomass alternated between *Microcystis aeruginosa* and non-toxic cyanobacteria throughout the season (Figure 3.5).

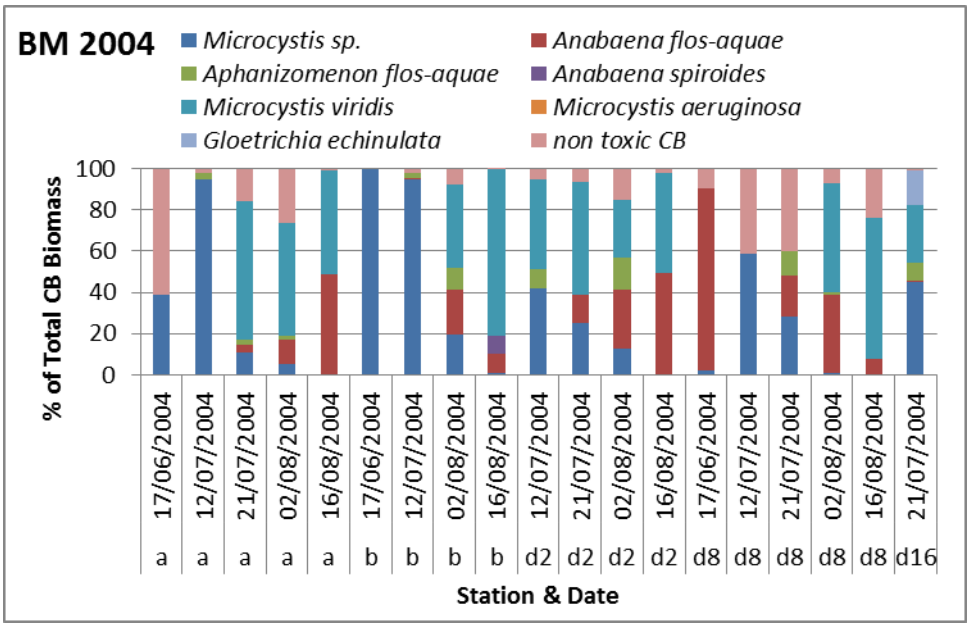


Figure 3.4: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Missisquoi Bay in 2004.

As previously mentioned, 22 of total 29 samples detected were dominated by toxic cyanobacteria, whose percentage of biomass was over 50 percent in 2006 at Missisquoi Bay. As shown in Figure 3.6, the Bay was dominated by *Microcystis flos-aquae, Anabaena spiroides* and *Aphanizomenon flos-aquae* which could potentially produce cyanotoxins. From mid-July (July 18) to early August (August 9), *Anabaena spiroides* was identified as the dominant genera with the peak of biomass reaching 146,345.0 mg/m^3 in August 2. The dominance of *Anabaena spiroides* sustained until September 27 (Figure 3.6) and was surpassed by *Aphanizomenon flos-aquae*. This distribution is very similar to 2001 that the dominance of *Aphanizomenon flos-aquae* always appeared in September.

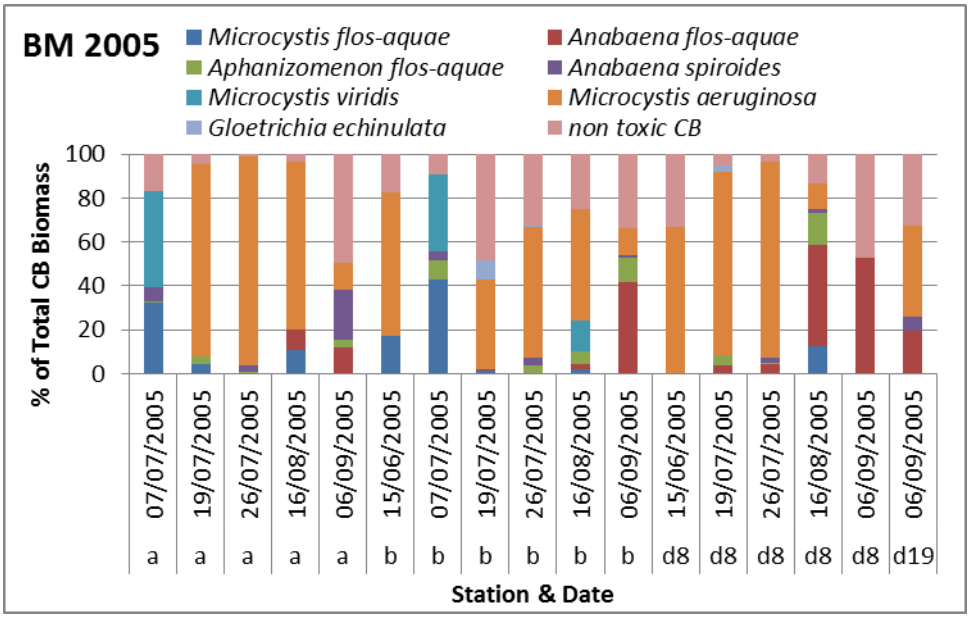


Figure 3.5: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Missisquoi Bay in 2005.

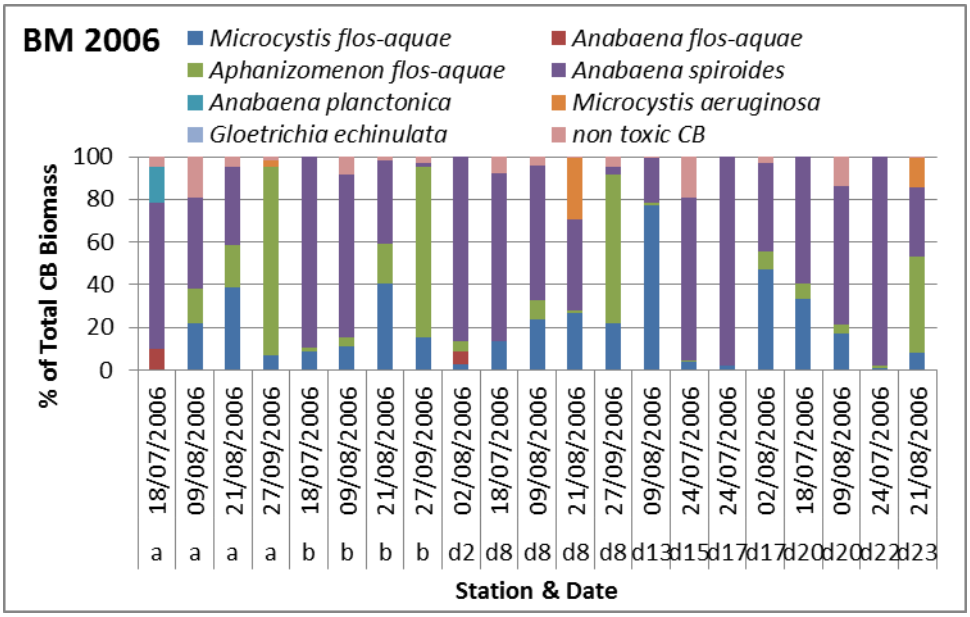


Figure 3.6: Distribution of cyanobacterial biomass of the most abundant species in water in different stations in Missisquoi Bay in 2006.

Microcystis flos-aquae were not found in surface water in 2007, as it was the case in 2002. Apparently, *Anabaena flos-aquae* play the most important role in the dominance of water, but exceptions were observed in August 18 in stations d1 and d2 (Figure 3.7). The Bay was dominated by *Anabaena spiroides* on that day. The only year that no scum occurrence was observed was in 2007. Although *Anabaena flos-aquae* were dominant, actual biomass was very low.

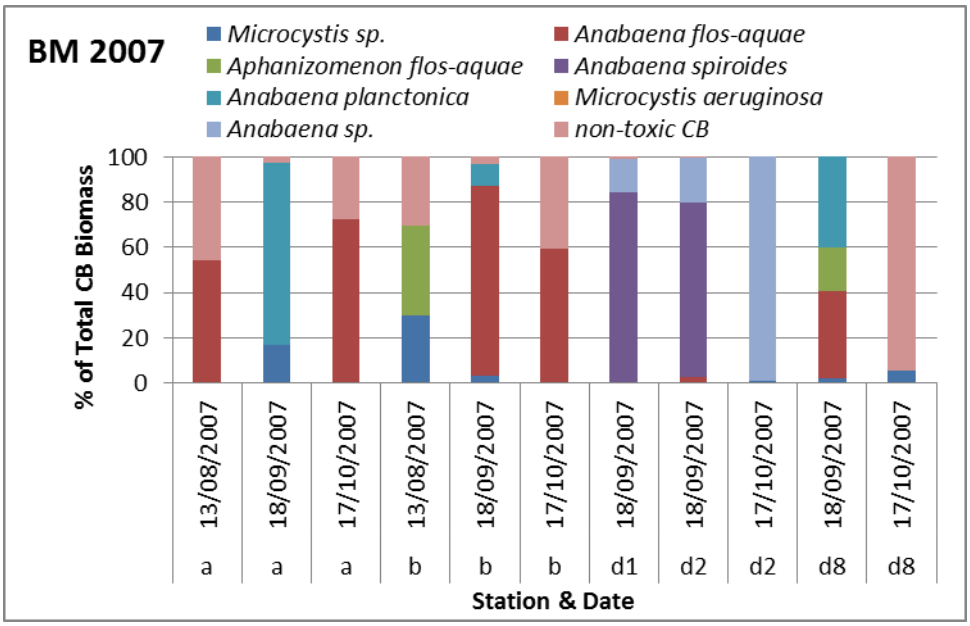


Figure 3.7: Distribution of cyanobacterial biomass of the most abundant species in water in different stations in Missisquoi Bay in 2007.

At last, *Microcystis aeruginosa* once again became the dominant species in this water resource in 2008. Due to the intermittent monitoring, the results of September and October were not included in the historical data. Therefore, the change of dominant species cannot be clearly observed.

There were often large variations of cyanobacterial abundance and dominant species for the same seasonal day but in different years at the Missisquoi Bay. However, no matter which species were present in the water, it can reproduce rapidly in a relatively short period of time and dominant the water. It should be noted that although dominant species were different from year to year, *Anabeana flos-aquae, Aphanizomenon flos-aquae* and *Microcystis spp.* were always present in every seasonal period, even with low biomass. The existence of these microcystins and anatoxin producing species indicates the production of cyanotoxins in the water. The relation between the concentration of cyanobacteria and cyanotoxins will be discussed in section 3.4.

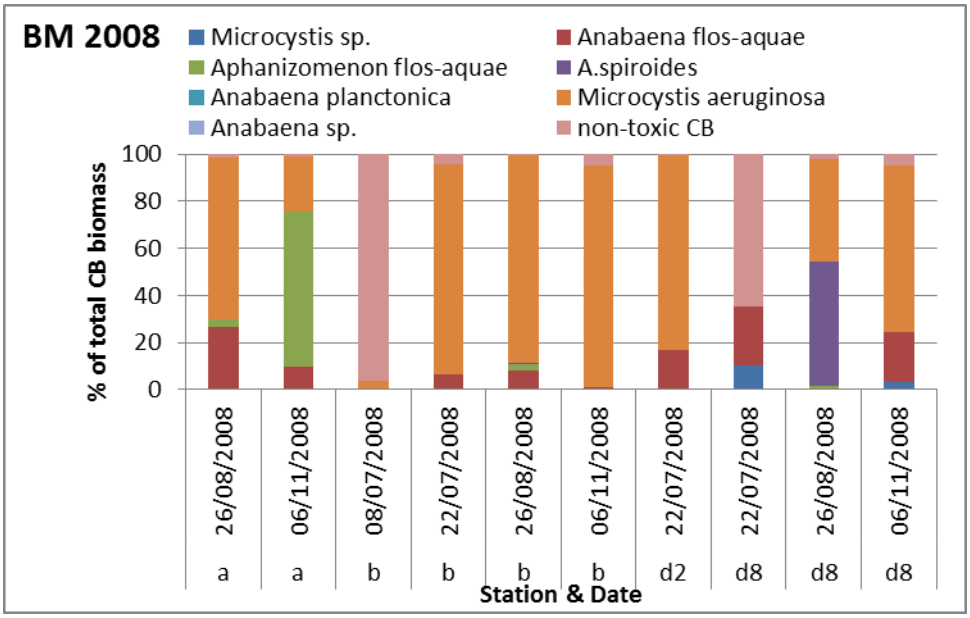


Figure 3.8: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Missisquoi Bay in 2008.

3.2.1.1 Spatial variation and consistency of dominant cyanobacterial species at the Missisquoi Bay

Over 20 stations were set up for cyanobacterial monitoring (map in Figure 3.1), but not every station was monitored in every year. Station A and station B were two sites always monitored in all years. Station d8, located near the supervised public beach in northwest of the Bay, is another point that had been monitored continuously from 2002 to 2008. It is worth mentioning that Station d6 is located near the intake of drinking water, where scum had always been observed in past years. Other stations monitored in just one or two years when the bloom was observed are not representative but have their specificity.

In 2002, station b, d8 and d10 were all dominated by *Anabaena spiroides* on the same day (August 13) with very similar composition of cyanobacteria (Figure 3.2). On August 5, the samples collected both in stations b and d10 contained *Aphanizomenon flos-aquae* and *Anabaena spiroides* with nearly the same fraction. Unfortunately, it lacks the data of station A; otherwise the hypothesis that the currents and wind can homogenize and cause the accumulation of a high concentration of toxic cyanobacteria can be demonstrated. However, the data showed in the following years provides convincing evidence.

Comparing the fraction of cyanobacteria in stations A and B on July 12, 2004, the compositions and fractions are nearly the same. The same situation was also found in stations b and d2 on August 2 and stations A and d2 on 16 (Figure 3.4). A similarity was always found between two stations on one day, making it reasonable to hypothesize that the wave and wind definitely plays an important role. This can be also illustrated by comparing the fractions between stations A and B on August 21 and on September 27 and between stations b and d20 on August 9 in 2006 (Figure 3.6). The same situation was also found in 2007.

3.2.2 Cyanobacterial analysis of Lake Nairne (2002-2008)

Compared to Missisquoi Bay, the situation in Lake Nairne is much simpler. The monitoring stations are concentrated in three points. *Anabaena flos-aquae* and *Aphanizomenon flos-aquae* were also the most important genera in the water bloom.

In 2002, only two days were monitored in both stations A and B. The lake was occupied by *Aphanizomenon flos-aquae* on October 17 in the two stations with biomass measuring over 20,000 mg/m^3 (Figure 3.9). However, *Aphanizomenon flos-aquae* were not the dominant species in the following three years. Monitoring in 2003 started on September. 15, abundant *Microcystis sp.* appeared in late September and sustained until October 14 with a highest biomass of 15,684 mg/m^3 in station B. Usually, the bloom of *Microcystis sp.* began in early summer and grew greatly in August, as we observed at Missisquoi Bay.

In contrast, *Microcystis sp.* was observed in very early seasonal period (Figure 3.11) in spite of a very low biomass of fewer than 100 mg/m^3 in 2004. Although the *Anabaena flos-aquae* took the place of *Microcystis sp.* in the samples collected on July 19 and August 2, their biomass was less than 20 mg/m^3 in both stations A and B. Then, like what was observed elsewhere, *Microcystis sp.* regained the dominant position but biomass never achieved the peak level measured in 2003.

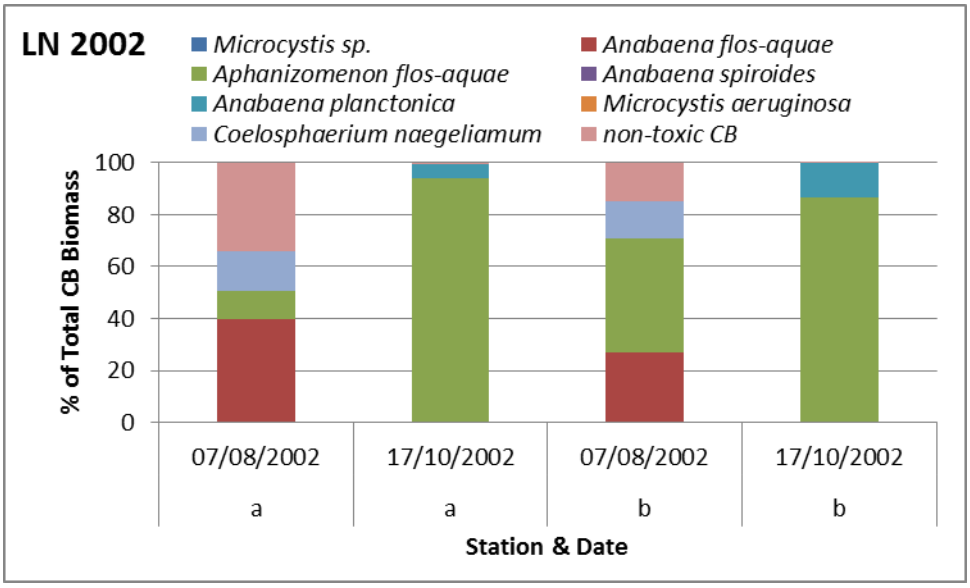


Figure 3.9: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Nairne in 2002.

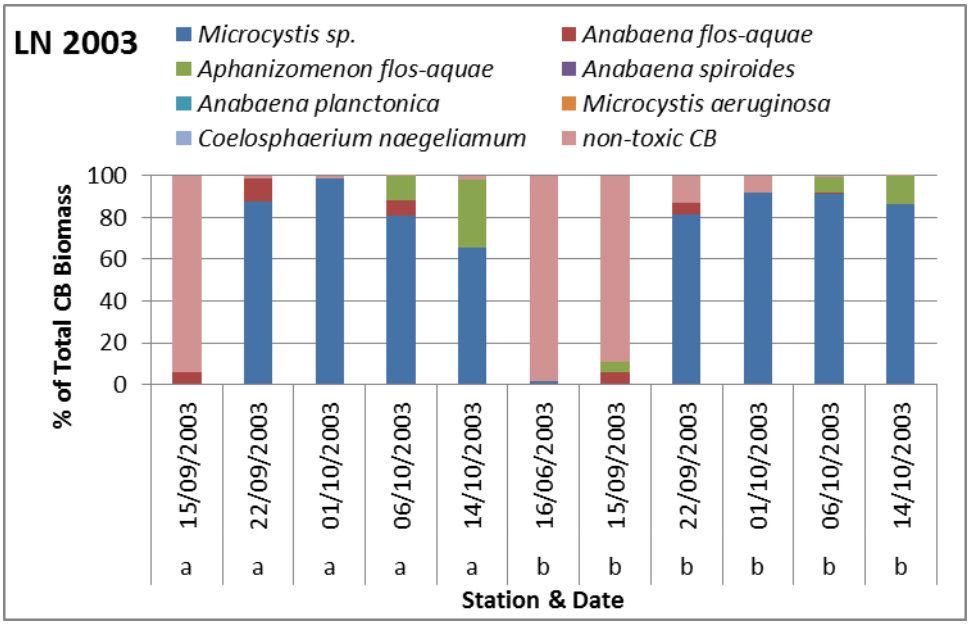


Figure 3.10: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Nairne in 2003.

Anabaena flos-aquae dominated Lake Nairne in 2005, but its concentration was as low as that in 2004. The highest biomass measurement of 165 mg/m^3 was recorded on August 15. Thus, it is reasonable to indicate that biomass of *Anabaena flos-aquae* was always very low when dominating this lake. The same phenomenon was found in the beginning of the seasonal period in 2006.

Anabaena planctonica and *Aphanizomenon flos-aquae* dominated the lake in August and September.

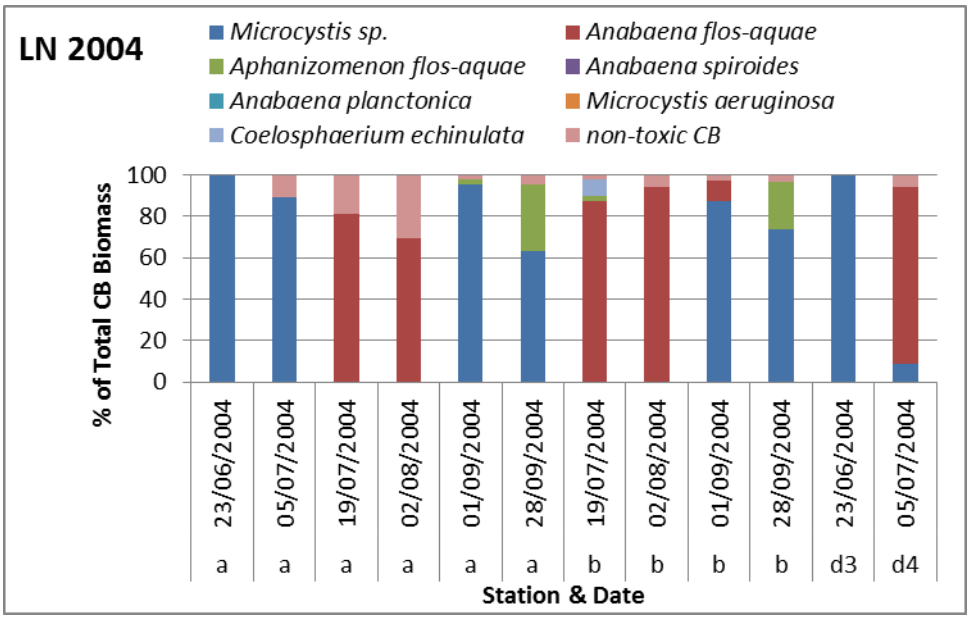


Figure 3.11: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Nairne in 2004.

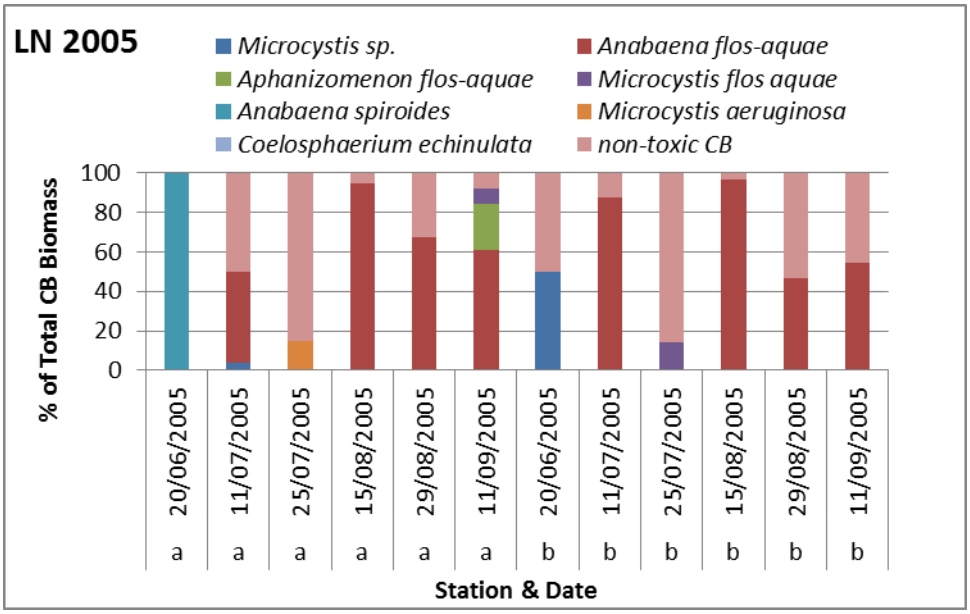


Figure 3.12: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Nairne in 2005.

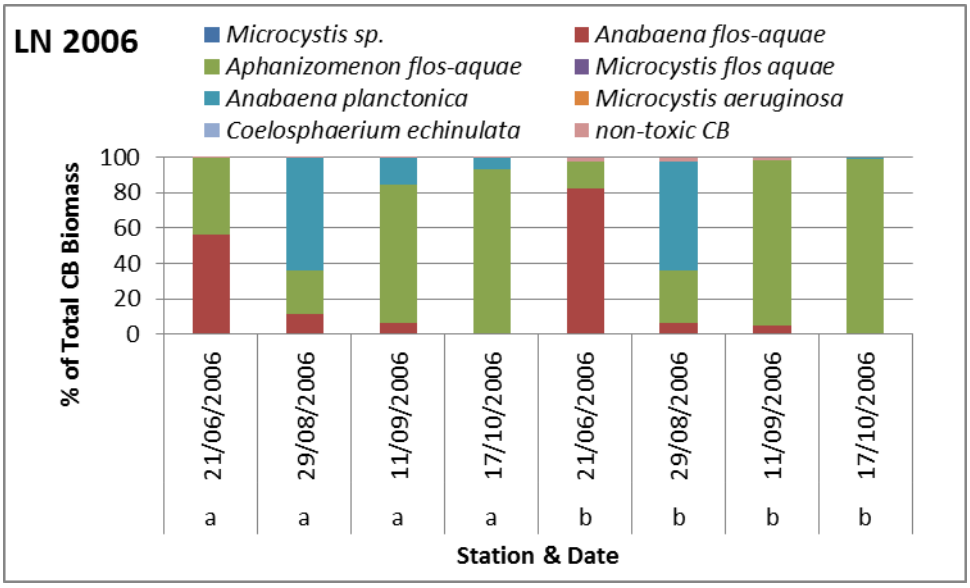


Figure 3.13: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Nairne in 2006.

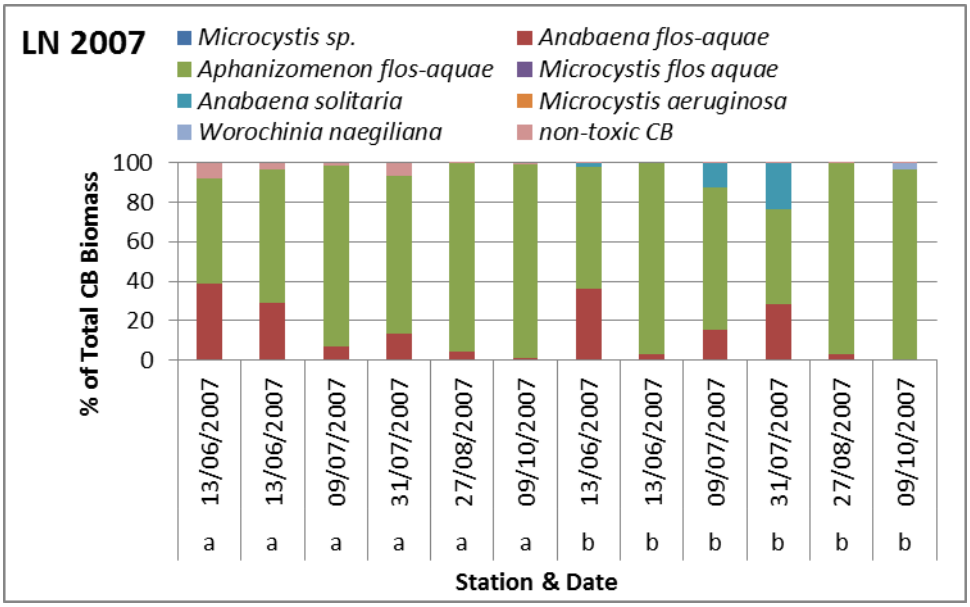


Figure 3.14: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Nairne in 2007.

Microcystis sp. were the most abundant species in 2003 and 2004, but they disappeared in the following three years. *Microcystis flos-aquae* were no longer detected in any water sample collected in 2006, 2007, and 2008. Lake Nairne was occupied by *Aphanizomenon flos-aquae* for the entire season in 2007. The highest concentration of *Aphanizomenon flos-aquae* was detected on October 9 in station A with a fraction over 98 percent (Figure 3.14). *Anabaena flos-aquae* were also in low quantity.

Unlike the past six years, *Worochinia naegiliana* was the only genus which was detected in 2008 in Lake Nairne. The bloom was observed on September 17 with a very high concentration of 363,758 mg/m^3.

Although cyanobacterial variation is also reflected in Lake Nairne due to the large historical monitoring data, the most abundant species were concentrated on *Aphanizomenon flos-aquae* and *Microcystis sp*. *Anabaena flos-aquae* did not bloom in this lake even in the absence of other species (2005; Figure 3.12). Its density still remained at a very low level. Potential inter-annual differences in precipitation and temperature are hypothesized to explain some of the distribution variability of cyanobacterial species.

3.2.2.1 Spatial variation and consistency of dominant cyanobacterial species at Lake Nairne

The figures presented above show a high uniformity in the distribution of dominant cyanobacterial species on the same monitoring day between two different stations. Station A was in the center of the lake and station B was located near the shore, where there was a supervised public beach.

In 2002, total biomass of *Aphanizomenon flos-aquae* detected in station A on October 17 was 94 mg/m^3 comparing to 87 mg/m^3 measured in station B on the same day. Apparently, the compositions of cyanobacteria in these two stations were very similar by comparing the fraction of cyanobacterial biomass in 2003 (Figure 3.10). However, the biomass of the most abundant species *Microcystis sp.* was 1,180 mg/m^3 in station A and much higher at 5,230 mg/m^3 in station B on October 6. The biomass of *Microcystis sp.* in station A reduced to 875 mg/m^3 on October 14, on the other hand the highest biomass 15,684 mg/m^3 detected in station B on the same day. This was not well proved in the following two years, probably due to the total cyanobacterial biomass remaining

at a very low level. In 2006, the spatial variation was identified again on August 29 and September 11.

3.2.3 Cyanobacterial analysis at the Lake Brome (2001-2003)

Lake Brome is the only lake among those studied where no scum occurred in surface water from 2001 to 2003. Three stations were located in the center, north, and south of the lake. The distribution of cyanobacteria was very similar to that of Missisquoi Bay. It is difficult to determine clearly which species dominated the lake. *Microcystis sp.* was found in all three stations on August 15 and September 24 in 2001, but biomass varied from five mg/m^3 to 60 mg/m^3. Thus, *Microcystis flos-aquae* were not the species dominating Lake Brome in 2001. Comparable to the situation in Lake Nairne, *Anabaena flos-aquae* appeared in early season. It did not reproduce quickly in the absence of other species. Nevertheless, *Anabaena solitaria* and *Aphanizomenon flos-aquae* were the most abundant species. Their biomass reached a maximum value in three stations at the same (September 24, 2001, Figure 3.15).

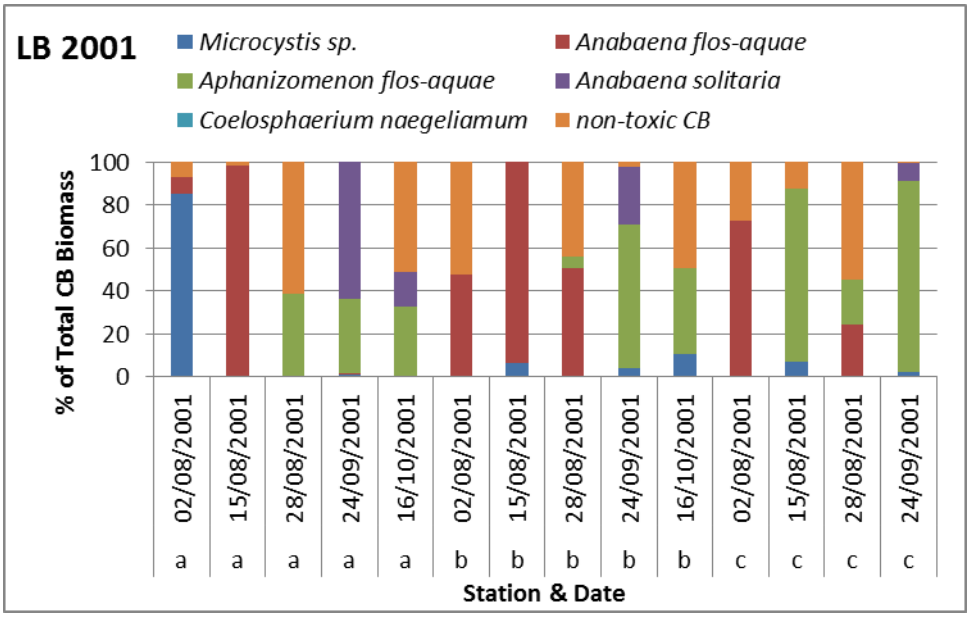


Figure 3.15: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Brome in 2001.

Samples from three stations collected on the same day (August 12, 2001) measured cyanobacterial biomass consisting of *Aphanizomenon flos-aquae* and *Anabaena solitaria* in 2002. The proportions of these two species with their corresponding biomass were uniform in all three

stations (Figure 3.16). The same composition was found on September 3. The dominant *Aphanizomenon flos-aquae* achieved a very high concentration of biomass: over 10,000 mg/m^3. At the same time, *Anabaena solitaria* followed *Aphanizomenon flos-aquae*, becoming the second species to dominate the lake with biomass variation from 3,552 mg/m^3 to 5,585 mg/m^3.

The distribution of cyanobacterial biomass in 2003 was somewhat similar to that seen in 2002. The bloom period was dominated by *Aphanizomenon flos-aquae* from mid-August to September. The most abundant biomass was observed on August 28 in station B.

On the other hand, the biomass of *Aphanizomenon flos-aquae* in station A and station C did not reach peaks on the same day. Their peaks were achieved on the next monitoring day, September 17). It must be noted that the levels of *Anabaena solitaria* were always less than those of *Aphanizomenon flos-aquae* in almost all samples monitored. However, it seems they followed the period dominated by *Aphanizomenon flos-aquae*. They reached their highest biomass of 7,970 mg/m^3 when *Aphanizomenon flos-aquae* reached its peak level of 9,175 mg/m^3. *Anabaena flos-aquae* once again appeared in the early bloom period with low biomass.

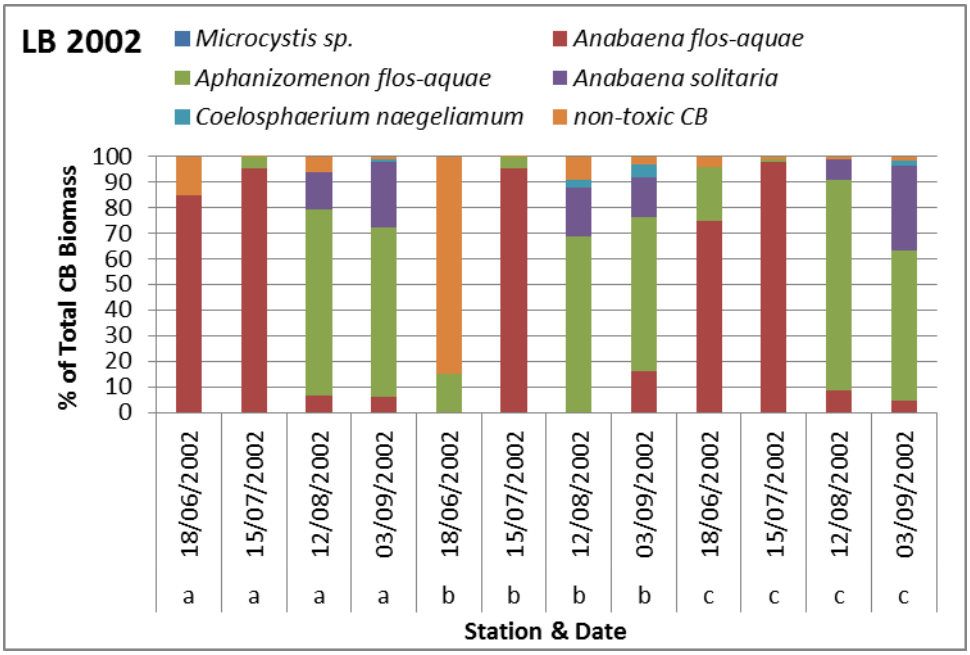


Figure 3.16: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Brome in 2002.

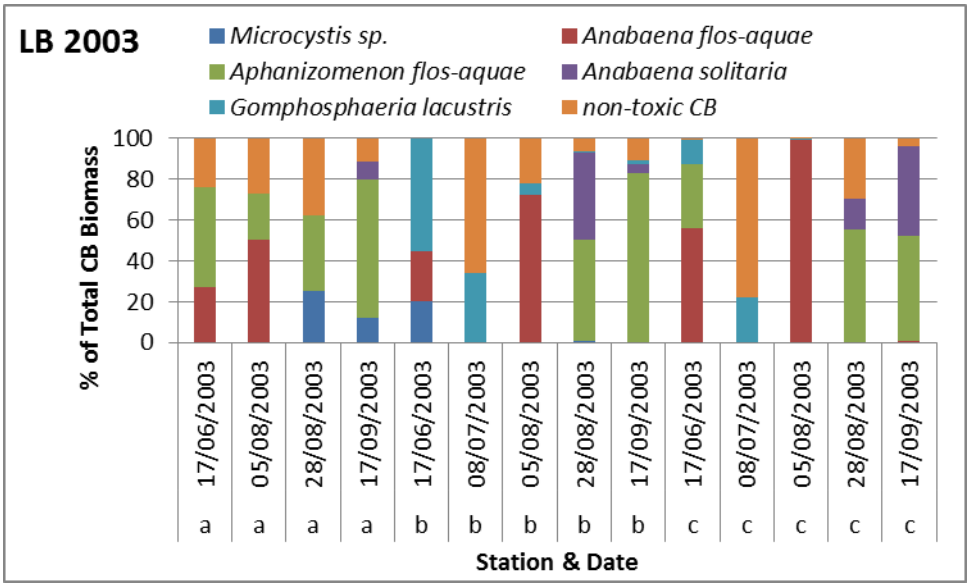


Figure 3.17: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake Brome in 2003.

3.2.3.1 Spatial variation and consistency of dominant cyanobacterial species at Lake Brome

As one of the most abundant species in Lake Brome, *Aphanizomenon flos-aquae* was detected in stations A, B, and C on September 24, though they were not the dominant species in station A. This uniformity was maintained until October 16, when their biomass dropped to 148 mg/m^3 and 150 mg/m^3 in both stations A and B.

In contrast, the concentration of *Anabaena solitaria* fluctuated significantly, from 113 mg/m^3 to 1,977 mg/m^3 between the three monitoring stations on the same sampling day (September 24).

On August 12, 2002, the detected biomass of *Aphanizomenon flos-aquae* in stations A, B, and C was 1,090 mg/m^3, 977 mg/m^3 and 1,146 mg/m^3 respectively. Comparing to the biomass of 9,248 mg/m^3, 4,884 mg/m^3 and 10,040 mg/m^3 measured in these three stations on September 3, it may be ascertained that the lowest concentration of *Aphanizomenon flos-aquae* was always in station B and the highest one in station C. This phenomenon can also be used with *Anabaena solitaria*.

According to the monitoring data and the location of these three stations, the direction of flow may drive the drift of species because station C is proximate to the junction of Lake Brome and the

Yamaska River (Figure 3.3). The assumption that the waves and wind can homogenize and accumulate the cyanobacteria into one direction is still applicable.

This hypothesis could also explain the situation on August 28, 2003, when the highest biomass of *Aphanizomenon flos-aquae* was detected in station B and the lowest in station C, but with variable wave and wind direction.

3.2.4 Cyanobacterial analysis at the Lake William (2000-2003)

There were several stations located on Lake William, but station A and station B were the main points for monitoring. Samples were collected continuously from 2001 to 2003. The results were measured in other stations only when bloom was observed.

The dominant cyanobacterial species in Lake William was very simple and clear. *Aphanizomenon flos-aquae* almost dominated the entire lake throughout the whole seasons in every year, although *Anabaena flos-aquae*, usually in the beginning of the seasonal period, surpassed the biomass of *Aphanizomenon flos-aquae* and became the first dominant species during a short period.

Taxonomic analysis shows that the appearance of *Aphanizomenon flos-aquae* started in early August and reproduced gradually in August and September. Then, the biomass began to reduce after mid-September. The fraction of *Aphanizomenon flos-aquae* remained at very high level (Figure 3.18) in late September and October due to the absence of other cyanobacterial species.

In 2000, the specific site where samples were collected is not shown in map, but it is obvious to identify the abundance of *Aphanizomenon flos-aquae* as we mentioned above during the seasonal period (Figure 3.18). However, maximum biomass was reported on July 24 with a high concentration of 12,220 mg/m^3 rather than in August. Nevertheless, *Aphanizomenon flos-aquae* still sustained a large biomass of 7,744 mg/m^3 and 3,349 mg/m^3 on August 7 and August 18.

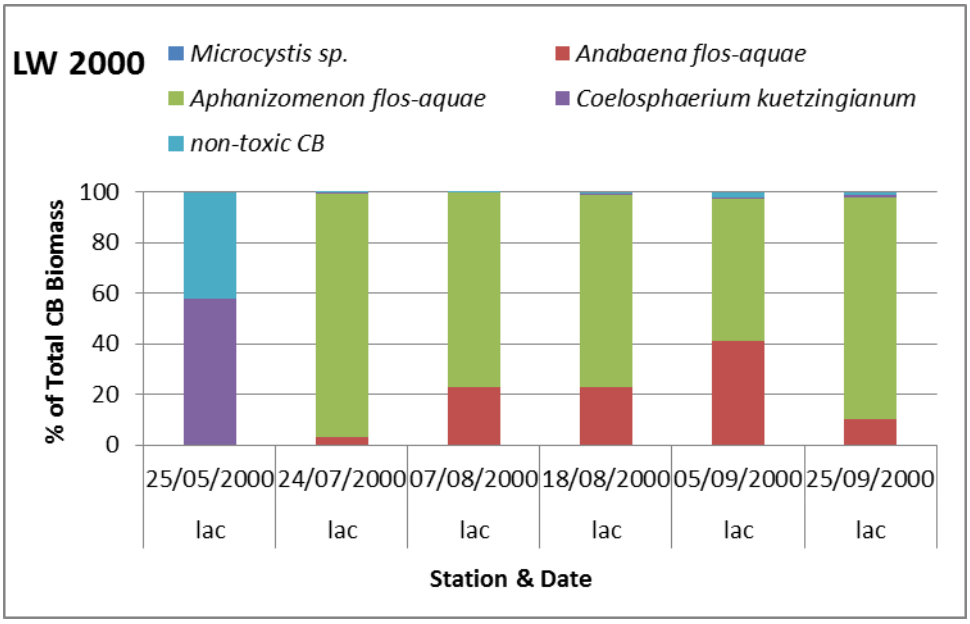


Figure 3.18: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake William in 2000.

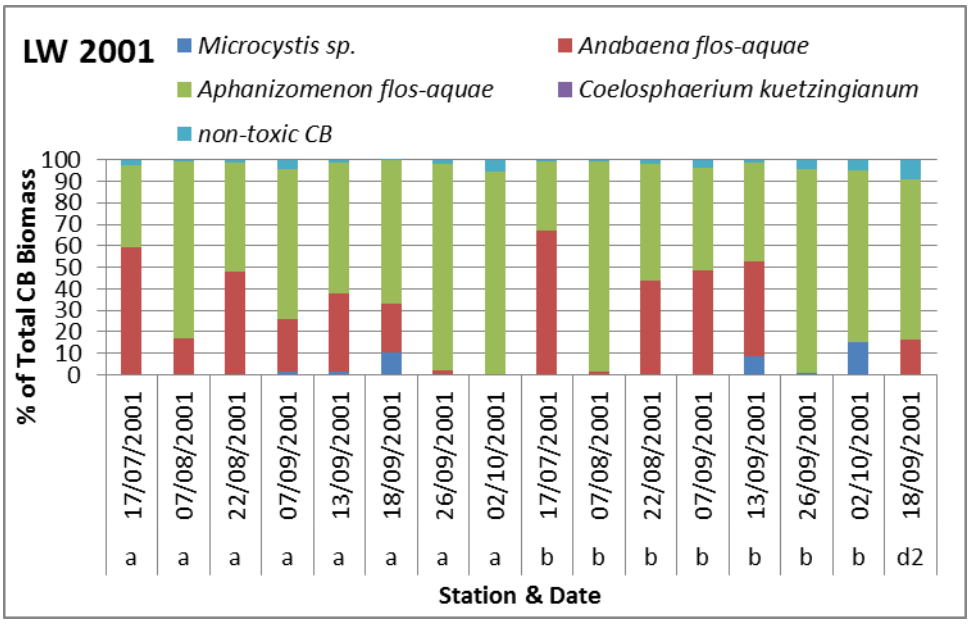


Figure 3.19: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake William in 2001.

Aphanizomenon flos-aquae dominated from July to September of 2001 except July 17 when *Anabaena flos-aquae* exceed all other species. Samples measured cyanobacterial biomass of

4,103 mg/m^3 and 835 mg/m^3 consisting of *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* on August 7, which were less than that detected on the same date in 2000 (Figure 3.18 and Figure 3.19).

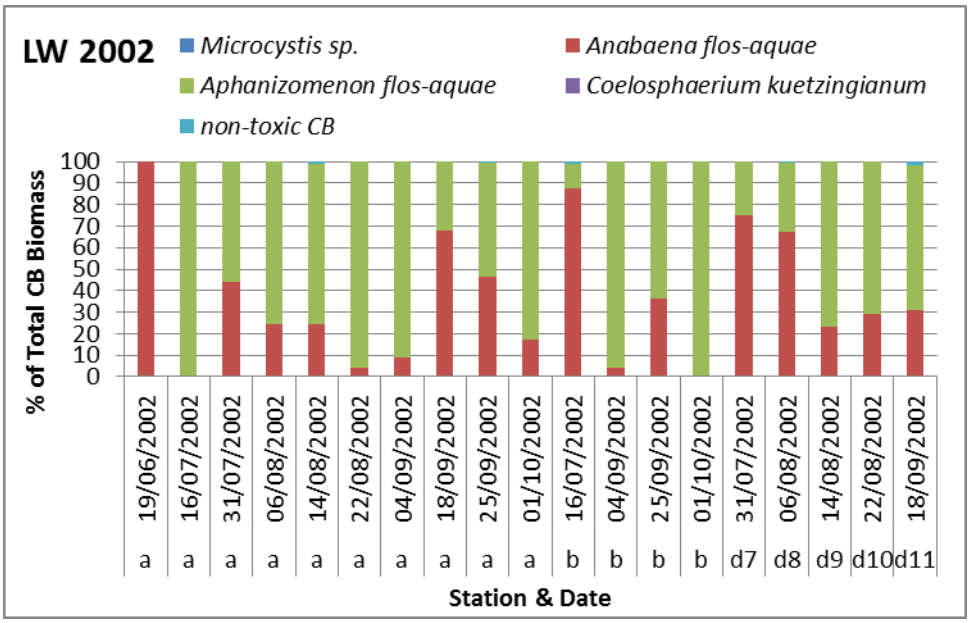


Figure 3.20: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake William in 2002.

The huge blooms of *Aphanizomenon flos-aquae* with biomass measurements of 19,910 mg/m^3, 12,241 mg/m^3 and 13,489 mg/m^3 appeared respectively in station A on August 14, August 22 and September 4, 2002. In addition, the biomass detected in station d9 on August 14 was even higher than 20,000 mg/m^3. *Anabaena flos-aquae* were detected in almost in every sample. In 2002, *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* achieved a certain consistency in biomass during the bloom period.

As Figure 3.21 shows, *Aphanizomenon flos-aquae* were definitely the dominant species in Lake William. The peak biomass of 15,510 mg/m^3 was detected on September 3 in station B.

3.2.4.1 Spatial variation and consistency of dominant cyanobacterial species in Lake Brome

The spatial variation of cyanobacterial distribution is relatively similar, due to the special shape of Lake William and the location of stations A and B (Figure 2.4). The most abundant biomass of *Aphanizomenon flos-aquae* appeared in stations A and B which occurred on the same day -- August 7 in 2001 -- and then decreased over time (Figure 3.18). In 2002, the data of August was not available in station B, so by comparing the distribution of *Aphanizomenon flos-aquae* in September, we can find that the proportions on September 4 and September 25 were very similar.

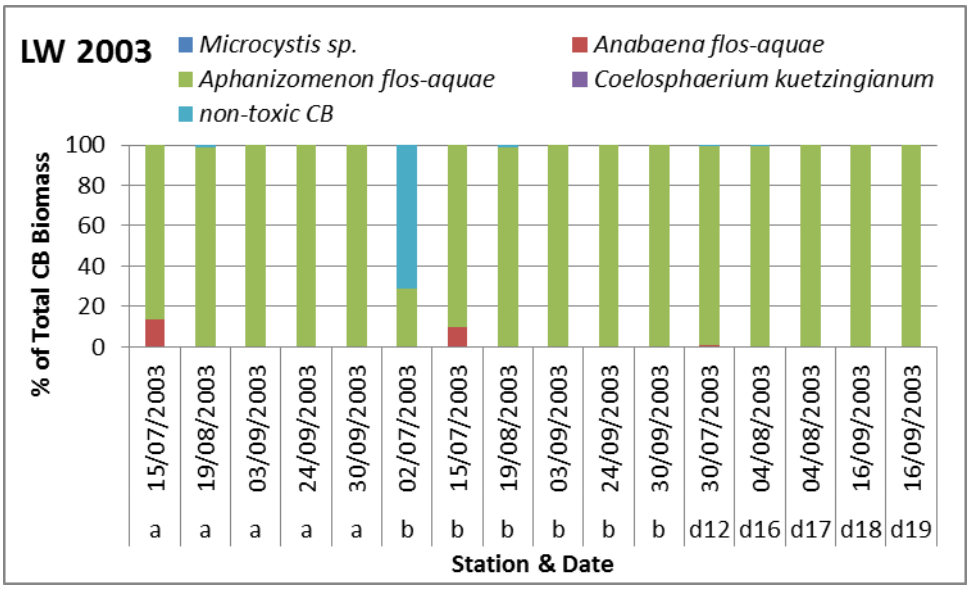


Figure 3.21: Distribution of cyanobacterial biomass of the most abundant species in water in different stations at Lake William in 2003.

However, the density of *Aphanizomenon flos-aquae* detected in station B was always a little higher than that detected in station A.

3.3 Determination of dominance of specific species of cyanobacteria in scum

The spatial-temporal variation of cyanobacteria in the scum is described in this section. The scum was always observed over one day or appeared in a very short term at a certain site. According to the analysis of the four lakes, the dominant cyanobacterial species varied significantly with the time and the site. Before comparing the differences among the four lakes, it is necessary to analyze the frequency of the presence of cyanobacterial species in every lake. Table 3.5 indicates

a frequent presence of certain potentially toxin-producing species in the scum sources of the four lakes. There was no scum appearing in Lake Brome during the monitoring years, thus Lake Brome will not be discussed in this section. Toxic cyanobacteria detected in Missisquoi Bay were much more diversified in terms of species than that in the other two lakes. However, *Anabaena flos-aquae, Microcystis sp. and Aphanizomenon flos-aquae* were the most frequent presence of cyanobacteria in both three lakes, and it is very similar to the situations in water.

Table 3.5: The frequency of presence of potential toxic cyanobacteria in four lakes.

	Missisquoi Bay	Lake Nairne	Lake Brome	Lake William
Total number of samples taken from 2000 to 2008 that are scum	**32**	**13**	**water**	**7**
Anabaena flos-aquae	**16 (50%)**	**7 (53.8%)**		**2 (28.6%)**
Microcystis sp.	**27 (84.4%)**	**9 (69.2%)**		**6 (85.7%)**
Aphanizomenon flos-aquae	**17 (53.1%)**	**7 (53.38%)**		
Anabaena spiroides	**12 (37.5%)**			
Gloeotrichia echinulate	**10 (31.25%)**			
Microcystis viridis	**7 (21.875%)**			
Microcystis aeruginosa	**6 (18.75%)**			
Anabaena circinalis	**1 (3.125%)**			
Microcystis mesenbergii	**1 (3.125%)**			
Anabaena planctonica		**3 (23.1%)**		
Worochina naegiliana		**1 (7.7%)**		
Oscillatoria utcrmoehlii				**1(14.3%)**
Aphanizonemon gracil				**1(14.3%)**

3.3.1 Cyanobacterial analysis in the Missisquoi Bay (2000-2008)

As Table 3.5 presents, nine toxic cyanobacteria have appeared in the Missisquoi Bay water. The frequency of the presence of cyanobacteria cannot represent its dominance in the Bay at a certain time.

There was no scum observed in 2000 in Missisquoi Bay. In 2001, scum was found on various days in stations d3, d6 and d7, which were located along the eastern shore of the Bay. The dominant species detected in these three stations were different, but comparing to water, it is easy to detect commonalities. *Anabaena flos-aquae* was the most abundant species in station d3 and the other stations on August 21, and *Aphanizomenon flos-aquae* replaced *Anabaena flos-aquae* to

become the dominant species on September 19, September 26 and October 2 (Figure 3.21 a)). The biomass detected in the scum was much higher than that measured in the water samples. The maximum biomass of *Aphanizomenon flos-aquae* was 25,624,764.0 mg/m^3 when it bloomed on September 26. *Microcystis sp.* was present in water with high biomass, but it was not the most abundant species.

Whereas, *Microcystis sp.* was dominant in early summer in 2002 with nearly 100 percent of total biomass in station d6 which was close to the intake of a water plant (station d6) and supervised public beach, and stations d11 and d12.

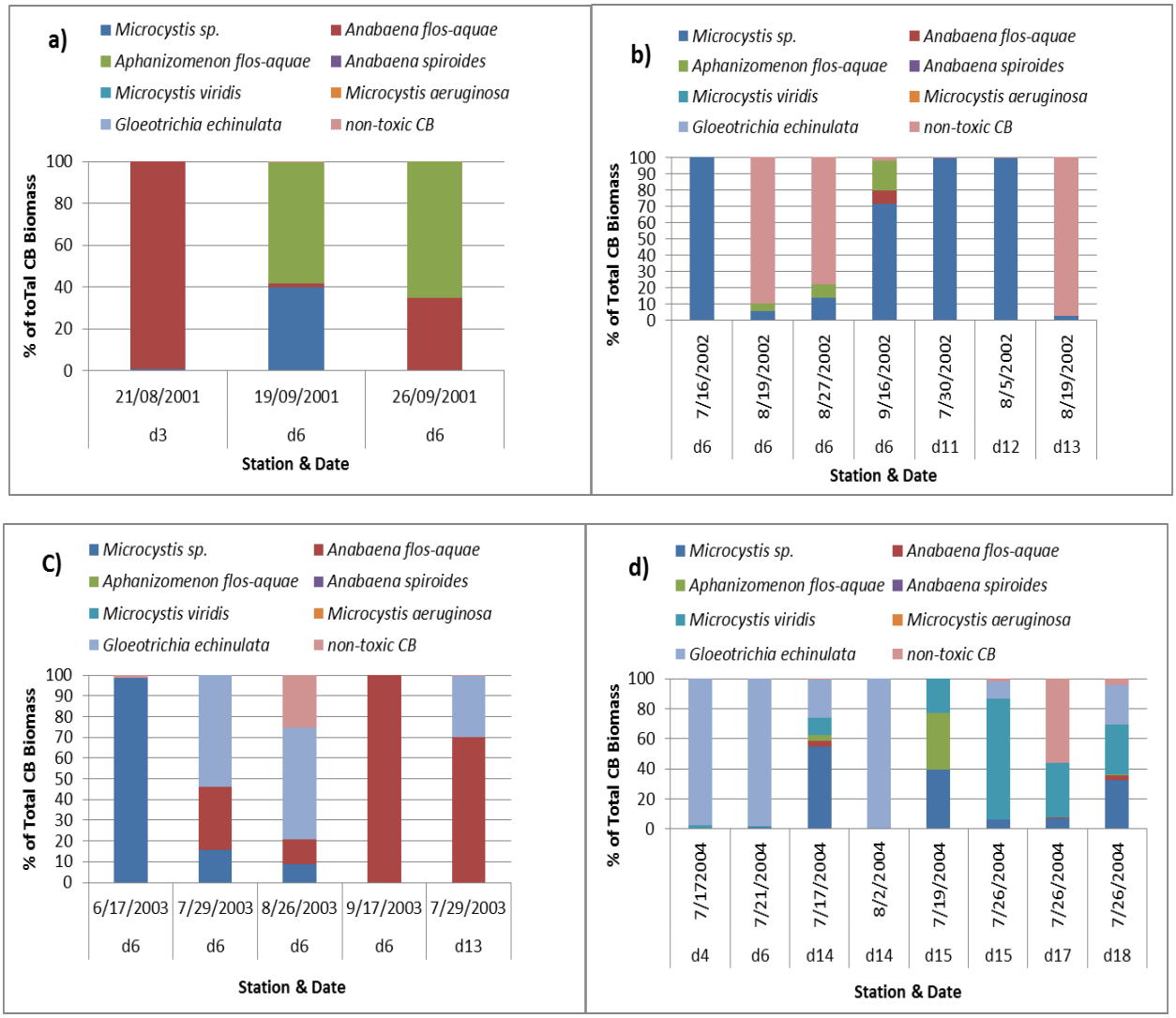


Figure 3.22: The distributions of different cyanobacterial species in Missiquoi Bay from 2001 to 2006: a) 2001; b) 2002; c) 2003; d) 2004; e) 2005 and f) 2006.

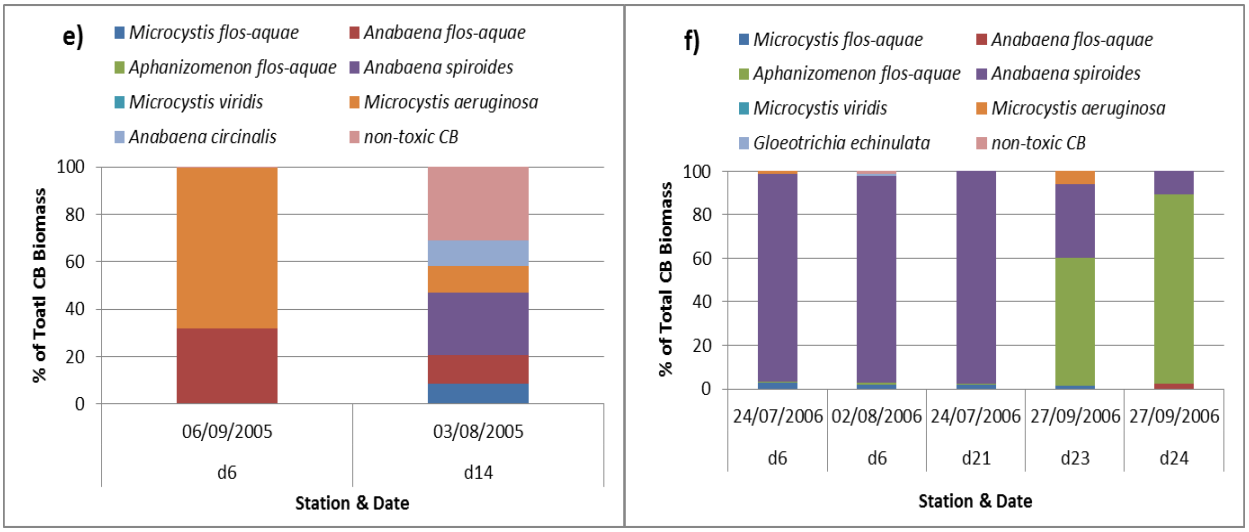


Figure 3.22: The distributions of different cyanobacterial species in Missiquoi Bay from 2001 to 2006: a) 2001; b) 2002; c) 2003; d) 2004; e) 2005 and f) 2006. (suite)

The biomass of *Microcystis sp.* detected on July 16, 2002 was 37,780,443 mg/m^3, so it is reasonable to predict the high concentration of cyanotoxin measured on this day. As the distribution in the water, *Anabaena spiroides* surpass *Microcystis sp.* became the dominant species at the Bay in 2002.

The compositions of dominant species detected on the same day in the scum and water samples show a strong similarity. In other words, the samples collected in different stations on the same monitoring day had similar compositions of cyanobacteria, but the cyanobacteria reproduced with a very high biomass in certain stations to form a scum in that area. On August 26, 2003, *Microcystis sp.* and *Anabaena flos-aquae* were the most abundant species in station d6 and the other stations (Figure 3.4 and Figure 3.22 c). The difference was that the biomass in station d6 was much higher than the other stations. The same situation was also found in the following years.

Gloeotrichia echinulata, whose potential toxicity is not identified, was found in 2003 in Missisquoi Bay and dominated the Bay in July and early August 2004 at three stations (Figure 3.22 d)). Then, *Microcystis viridis* and *Microcystis sp.* alternatively dominated the Bay.

In 2005, the scum was observed on only two days (Figure 3.22 e). One was dominated by *Anabaena flos-aquae* and *Microcystis aeruginosa,* and the other day the Bay was dominated by multiple species. Same as the water in 2006, *Anabaena spiroides* was the most abundant species in

July and August and was replaced by *Aphanizomenon flos-aquae* in September. However, the density of dominant species in the scum was hundreds of times than that detected in the water.

No scum appeared in 2007 and the scum of *Microcystis aeruginosa* was found only once in station d6 on July 22, 2008.

Station d6, the monitoring site located above the intake of drinking water plant, was the only monitoring station where the scum was observed in almost every year. Almost all the high accumulations of cyanobacteria were found near the shore of the Bay, some stations with scum observed were near the public beach where the humans could directly the toxic cyanobacteria.

3.3.2 Cyanobacterial analysis in Lake Nairne (2002-2008)

The scum appearing in Lake Nairne was less than that in Missisquoi Bay during the monitoring years. An occurrence of scum consisting of *Anabaena flos-aquae* and *Microcystis sp.* was detected on September 10, 2002 in Lake Nairne, and then the dominant species changed to *Aphanizomenon flos-aquae* and *Microcystis sp.* one week later (Figure 3.23). However, in 2003 and 2004, the prevailing species was *Microcystis sp.*, and in 2005, October 2006 and 2007 *Aphanizomenon flos-aquae* dominated the cyanobacterial fraction of phytoplankton. Hence, the inter-annual variation of the dominant species of cyanobacteria is variable and unpredictable. The same conclusion can be applied to Missisquoi Bay.

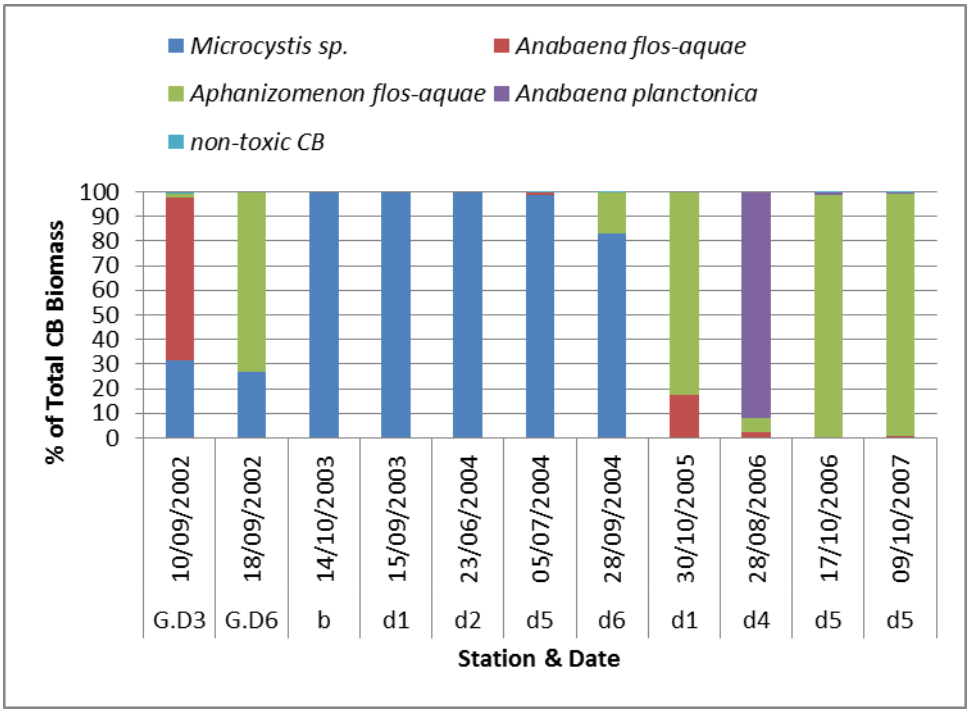


Figure 3.23: The distribution of cyanobacteria in scum in Lake Nairne from 2002 to 2008.

On the other hand, the scum rarely appeared in the center of the lake. Almost all scum was found in stations near the shore. One hypothesis to explain this is that the high concentration of cyanobacteria was accumulated by wind and wave towards the shore. As the results presented in Missisquoi Bay, the compositions of cyanobacteria were very similar by comparing cyanobacteria identified in scum to that detected in the water on the same day among different stations.

3.3.3 Cyanobacterial analysis in the Lake William (2000-2003)

In 2000 and 2002, no scum was found in Lake William and in 2001and 2003, *Aphanizomenon flos-aquae* was the only species dominating the scum in Lake William. Contrary to the unpredictable dominant species in Lake Nairne and Missisquoi Bay, the cyanobacterial species present in Lake William demonstrated the presence of uniqueness in certain conditions which have not yet been tested.

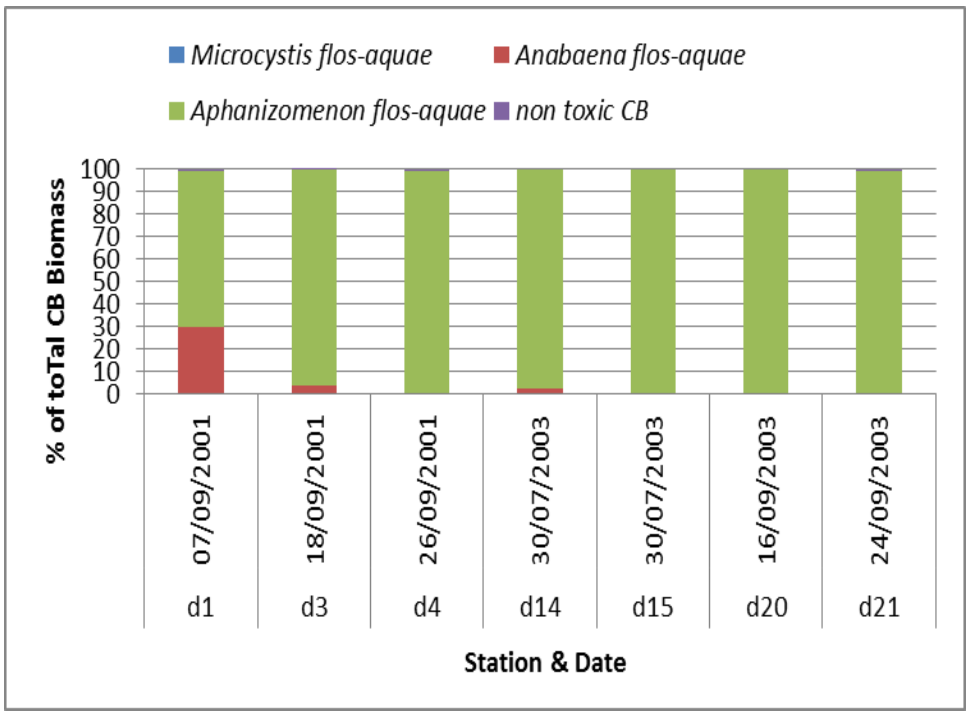


Figure 3.24: The distribution of cyanobacteria in the scum in Lake William from 2000 to 2003.

3.4 Relationship between biomass of cyanobacteria and their toxins produced

The aim of section 3.4 is to analyze the relationship between the biomass of cyanobacteria and its toxins produced in these four lakes in the past years. The lakes were dominated by several species of toxin-producing cyanobacteria both in the scum and water. It is reasonable to predict a risk of having high concentration of cyanotoxin produced when large number of cyanobacteria bloomed. Actually, the cyanotoxins were detected at very high concentrations in the past years in these four lakes, especially in Missisquoi Bay. Microcystins and anatoxin were the main cyanotoxins measured in these four lakes by MDDEP.

3.4.1 Missisquoi Bay (2000-2008)

High MC-LR eq concentrations were measured in Missisquoi Bay in the nine years of monitoring. The maximum level of MC-LR was always found in the scum samples dominated by potentially microsystin-producing species when its biomass also achieved the peak amount, such as *Microcystis sp.* etc. In 2002, the highest concentration of MC-LR detected was 33,540 µg/L with 37,780,443 mg/m^3 biomass measured on July 16 in station d6 where 100 percent dominated by *Microcystis sp.* (Figure 3.25). The extremely high concentrations detected near the intake of

drinking water treatment plant can pose a risk to human health if the physical removal or the treatment of dissolved toxins is unavailable (Natasha McQuaid, 2009).

According to historical data, the concentration of MC-LR eq was relatively lower in water samples than that in scum samples. But in scum samples, when anatoxin producing species dominated the Missisquoi Bay on August 21, 2001, July 29, 2003 and September 27, 2006, the concentrations of MC-LR eq were lower than 1 µg/L. So it is reasonable to assume the existence of a clear association between the cyanotoxin produced and the toxic cyanobacterial biomass.

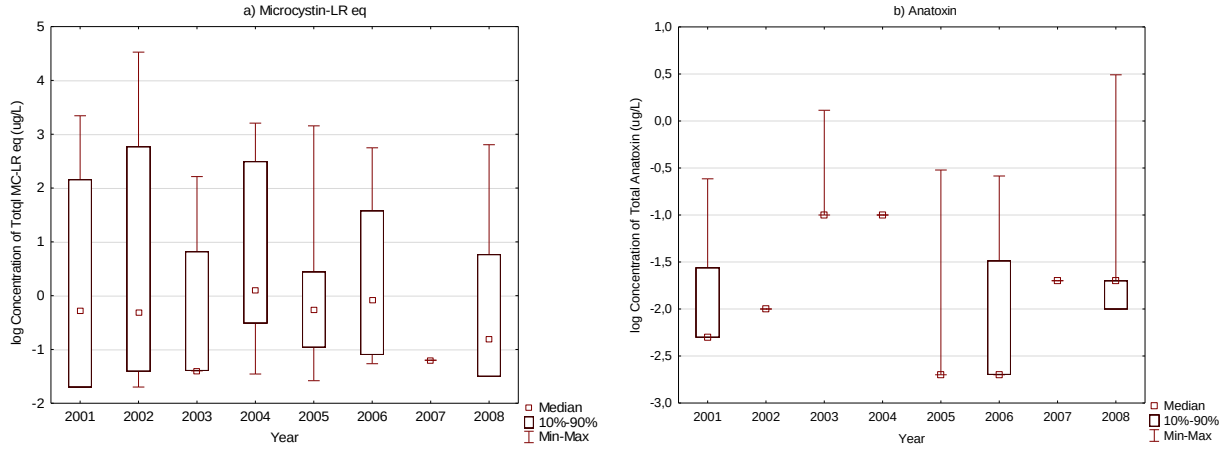


Figure 3.25: Total cyanotoxins measured in all samples from 2000 to 2008 at Missisquoi Bay: a) Total microcystin-LR eq; b) Anatoxin.

Unlike the high concentration of MC-LR detected in Missiquoi Bay, the anatoxin detected was very low, even lower than the detection limit. Even the anatoxin-producing species dominated the Bay, the concentration of anatoxins detected does not seem to have a relationship with the biomass.

3.4.1.1 Cyanobacterial biomass and cyanotoxin in scum in Missisquoi Bay

As mentioned above, the high concentrations of MC-LR eq were detected when MC-producing species dominated the scum samples. At the same time, the corresponding biomass reached maximum levels. The correlation between the concentration of MC-LR eq (µg/L) and the biomass of MC-producing cyanobacteria (mg/m^3) of all scum samples collected in Missisquoi Bay is not very linear (R^2=0.32; p=0.00014), but the results suggest an association between these two parameters (Figure 3.26).

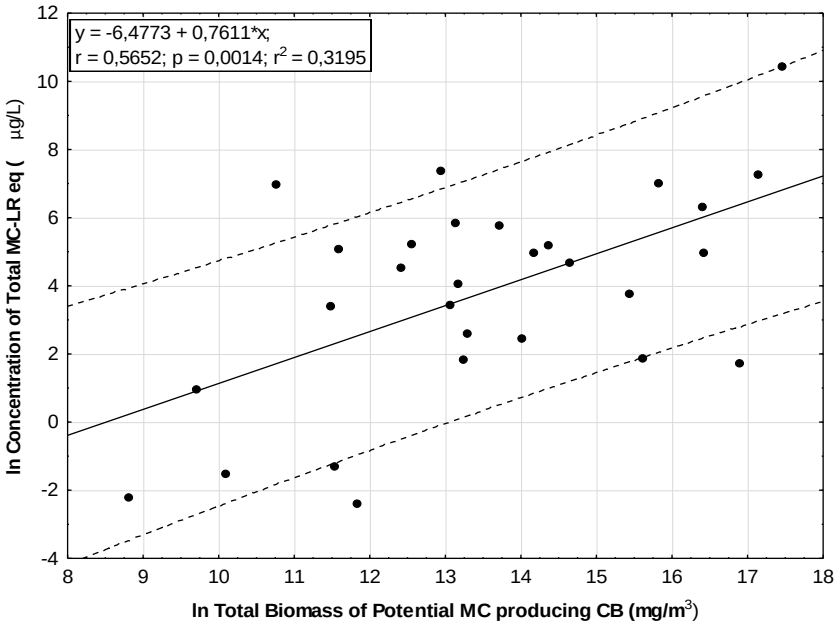


Figure 3.26: Relationship between total biomass of MC-producing cyanobacteria and total MC-LR eq measured of Missisquoi Bay.

Some scum samples were populated with *Anabaena flos-aquae* and *Aphanizomenon flos-aquae*, which potentially produce anatoxin. By eliminating the samples dominated by *Aphanizomenon flos-aquae* and *Anabaena flos-aquae*, the correlation between the concentration of MC-LR eq and the cyanobacterial biomass is poor (R^2=0.24; p=0.0228) (Figure 3.27). The samples included in Figure 3.27 were mainly dominated by *Microcystis sp.*, *Microcystis viridis* and *Anabaena spiroides*, but *Anabaena spiroides* is identified to produce potential microcystin and anatoxin. However, most of the time, scum samples were dominated by several species, including microcystin and anatoxin producing species.

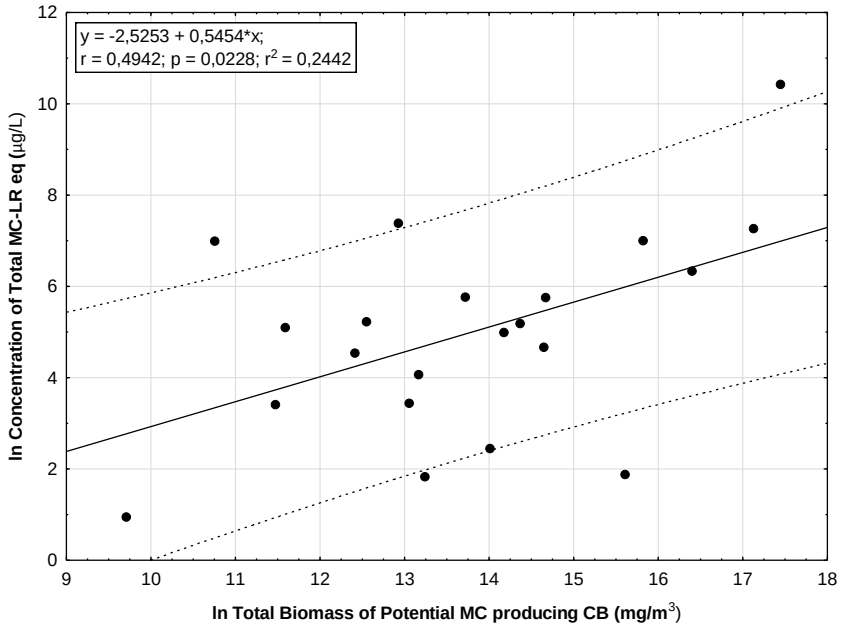


Figure 3.27: Relationship between total biomass of MC-producing cyanobacteria and total MC-LR eq measured in scum samples dominated *by Microcystis sp., Microcystis viridis* and *Anabaena spiroides*.

Scum samples of Missisquoi Bay were separated according to which was dominated by only one species with proportion of total cyanobacterial biomass over 90 percent and by multiple species. By comparing Figure 3.28 a) and Figure 3.28 b), the correlation between the concentration of total MC-LR eq and biomass when species with biomass over 90 percent dominated the samples was much stronger than that dominated by multiple species. This can be explained by different quantity of MC produced by a variety of MC-producing species when multiple species dominated the Bay. The variable distributions of biomass of species also can affect the levels of MC produced. In contrast, the relatively concentrated linear relationship shown in Figure 3.28 a) demonstrates that the unity of species contributes a better dynamic of cyanobacterial biomass and cyanotoxins.

The exceptions of results measured on August 21, 2001 and September 17, 2008 when *Anabaena flos-aquae* dominated the Bay were marked in Figure 3.28 a). It is understandable that *Anabaena flos-aquae* not only contributed the production of microcystins but also anatoxins in different water conditions. The linear relationship will be stronger when excluding these two points.

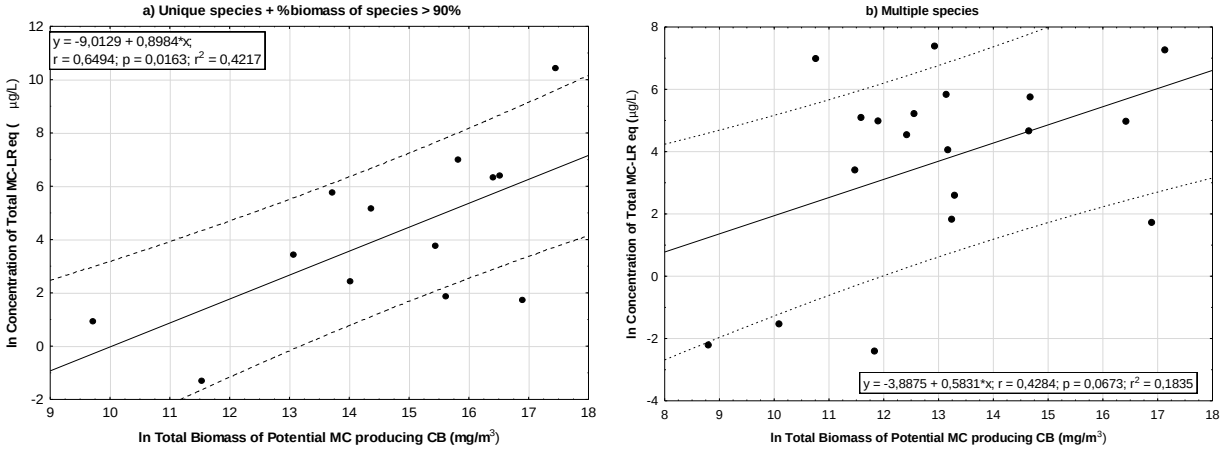


Figure 3.28: The relationship between the concentration of MC-LR eq (µg/L) and total biomass of MC producing cyanobacteria (mg/m^3) when scum samples dominated at Missisquoi Bay by: a) unique species and %biomass of species>90%; b) multiple species.

The concentration of anatoxins (µg/L) detected in scum samples in Missisquoi Bay from 2000 to 2008 varied from 0.002 µg/L to 3.1 µg/L. The maximum was measured on July 22, 2008 when *Microcystis aeruginosa* dominated the scum, but on the same day *Anabaena flos-aquae* was detected with high density of 309,741 mg/m^3. The relationship between the concentration of anatoxins and the biomass of anatoxin-producing species cannot be successfully established due to low quantity of anatoxins (even lower than the minimum measuring level) produced in Missisquoi Bay.

3.4.1.2 Cyanobacterial biomass and cyanotoxin in water samples at Missisquoi Bay

The concentrations of microcystins detected in water samples were much lower than that in scum samples in Missisquoi Bay. It is difficult to determine a clear linear relation between the MC produced and the correspondent biomass in Figure 3.29. According to the analysis of historical data, the results could be explained by several factors. At first, although MC-producing species like *Microcystis sp.* exist in non-scum samples and occasionally dominated the Bay, *Anabaena flos-aquae, Aphanizomenon flos-aquae* and *Anabaena spiroides* were the most frequent present and most dominant species in water, which potentially produce sanatoxins. At the same time, the toxin produced by *Anabaena spiroides* and *Anabaena flos-aquae* was uncertain according to different environmental situations. They also probably produce microcystins in certain situations.

Additionally, the majority of water samples were dominated by multiple species. The variation in the proportion of cyanobacterial biomass could explain the quantity of MC produced.

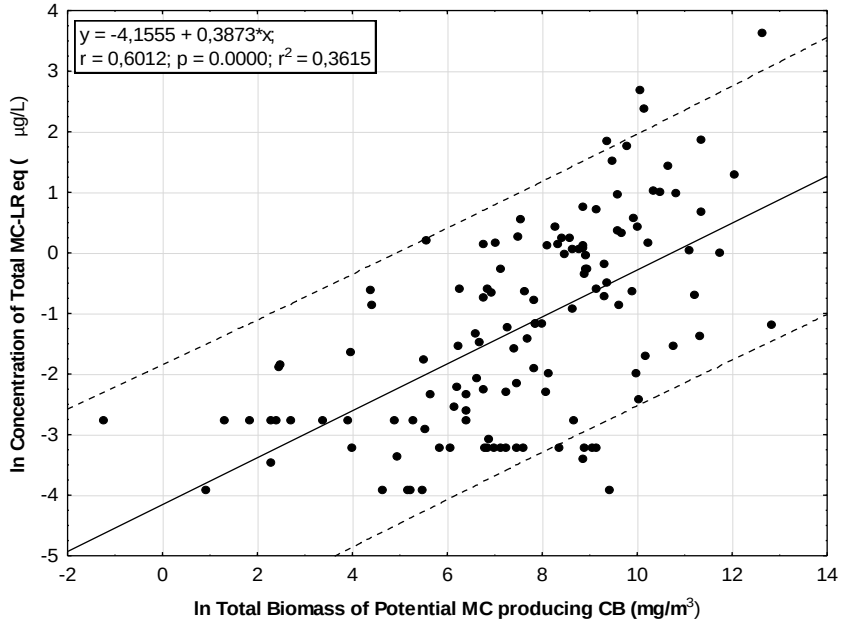


Figure 3.29: The relationship between the concentration of MC-LR eq and the biomass of MC producing cyanobacteria in water samples at Missisquoi Bay.

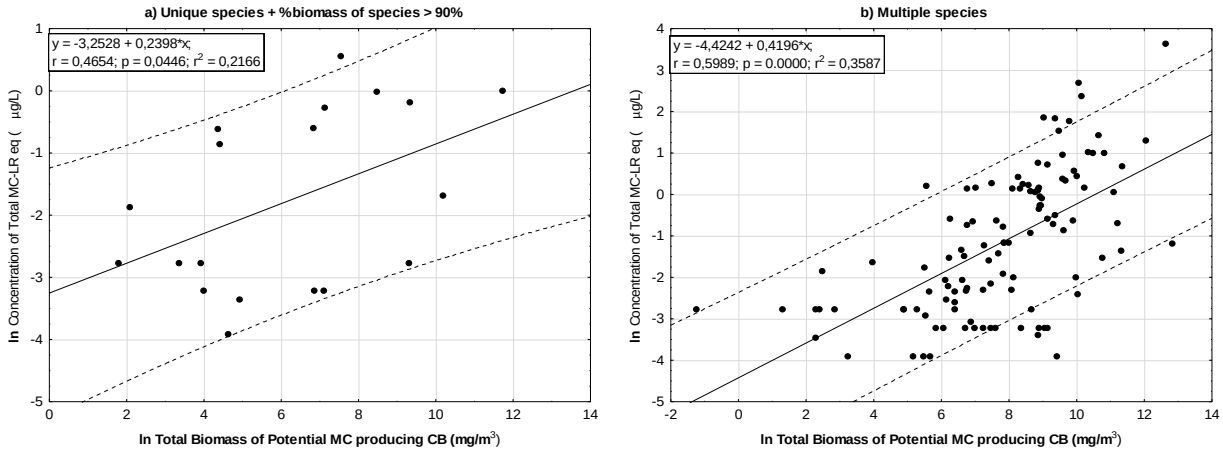


Figure 3.30: The relationship between the concentration of MC-LR eq (µg/L) and total biomass of MC producing cyanobacteria (mg/m^3) when water samples dominated by: a) unique species and %biomass of species>90%; b) multiple species.

Although anatoxins-producing species were the main dominant species in water samples collected in Missisquoi Bay over the past years, the anatoxins detected were as low as the detection limit (varied with years). Consequently, the dynamic of anatoxins production was nil or very low.

The biomass and the concentration of MC-LR eq measured in water samples were always lower than that detected in the scum. The regression of MC-LR eq concentration and the biomass were so different between the scum and water in both single and multiple dominant species situations by comparing Figure 3.28 a) with Figure 3.30a) and Figure 3.28b) with Figure 3.30 b). Their regression of scum samples is obviously higher than that of water samples. It could illustrate that the species produced much more cyanotoxins when the water was scum.

From the results showed in Figure 3.28 and Figure 3.30, the regression of MC-LR eq concentration and the biomass when samples dominated by multiple species was slightly higher than that when dominated by simple species. This could be due to the presence of species which could produce uncertain types of cyanotoxins.

3.4.2 Lake Nairne (2002-2008)

The concentrations of total MC-LR eq detected in Lake Nairne were lower than that of Missisquoi Bay. The maximum concentration was measured on October 14, 2003 with 173 µg/L MC-LR eq and the lake was 100 percent dominated by *Microcystis sp.* (Figure 3.31 a). The high concentrations of MC-LR eq were detected in Lake Nairne in 2003 and 2004 and they decreased since 2005 to less than 1 µg/L when there was scum on the lake. This decrease could be due to a change of dominant species in Lake Nairne, because the main species dominated the lake changed from *Microcystis flos-aquae* to *Aphanizomenon flos-aquae* since 2005.

Few anatoxins were detected in Lake Nairne from 2002 to 2008, and almost all results recorded were below the detection limit. Therefore, the relationship between the biomass and the concentration of anatoxins cannot be well established in Lake Nairne. Although *Aphanizomenon flos-aquae* became the dominant species even with high proportion of biomass, the concentration of anatoxins still remained on a very low level. It is reasonable to assume that when *Aphanizomenon flos-aquae* were the most dominant species; the anatoxins produced by it were always low. This point will continue to be discussed in the following lakes.

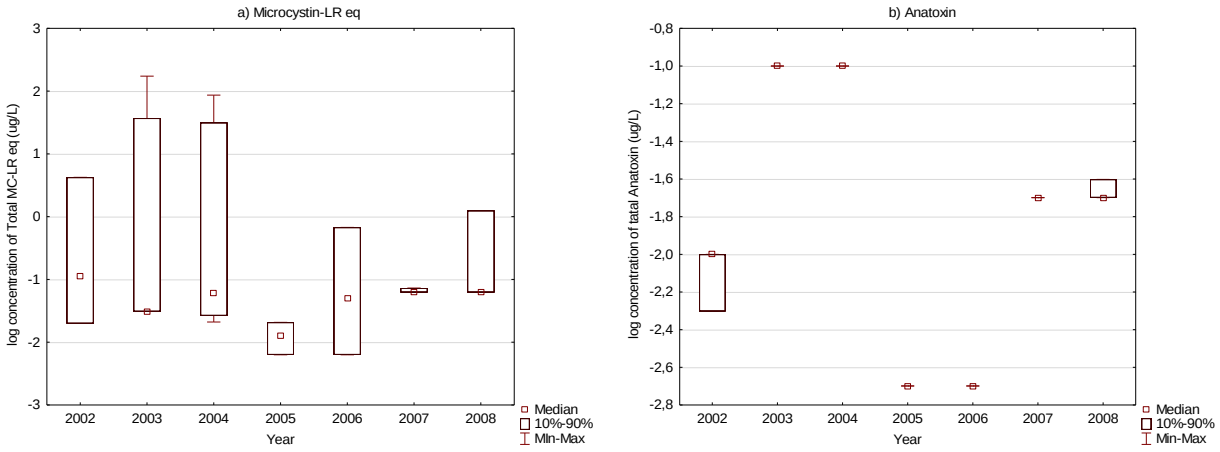


Figure 3.31: Total cyanotoxins measured from 2002 to 2008 at Lake Nairne: a) Total microcystin-LR eq; b) Anatoxins.

3.4.2.1 Cyanobacterial biomass and cyanotoxin in scum in Lake Nairne

The ratio of concentration of MC-LR eq and the MC-producing cyanobacterial biomass showed in the scum of Lake Nairne was 0.82 (Figure 3.32). This is very similar to the result of 0.76 in Missisquoi Bay (Figure 3.26 a), also in scum samples. The results could prove a strong correlation existing between the concentration of MC-LR eq and total MC-producing cyanobacterial biomass. Yet, it is necessary that this be further tested in the future.

As we mentioned above, the concentrations of anatoxins were so low and below the detection limit. The corresponding biomass and anatoxins concentration were disproportional in Lake Nairne.

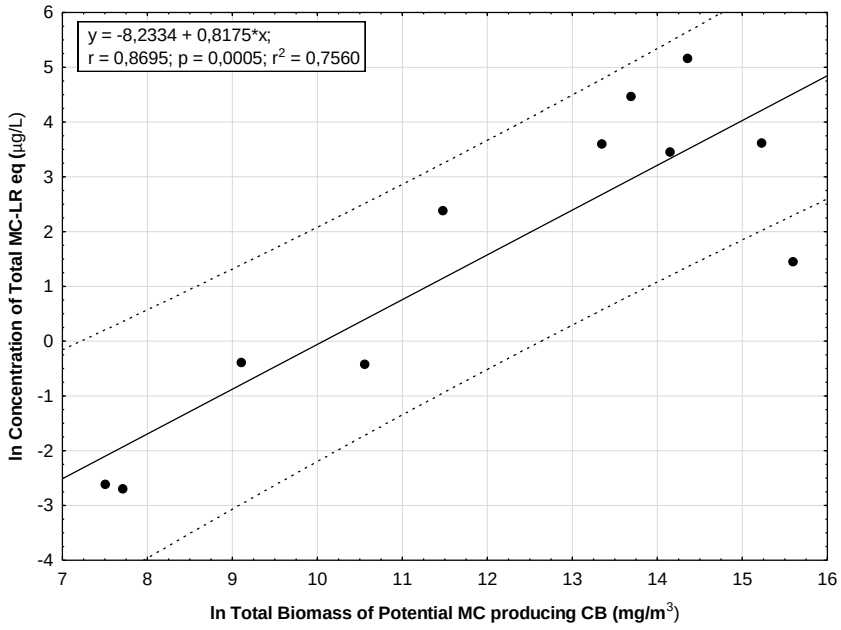


Figure 3.32: Relationship between total biomass of MC-producing cyanobacteria and total MC-LR eq measured in scum sample collected in Lake Nairne.

The highly correlated relationship between the concentration of MC-LR eq and the biomass of MC-producing species was found in scum samples dominated by unique species and the species with over 90 percent biomass (Figure 3.33a). In contrast, the linear relationship showed in scum samples dominated by multiple species was poor (Figure 3.33b). The reasons mentioned at Missisquoi Bay may also explain why the relationship in Lake Nairne was invalid. The compositions of cyanobacteria and their proportions may be the main explanation.

The ratio of concentrations of MC-LR eq and the biomass of scum samples dominated by simple species obtained in Lake Nairne was much higher than that showed in Missisquoi Bay. In other words, when biomasses were the same, the MC detected in Lake Nairne was more than that detected at Missisqoi Bay. This could be due to the majority of the samples collected in Lake Nairne were 100 percent dominated by *Microcystis sp.* which produced only microcystins. However, the samples of Missisquoi Bay in Figure 3.28 a) were dominated by *Microcystis sp.* or *Anabaena spiroides*, the later potentially produce MC and anatoxins with varied conditions.

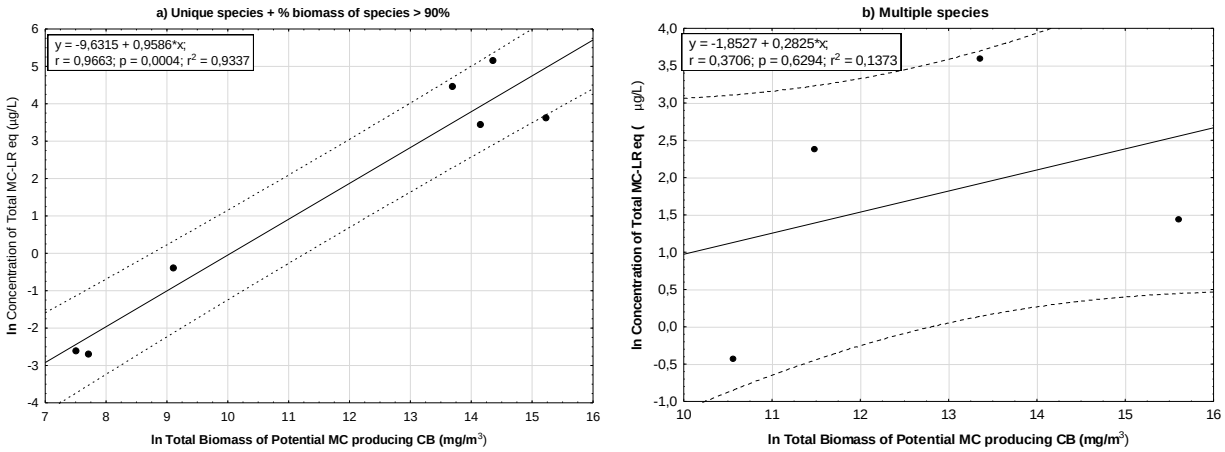


Figure 3.33: The relationship between the concentration of MC-LR eq (µg/L) and total biomass of MC producing cyanobacteria (mg/m^3) when samples dominated in Lake Nairne by: a) unique species and %biomass of species>90%; b) multiple species.

3.4.2.2 Cyanobacterial biomass and cyanotoxin in non-scum in Lake Nairne

The biomass and the concentrations of MC-LR eq and anatoxins measured in water samples in Lake Nairne were very low. The concentrations of total MC-LR eq varied from 0.02 µg/L to 0.335 µg/L (less than 1 µg/L) (Figure 3.34), although *Microcystis sp.* dominated the Lake Nairne with high proportions of biomass in 2003 and 2004. The low biomass of *Microcystis sp.* determined the low concentrations of MC produced into the lake. All the concentrations of anatoxins detected were always recorded in the same value. Thus, same as the analysis above, a relationship between the biomass and the concentration of anatoxins could not be established.

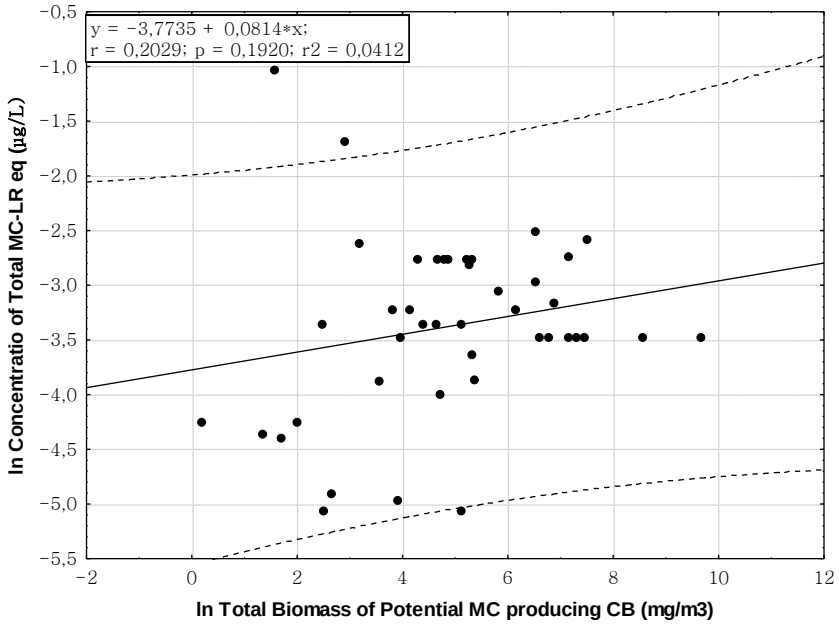


Figure 3.34: Relationship between total biomass of MC-producing cyanobacteria and total MC-LR eq measured in water samples in Lake Nairne.

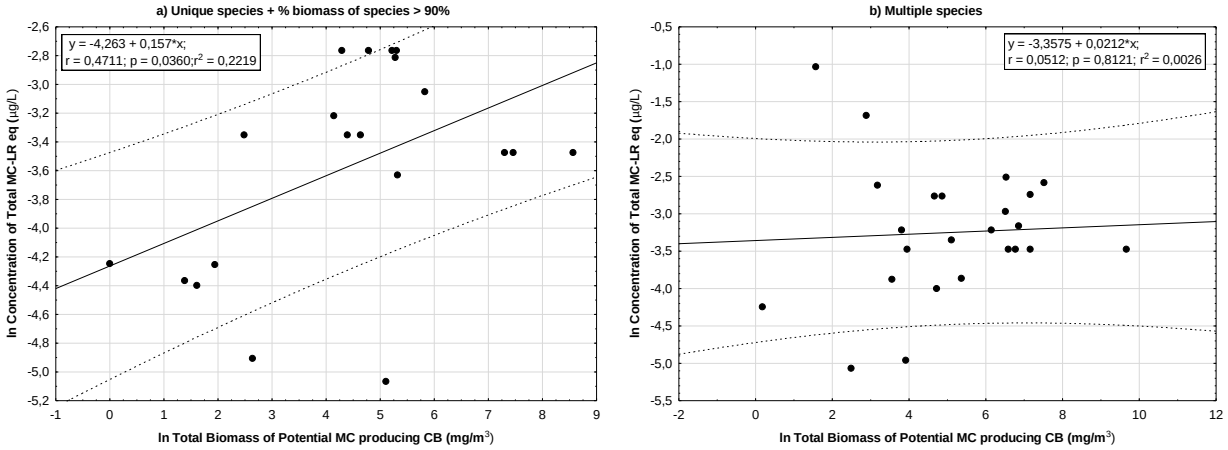


Figure 3.35: The relationship between the concentration of MC-LR eq (µg/L) and total biomass of MC producing cyanobacteria (mg/m3) when water samples dominated in Lake Nairne by: a) unique species and %biomass of species>90%; b) multiple species.

Due to the low concentrations of MC-LR eq detected in Lake Nairne, the correlation between the biomass and the concentrations of MC-LR eq was not strong, neither in samples dominated by simple species nor by multiple species. However, the relationship showed in Figure 3.35a) was slightly stronger than that in Figure 3.35b). In other words, when samples were dominated by one

species with a high proportion of biomass, the concentration of MC-LR eq had a stronger correlation with the biomass.

3.4.3 Lake Brome (2001-2003)

Lake Brome was the only lake where no scum was observed during the monitoring years. There were only two values of concentrations of MC-LR eq detected in Lake Brome which were 0.02 µg/L and 0.04 µg/L. The measurement of concentrations determined that the relationship between the concentrations of MC-LR eq and the biomass cannot be well identified, because the biomass varied on every monitoring day.

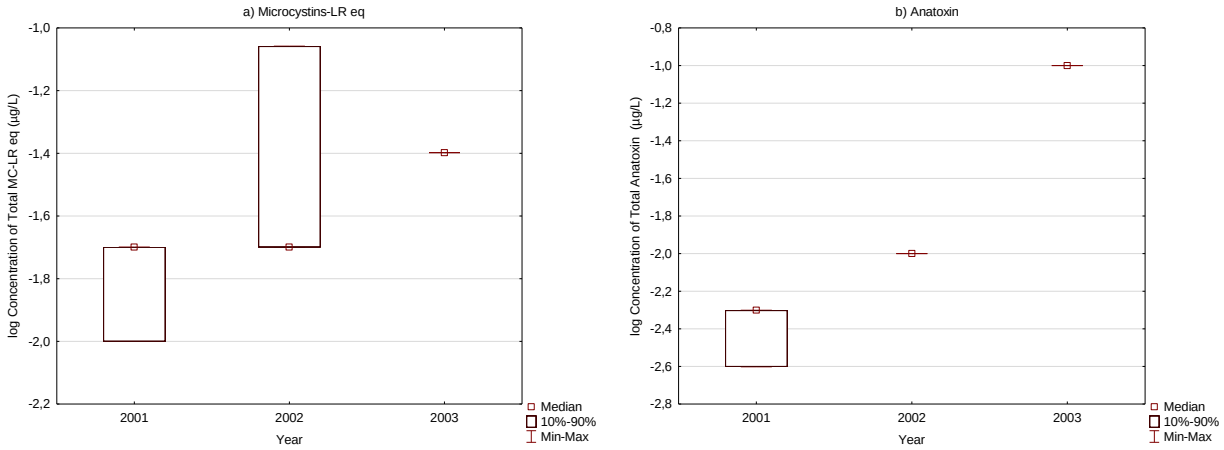


Figure 3.36: Total cyanotoxins measured from 2001 to 2003 in Lake Brome: a) Total microcystin-LR eq; b) Anatoxin.

The concentrations of anatoxins detected in Lake Brome also remained at a minimum value (detection limit). Consequently, there was no well-defined relationship between the biomass and the concentrations of MC-LR eq or concentrations of anatoxin in Lake Brome.

3.4.4 Lake William (2000-2003)

Potential anatoxins-producing species *Aphanizomenon flos-aquae* dominated Lake William in almost all monitoring years. There is no doubt that the concentrations of MC-LR eq were at a very low level. Contrary to the MC-LR, although the concentrations of anatoxins were still low other than those in 2001, this is the only lake where the concentrations of anatoxin surpassed the value of MC-LR (Figure 3.37). The maximum concentration of anatoxin was 8.22 µg/L on July 17, 2001 which was the highest anatoxin detected in all the lakes.

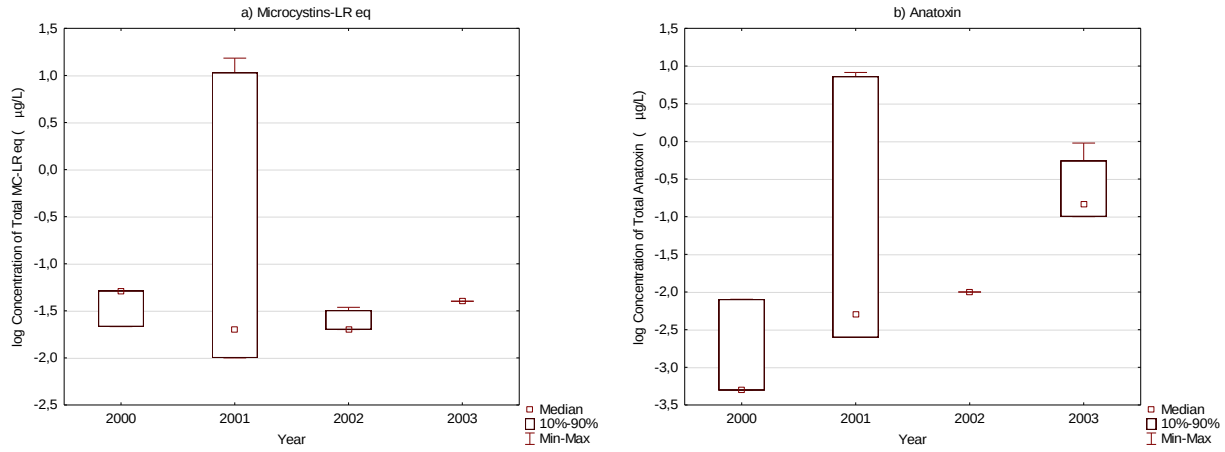
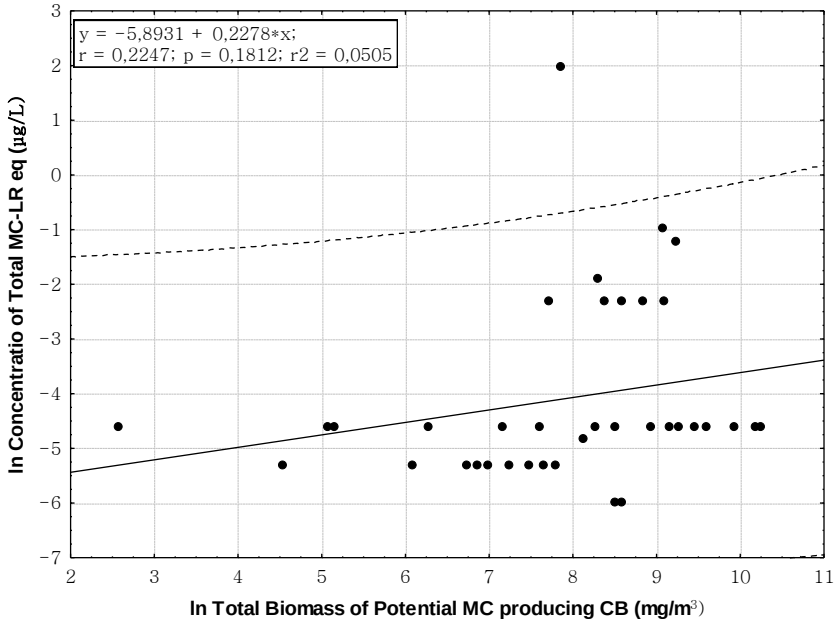


Figure 3.37: Total cyanotoxins measured from 2000 to 2003 in Lake William: a) Total microcystin-LR eq; b) Anatoxin.



Figur 3.38: The relationship between the concentration of anatoxin (µg/L) and the biomass of anatoxin-producing cyanobacteria in water samples in Lake William.

Scum samples from Lake William were all dominated by *Aphanizomenon flos-aquae*, but the maximum concentration of anatoxin was not detected in scum samples. On the contrary, the maximum concentration of anatoxin was found in the water sample which was dominated by *Anabaena flos-aquae*. It illustrated that *Anabaena flos-aquae* were more toxic than *Aphanizomenon flos-aquae*. On the other hand, it is reasonable to assume that the presence of

Aphanizomenon flos-aquae will not affect the relationship between the concentrations of MC-LR eq and the biomass of MC producing species. Figure 3.38 showed the relationship between the concentrations of anatoxin and the biomass of anatoxin-producing cyanobacteria in water samples in Lake William.

3.5 Comparison of different potentially toxic cyanobacteria dominated in four lakes

Due to the low concentrations of anatoxin detected in four lakes, the goal of this section is to analyze and compare the relationships between the concentrations of MC-LR eq and the biomass of MC producing species when different potentially toxic cyanobacteria dominated the lakes. At first, the analysis was separated by species. Samples were distinguished according to the most dominant species and probably other toxic species present in samples.

The strongest correlation between the concentrations of MC-LR eq and the biomass of MC producing cyanobacteria was identified when the samples were dominated by *Microcystis sp*. (*Microcystis flos-aquae, Microcystis viridis and Microcystis aeruginosa*, etc.) in scum samples of four lakes (Figure 3.39a). Although *Aphanizomenon flos-aquae* which potentially produce anatoxin dominated the samples, as we assumed above, the presence of *Aphanizomenon flos-aquae* seems to have no influence on other MC-producing species with their production of microcystins. Thus, the linear relationship in Figure 3.39 c) showed a relatively high correlation. *Anabaena flos-aquae* was microcystins and anatoxins-producing species. The variations of cyanotoxins in certain situations are reflected in the low linear relationship with relatively poor correlation between the concentrations of MC-LR eq and the biomass as illustrated in Figure 3.39 b.

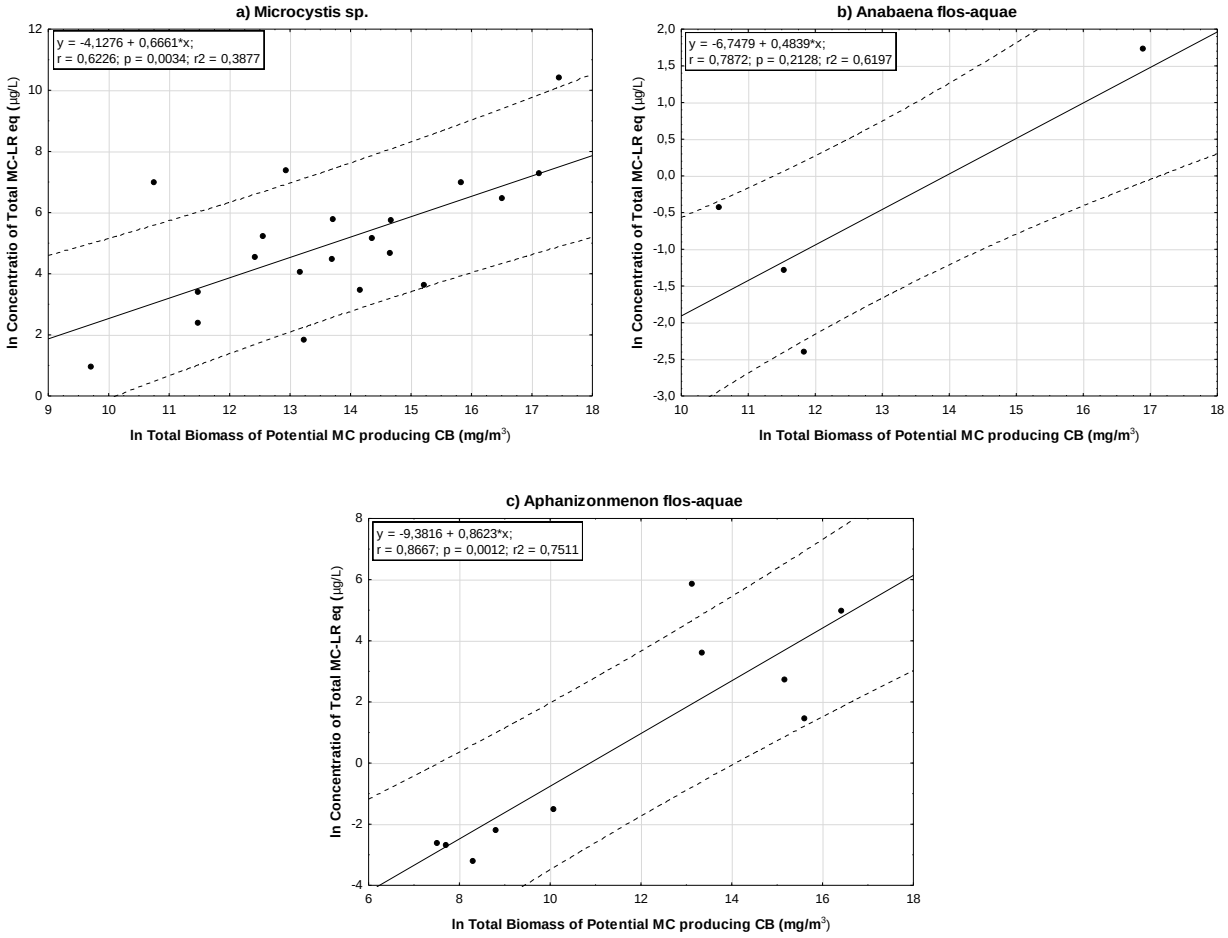


Figure 3.39: The relationship between the concentrations of MC-LR eq and the biomass of MC producing cyanobacteria when the scum samples dominated by: a) *Microcystis sp.* (*Microcystis flos-aquae, Microcystis viridis* and *Microcystis aeruginosa*, etc.); b) *Anabaena flos-aquae* and c) *Aphanizomenon flos-aquae*.

Similarly, the best correlation between the concentrations of MC-LR eq and the biomass with relatively high linear relationship (R2=0.41; p=0.000000) was shown in Figure 3.40a when water samples dominated by *Microcystis sp.* Comparing Figure 3.39 with Figure 3.40, the relationship between the concentration of MC-LR eq and the biomass of MC-producing cyanobacteria has a high similarity in scum samples and water samples, regardless of which species dominated the samples.

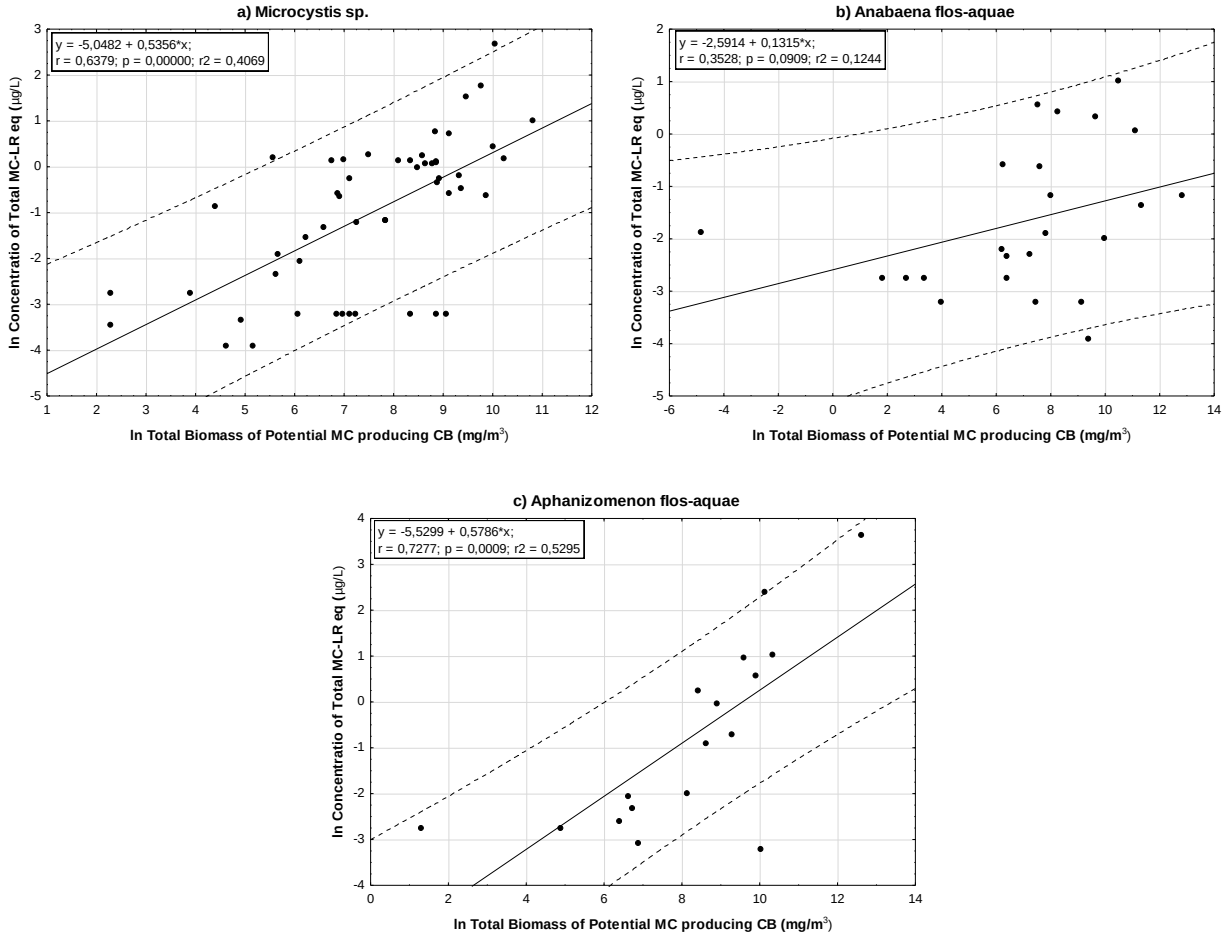


Figure 3.40: The relationship between the concentrations of MC-LR eq and the biomass of MC producing cyanobacteria when the water samples dominated by: a) *Microcystis sp*. (*Microcystis flos-aquae, Microcystis viridis* and *Microcystis aeruginosa*, etc.); b) *Anabaena flos-aquae* and c) *Aphanizomenon flos-aquae*.

3.6 The advantages of probes application

The data of concentration of cyanobacteria provided by MDDEP was analyzed in cyanobacterial biomass (mg/m^3) and cell density (cells/m). By using biomass, results are showing a clearer relationship of cyanobacteria abundance and the microcystins. However, because cyanobacteria cell volumes can vary significantly in size, the use of cyanobacterial biovolume rather than cyanobacterial biomass are more standardized regardless of cell size (Brient et al., 2008).

Threshold values used by the authorities are expressed in the number of CB cells, the biovolume of CB and the pigment concentration (e.g. World Health Organization thresholds for Alert Level 2 are 100,000 cells/mL, biovolume of 10 mm^3/L or 50 µg/L Chl*a*) (Ingrid Chorus & Jamie Bartram, 1999; Zamyadi et al., 2011). Biovolume measurement is the most common approach to determining the risk provoked.

Furthermore, the WHO estimates that Microcystis's maximum potential microcystin content is 200 fg/cell (or 0.2 pg/cell), based on field samples when a bloom is dominated by the genus and has a density higher than 100 000 cells/mL (J. B. Falconer et al.). This is the highest documented cellular quota in the literature and was therefore used to calculate the 'worst case scenario' of microcystin production (N. McQuaid, et al., 2011). The maximum potential microcystin concentration (MPMC) of each water sample was determined by multiplying the maximum microcystin production per Microcystis biovolume (pg μm^{-3}) (eqn (1)) by the Microcystis biovolumes sampled (N. McQuaid, et al., 2011).

(0.2 pg of *microcystin*)/(1 cell *Microcystis sp.*)*(1 cell *M. flos-aquae*)/(14.1 mm^3)= 0.014 pg μm^{-3} (1)

Table 3.6 indicates the maximum potential microcystin concentrations corresponding to cyanobacterial biovolume thresholds.

Table 3.6: Maximum potential microcystin concentrations corresponding to cyanobacterial biovolume thresholds (adapted from (N. McQuaid, et al., 2011)).

	Cyanobacterial biovolume threshold (mm^3/L)	Maximum potential microcystin concentration (µg/L)
Alert Level 1 (biovolume)*	**0.2**	**2.6**
Alert Level 2 (biovolume)*	**10**	**130**

*(I. Chorus & J. Bartram, 1999).

According to Table 3.6, the maximum potential microcystin concentration (µg/L) is correlated to the biovolume measured. In addition, the biomass of each species is not easily determined. Therefore, the biomass used is not a good indicator for estimating the concentration of potential microcystin. Further research is needed to give considerations to the correlation between the

biovolume and the concentration of microcystin. Given that, the estimation of microcystin is more accurate by using the probes which measure biovolumes in *situ* quicklys.

CONCLUSION

The variation in cyanobacterial-dominant species shows a large spatial-temporal difference, thus making it difficult to determine which species dominate one lake in a certain seasonal cycle. The dominant species were changing even in the same lake in different years. However, several species were always present in these four lakes with abundant biomass based on historical monitoring data.

The main species present in the four lakes were *Microcystis sp., Anabaena flos-aquae and Aphanizomenon flos-aquae*. *Anabaena flos-aquae* usually appeared in the beginning of season in a very low biomass. Subsequently, *Microcystis sp*. and *Aphanizomenon flos-aquae* proliferate rapidly in mid-August. *Aphanizomenon flos-aquae* substained in September, even persisting until October. The frequency of these three species is relatively high, but the dominant species changed in different waters and seasonal cycles. However, the dominant species in scum is simpler and clearer than that in water according to the composition measured. The composition of cyanobacterial species in water was complex and without stability as compared to scum. The bloom of one or a few species dominating in scum sample was always accompanied by a massive abundance of biomass and suppressed the propagation of other cyanobacterial species.

When the water was dominated by one species, the biomass of toxic cyanobacteria and cyanotoxin detected showed a relatively high linear relationship. Especially in scum samples, when *Microcystis sp.* or other MC producing cyanobacteria dominated the water, the relationship between the biomass and the concentration of MC-LR eq measured was high and worth mentioning. *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* were both anatoxin-producing cyanobacteria and the main dominant species in the four lakes, but the concentration of anatoxin was always low, sometimes even lower than the limit of detection. This demonstrates that the toxicity of *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* are not strong.

The cyanobacterial biomass and the concentration of cyanotoxins detected along the shore were much higher than those measured in the center of the lake. This was likely caused by waves and wind, or the direction of water flow. When the water resource was dominated by MC-producing cyanobacteria with a high abundance, the concentration of MC-LR eq detected was also high. The biomass of MC-producing cyanobacteria monitored can indicate the concentration of MC-LR eq to a certain extent. This conclusion cannot be used for anatoxin-producing

cyanobacteria, because of high anatoxin-producing cyanobacteria biomass and low anatoxin detection.

The dominant cyanobacterial species can be further studied with more complete monitoring parameters such as turbidity, pH, and temperature of water resources, all of which can probably indicate the environment most suitable for certain species. The biovolume can be considered as an indicator to discuss the microcystins detected in the future studies.

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APPENDICES

APPENDIX 1 – Potentially toxic species of cyanobacteria and their detected toxins.

List of potentially toxic species of cyanobacteria and their associated toxins (N.I : Toxin was present but not identified) (adapted from : (Zamyadi & Prévost, 2007)) (Agence Française de Sécurité Sanitaire des Aliments (AFSSA) & Agence Française de Sécurité Sanitaire de l'Environnement et du Travail (AFSSET), 2006)

Species	Toxins	Species	Toxins
Anabaena affinis	N.I.	*Nodularia spumigena*	Nodularins
Anabaena circinalis	Anatoxin-a, Saxitoxins, Microcystins	*Nostoc paludosum*	N.I.
Anabaena flos-aquae	Anatoxin (-a, -a(s), -b,-b(s), -c, -d), Microcystins	*Nostoc rivulare*	N.I.
Anabaena hassallii	N.I.	*Nostoc sp.*	Microcystins
Anabaena lemmerman	Microcystins, Anatoxin-a(s)	*Oscillatoria Formosa*	Homoanatoxin-a
Anabaena planktonica	Anatoxin-a	*Oscillatoria lacustris*	N.I.
Anabaena spiroides	Anatoxin-a, Microcystins	*Oscillatoria limosa*	Microcystins
Anabaena torulasa	N.I.	*Oscillatoria tenuis*	Microcystins
Anabaena variabilis	N.I.	*Oscillatoria nigroviridis*	Oscillatoxin-a
Anabaena sp.	Anatoxin-a	*Oscillatoria sp.*	Anatoxin-a
Aphanizomenon flos-aquae	Anatoxin-a, Saxitoxins	*Phormidium favosum*	Anatoxin-a
Aphanizomenon ovalisporum	Cylindrospermopsin	*Planktothrix agardhii*	Microcystins
Aphanizomenon sp.	Anatoxin-a	*Planktothrix mougeotii*	Microcystins
Coelosphaerium naegelianum	Hepatoxin	*Planktothrix rubescens*	Microcystins
Cylindrospermopsis raciborskii	Cylindrospermopsin, Saxitoxins	*Planktothrix sp.*	Anatoxin-a
Cylindrospermum sp.	Anatoxin-a	*Pseudanabaena sp.*	Neurotoxin
Fischerella epiphytica	N.I.	*Raphidiopsis sp.*	Cylindrospermopsin

List of potentially toxic species of cyanobacteria and their associated toxins (N.I : Toxin was present but not identified) (adapted from : (Zamyadi & Prévost, 2007)) (Agence Française de Sécurité Sanitaire des Aliments (AFSSA) & Agence Française de Sécurité Sanitaire de l'Environnement et du Travail (AFSSET), 2006) (suite)

Species	**Toxins**	**Species**	**Toxins**
Gloeotrichia echinulata	N.I.	*Schizothrix calciola*	Aplysiatoxin
Gloeotrichia pisum	N.I.	*Scytonema hofmanni*	Scytophycins a et b
Hapalosiphon hibernicus	Microcystins	*Scytonema pseudohofmanni*	Scytophycins a et b
Lyngbya birgei	N.I.	*Spirulina subsalsa*	N.I.
Lyngbya gracilis	Debromoaplysiatoxin	*Symploca hydnoides*	N.I.
Lyngbya major	N.I.	*Symploca muscorum*	Aplysiatoxin
Lyngbya majuscule	Lyngbyatoxin-a	*Synechococcus sp.*	N.I.
Lyngbya wollei	Saxitoxins	*Trichodesmium erythraeum*	Neurotoxin
Microcoleus lyngbyaceus	N.I.	*Umezakia natans*	Cylindrospermopsin
Anabaenopsis milleri	Microcystins	*Woronichinia naegeliana anciennement* *Gomphosphaeria naegelianum*	Anatoxin-a

APPENDIX 2 – Limit concentration (µg/L) of detection (LOD) of the toxins analyzed per year of sampling DSEE (MDDEP).

Years of sampling / Toxins	2001	2002	2003	2004 (before 9th August)	2004 (Since 19th August)
MC-LR	0,005	0,005	0,02	0,02	0,010
MC-RR	0,1	0,1	0,1	0,05	0.1
MC-YR	0,005	0,005	0,01	0,01	0.1
Anatoxin-a	0,005	0,01	0,1	0,10	0,10