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CYANOBACTERIA IN SURFACE AND BANK FILTERED DRINKING WATER SOURCES: APPLICATION OF PHYCOCYANIN PROBES FOR MONITORING BLOOMS

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DEDICATION

To Majzoob.

My dad, my mom

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RÉSUMÉ

Les cyanobactéries sont une préoccupation pour les sources d'eau potable étant donné les impacts anticipés des changements climatiques et de l'eutrophisation des plans d'eau. L'objectif principal de ce projet de Maitrise était de proposer un système rapide, à faible coût pour le suivi des cyanobactéries dans les sources d'eau potable. Plus précisément, l'objectif était d'évaluer la précision des sondes à phycocyanine (PC) pour le suivi des cyanobactéries en continu. Pour valider la sonde de PC pour mesurer les concentrations des cyanobactéries et l'abondance du phytoplancton, les résultats de sonde ont été comparés à des comptes cellulaires taxonomiques provenant d'échantillons choisis de l'eau brute.

Le système de suivi a été validé à deux sites, l'un sur le lac Érié en Ontario et l'autre au sud du Québec. Les deux sites sont des sources d'eau potable touchées par des fleurs de cyanobactéries récurrentes. La validation des mesures de la sonde (en Ratio d'Unités de Fluorescence ou « Ratio Fluorescence Units » en Anglais (RFU)) a été effectuée pour comparer les concentrations et biovolumes de cyanobactéries et de phytoplancton. Les échantillons choisis provenaient de l'eau brute d'une prise d'eau sur le lac Érié et un système d'approvisionnement en eau potable traitée par un massif filtrant au sud du Québec. Les analyses de corrélation et de régression ont été réalisées en comparant les biovolumes de cyanobactéries et les résultats des mesures à l'aide de la sonde de PC. Les mesures de la sonde étaient significativement corrélées aux biovolumes cyanobactériens aux deux sites ($r > 0,6$, $p < 0,00001$).

Les résultats des deux sites montrent que les concentrations de cyanobactéries étaient souvent faibles. Un seuil d'alerte a été déterminé en fonction des résultats de cette étude et comparé aux résultats d'études précédentes. La sonde de PC a également été utilisée pour estimer l'efficacité du massif filtrant par la mesure des valeurs RFU de deux lacs et l'eau de puits passée par le massif filtrant. Les sondes de PC sont des outils de suivi utiles qui peuvent être appliqués pour le contrôle de la qualité de l'eau potable lors de proliférations de cyanobactéries menant à des conditions d'eau brute variant rapidement.

ABSTRACT

Cyanobacteria are a growing concern in drinking water sources given rising temperatures from global climate change and increasing eutrophication of water bodies. The main goal of this project was to propose a rapid, low cost monitoring system by applying an in situ phycocyanin in drinking source waters. More specifically, the objective was to evaluate the accuracy of phycocyanin (PC) probes for monitoring cyanobacteria in source waters. Continuous monitoring was conducted using an online fluorescence probe to measure phycocyanin. To validate the PC probe for cyanobacteria and phytoplankton abundance results were compared to traditional taxonomic cell counts from raw water grab samples. Two field sites, one on Lake Erie in Ontario and the other in Southern Québec, Canada were used to test the proposed monitoring system. Validation of probe measurements (in Ratio Fluorescence Units (RFU)) was conducted to compare cyanobacteria density, cyanobacteria biovolume, phytoplankton density and phytoplankton biovolume using grab samples from field sites that included intake water (Lake Erie) and surface waters and well water that passed through bank filtration (Southern Québec). Correlation and regression analyses were conducted between cyanobacterial cell biovolumes and *in situ* cyanobacteria measurement results using the PC probe. Probe measurements were significantly correlated to cyanobacterial biovolumes at both field sites ($r>0.6$, $p<0.00001$). Results from both sites show that cyanobacteria concentrations were often low, and an alert threshold was determined based on results from this study and compared to results from previous investigations. At the field site in Québec, the PC probe was also used to estimate the efficiency of bank filtration through the measurement of RFU values from two lakes and bank filtered well water. PC probes are useful monitoring tools that can be applied to monitor the quality of drinking water sources and their rapidly varying raw water conditions during blooms.

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LIST OF SYMBOLS AND ABBREVIATIONS

The list of symbols and abbreviations presents the symbols and abbreviations used in the thesis or dissertation in alphabetical order, along with their meanings. Examples:

CB: Cyanobacteria

BF: Bank Filtration

MC-LR: Microcystin-LR

PC: Phycocyanin

MPMC: Maximum Potential Microcystin Concentration

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CHAPTER 1 INTRODUCTION

As a result of global change and the disruption of key elemental cycles (C, N, P), large blooms of cyanobacteria in freshwater systems have become more frequent (Taranu et al., 2015) Of particular concern are the toxins produced by cyanobacteria; however, their presence can also disrupt drinking water treatment and lead to non-consumption advisories (Zamyadi et al., 2014). In order to respond quickly to the presence of large numbers of cyanobacterial cells in a drinking water source, monitoring tools and strategies are needed. Since the identification and quantification of cyanobacteria are highly costly and time consuming, on-line continuous monitoring using phycocyanin probes have been conducted recently (Brient et al., 2008; Kahlert & McKie, 2014; Kong et al., 2014; McQuaid et al., 2011; Zamyadi et al., 2012 b). Online monitoring tools also enable rapid and reliable process monitoring. A phycocyanin alert level threshold has been proposed for early warning systems for the operation of drinking water treatment plants (McQuaid et al., 2011). However, these probes and thresholds have only been tested at a limited number of sites and their more widespread use requires further testing.

In recent years, cyanobacteria blooms have been occurring in the western basin of Lake Erie. The presence of cyanobacteria and their toxins in drinking water sources has raised the question of the vulnerability of treatment plants to cyanobacteria blooms in the western basin of Lake Erie. This research is part of a study funded by the Canadian Water Network (CWN) and aims to provide information that can be used for the vulnerability assessment of a drinking water treatment plant affected by cyanobacterial blooms using phycocyanin probes.

Cyanobacteria and cyanotoxins are generally a problem for surface water sources of drinking water. However, groundwater systems could potentially also be vulnerable to cyanobacteria if they are under the influence of surface waters. Bank filtration is an example of a groundwater source of drinking water that could potentially be impacted by cyanobacteria. This Master's thesis also explores the use of probes for monitoring bank filtered drinking water sources.

Toxin-producing cyanobacteria have become dominant members of bacterial communities during both the summer and early fall period as visible blooms have appeared in surface waters. This has led the public health advisories and a guideline value for microcystin concentrations for drinking water. In Canada, Federal-Provincial-Territorial Committee on Drinking Water proposed, last updated in 2002, a maximum acceptable concentration of 0.0015 mg/L (1.5 µg/L) of total

microcystins in drinking water (Health Canada, 2016). The U.S. Environmental Protection Agency has introduced regulations for microcystins based on drinking water ingestion rates (L/kg/day) for each age group. The EPA has developed two health advisory values related to body weight and drinking water intake by age. The estimated ten-day health advisory values for children less than 6 years old is 0.3 µg/L and 1.6 µg/L for children older than or equal to 6 years (U.S. Environmental Protection Agency, 2015).

Thesis organization

In this thesis, a summary of the literature on the occurrence of cyanobacteria and the application of *in vivo* fluorescence probes is provided (Chapter 2). Chapter 3 presents the objectives of the research. Two chapters are issued to the methodology and results. The methodology of the research project is presented in Chapter 4. Chapter 5 presents the results from the Lake Erie monitoring project and Chapter 6 present an article submitted for publication on the breakthrough of cyanobacteria in bank filtration and the use of probes for monitoring bank filtered drinking water sources. Chapter 7 provides a general discussion of results from both field sites, notably, the performance of online monitoring using phycocyanin probes to establish local alert levels for taking action to avoid the breakthrough of cyanotoxins in treated drinking water. Finally, Chapter 8 provides the conclusions and recommendations for monitoring cyanobacteria in vulnerable drinking water sources.

CHAPTER 2 LITERATURE REVIEW

The occurrence of cyanobacteria in drinking water sources challenges water treatment utilities, as they can disrupt conventional drinking water treatment processes, cause taste and odour problems through the production of geosmin and methylisoborneol (MIB) (Graham et al., 2012) and lead to toxins breaking through into treated water (Zamyadi et al., 2012 a). Cyanobacteria blooms have a major impact on the quality of surface waters and have been shown to be becoming more frequent, largely related to changes in nitrogen and phosphorus concentrations (Taranu et al., 2015). There are many types of harmful algae or phytoplankton with major groups belonging to dinoflagellates, diatoms and cyanobacteria. Almost forty species of cyanobacteria have the ability to produce various types of toxins (Codd et al., 2005). The production of cyanotoxins in water bodies depends on environmental variables such as temperature, light intensity, nutrient enrichment, the wind and turbulence of water (Muruga et al., 2014; Paerl et al., 2011) and the formation of high density colonies in scums has been associated with the upregulation of cyanotoxin production genes (Wood et al., 2011). In eutrophic freshwater bodies, the cyanobacteria group can be the dominant phytoplankton, although eutrophic conditions are not required for their dominance (Molot, 2010).

Although human deaths from direct cyanotoxin exposure have only been noted in one instance attributed to cyanotoxins in water used for dialysis in Brazil (Azevedo et al., 2002), numerous companion animal and livestock deaths have been documented (Hilborn & Beasley, 2015) Cyanotoxins are capable of affecting the nervous system, the liver and the skin of exposed individuals (Davis et al., 2009; Komárek & Komárková, 2002; Long et al., 2001; Pantelić et al., 2013). In Appendix A, a list of common toxin-producing cyanobacteria species and their health effects is provided. More than 80 types of microcystins have been characterised and among all of them, microcystin-LR has been most commonly identified in the literature (WHO, 1999). Other common types of cyanotoxins include anatoxin-a and saxitoxins, as well as cylindrospermopsin (Otten & Paerl, 2015).

Cyanotoxins can be present in both intracellular and extracellular form, with most microcystins generally observed to be intracellular (McQuaid et al., 2011), although the ratio of extracellular to intracellular increases during a bloom season (Grützmacher et al., 2002). In addition, extra-cellular toxins can be adsorbed to suspended sediments and would therefore be found in the particulate phase of natural systems (Maghsoudi et al., 2015). Even low concentrations of cyanobacterial cells

have been shown to accumulate within various drinking water treatment processes leading to high toxin concentrations (Zamyadi et al., 2013 d).

The limitation of various treatment processes to reduce the occurrence of cyanotoxins and cyanobacteria has been observed (Newcombe et al., 2010; Zamyadi et al., 2013 c). Various physiochemical and biological approaches are being used to monitor the efficiency of drinking water treatment processes for the removing cyanobacterial cells and toxins. In this field, There are five common methods for quantifying toxic cyanobacteria: measuring toxin concentrations, chlorophyll *a* (Chl *a*) concentrations, cell counts, quantitative real-time PCR (qPCR), and remote sensing (Bridgeman et al., 2013). The gold standard approach involving the measurement of cyanobacterial cells through microscopy is time consuming and expensive. Thus, other approaches such as the use of probes are growing more common (Srivastava et al., 2013).

2.1 Cyanobacteria monitoring guidelines for drinking water production

A review and summary of guidance documents that consider cyanobacteria monitoring and risk assessment are provided in Table 2.1. The guidelines provide a risk management framework for improving the safety of drinking water with regards potentially hazardous concentrations of cyanotoxins. Table 2.1 summarizes alert level frameworks based on three type of measurements of cyanobacterial concentrations including cell numbers, biovolumes or pigment concentrations (e.g. chlorophyll *a* attributable to cyanobacteria or a specific cyanobacterial pigment detected by fluorometry such as phycocyanin pigment) (Preez & Van Baalen, 2006). The alert levels, their thresholds and actions to be taken are also provided and consider various monitoring approaches including taxonomic counts and the use of phycocyanin probes. The main actions to reduce the risk of cyanotoxins in drinking water focus on the characterisation of the source waters before and after the abstraction, and the optimization of subsequent treatment processes (du Preez et al., 2006).

The World Health Organization (WHO)'s framework is the basis of several others and provides three alert levels and strategies consisting of Vigilance Level, Alert Level 1 and Alert Level 2 (WHO, 1999). Following the WHO management framework, other organizations and authors have developed or suggested action plans to be implemented before and during a cyanobacteria bloom

in source waters. Some actions include cyanobacteria identification and enumeration, measurement of cyanotoxins, and mouse test bioassays (Ingrid Chorus, 2012).

Table 2-1: Alert level frameworks for cyanobacteria in source and recreational waters

Alert level	Threshold Definition	Recommended Actions	Reference
Detection Level	$\geq$ 500 & < 2,000 cells mL cyanobacteria (Individual species or combined total of any cyanobacteria)	Regular monitoring, Weekly sampling and cell counts	(Newcombe et al., 2010)
Alert Level 1 MEDIUM ALERT (30-50% of total CB are potentially toxic)	2,000 cells $mL^{-1} \leq$ CB < 6,500 cell mL^{-1} *Microcystis aeruginosa* or, 0.2 $mm^3L^{-1} \leq$ CB total biovolume < 0.6 mm^3L^{-1} (as dominated by CB potentially toxic)	2 weekly sampling at off take and at representative locations in reservoir, toxin monitoring	
Alert Level 2 HIGH ALERT (the cyanobacteria present are known toxin-producers.) (Established bloom of cyanobacteria with the potential for toxin concentration)	6500 cells/mL $\leq$CB <65000 cells/mL of *Microcystis aeruginosa* or total CB biovolume 0.6 mm^3L^{-1} < CB <6 mm^3L^{-1} (as dominated by CB potentially toxic), visible CB bloom with high concentration of potentially toxic CB and exceeded toxin concentration guideline, Ineffective treatment	health risk assessment considering toxin monitoring data, assessing the effectiveness of available treatment, continue monitoring as per Level 1, Toxin monitoring of treated water may be required	
Alert Level 3 VERY HIGH ALERT toxin concentration > 10x guideline concentration for microcystin.	$\geq$ 65,000 cells m L^{-1} *Microcystis aeruginosa* or the total biovolume of all cyanobacteria > 6 mm^3/L, May exposed in treated water without effective treatment	Immediate notification of health authorities if this has not already occurred at Level 1 or 2, Advice to consumers if the supply is unfiltered, toxin monitoring in source water and drinking water supply	
Alert Level 1	500 to 2000 cells mL^{-1}	Altering off-take depth	(Burch et al., 1993)
Alert Level 2	2000 to 15000 cells mL^{-1}	Deployment of booms	
Alert Level 3	> 15000 cells mL^{-1} (potentially toxic CB)	Use of PAC	

Table 2-1: Alert level frameworks for cyanobacteria in source and recreational waters. (continued)

Alert level	Threshold Definition	Recommended Actions	Reference
Vigilance Level	> 2000 cells/mL CB, Chl *a* >1 µg L^{-1}, or > 0.2 $mm3L^{-1}$	Increase in monitoring activities and inspection of the source water at the intakes	(WHO, 1999)
Alert Level 1	2000 cells/mL< CB<100000 cells/mL, 1 µg L-1 <Chl a <50 µg L-1, or 0.2 $mm3L^{-1}$< CB<10 $mm3L^{-1}$	Cyanotoxin monitoring, assessment of the capacity of treatment to remove cyanobacteria and cyanotoxins, early communications with public health authorities	
Alert Level 2	CB >100000 cells/mL, Chl a >50 µg L^{-1}, or CB>10 $mm3L^{-1}$ CB are potentially toxic	Monitoring program, treatment optimizations, consideration of activating alternative water supply plans, more extensive media releases	
Detection Level	CB > 500 cells mL^{-1}	-	(Burch et al., 2003)
Alert Level 1	2000 cells mL-1< CB < 5000 cells mL-1	-	
Alert Level 2	5000 cells mL^{-1}< CB < 50000 cells mL^{-1}	-	
Alert Level 3	CB > 50000 cells mL^{-1}	-	
Vigilance Level	Cyanobacteria > 2000 cells/mL	Routine monitoring	(Preez & Van Baalen, 2006)
Alert Level 1	2000 cells/mL< CB<100000 cells/mL	Phytoplankton identification and enumeration cyanotoxin analysis, mouse bioassay	
Alert Level 2	CB >100000 cells/mL 0.8 µg L^{-1} <microcystin <2.5 µg L^{-1}	Treatment optimisation, continuation of the monitoring program (daily monitoring of cyanobacteria and cyanotoxins), mouse test bioassays and Response Committee meetings	

2.1.1 Use of submersible fluorescence probes for cyanobacterial monitoring

Most current alert levels focus on chlorophyll *a* concentration and cyanobacteria cell counts (Ahn et al., 2007). Due to potential insufficiency of conventional drinking water treatment for removing taste and odour compounds and cyanotoxins, probes have been proposed as useful tools for early warning systems for monitoring cyanobacteria (Izydorczyk et al., 2005). Alert systems based on cyanobacterial enumeration in raw water are costly, time consuming and therefore

Table 2-2: Spectral groups of microalgae (Beutler et al., 2002)

Spectral group	Peripheral antenna	Division	Light Emitting Diodes (LED)
Green	Chlorophyll *a/b* carotenoids	Chlorophyta	450 nm
Blue	Phycobilisomes (Phycocyanin)	Cyanobacteria Glaucophyta	525 nm
Brown	Chlorophyll *a/c*	Heterokontophyta, Haptophyta, Dinophyta	570 nm
Red	Phycobilisomes (phycoerythrin)	Rhodophyta (some Cyanobacteria)	590 nm
Mixed	Chlorophyll *a/c* phycobiliprotein	Cryptophyta	610 nm

cannot be used to monitor rapid variations in raw water quality from cyanobacterial blooms (Zamyadi et al., 2012 b). The new *in situ* probes can also be used for rapidly monitoring the spatial distribution and temporal fluctuations of phytoplankton and cyanobacteria in water bodies (Gregor & Maršálek, 2004). The new method uses fluorescence to absorb light at one specific wavelength and emit it at longer wavelength (Lorenzen, 1966). Photosynthetic pigments yield specific fluorescence signals within different wavelength ranges (Beutler et al., 2002; Campbell et al., 1998). Table 2-2 presents the pattern for each type of spectral group of phytoplankton. For instance,

in cyanobacteria the principal light-harvesting complexes are phycobilisomes rather than Chlorophyll *a* or *b* (Campbell et al., 1998).

The application of *in vivo* probes improves the rapid monitoring of the distribution of cyanobacterial cells in source water surveys. Various environmental factors are the driving forces of changes in cyanobacteria concentrations in the ecosystem and can lead to important spatio-temporal heterogeneity in source waters and phytoplankton composition (Rychtecký & Znachor, 2011). Phycocyanin probes are able to measure dynamic profiles of depth and can be used simultaneously with other probes to measure other environmental variables such as temperature, turbidity, pH, and chlorophyll *a* concentration at many locations within the source waters (Kahlert & McKie, 2014).

Conventional quantification of cyanobacterial species can be in the form of cyanobacteria cells (units of cell mL^1 or abundance) or cyanobacteria biovolume (mm^3 L^{-1}). Various units have been used to express the density of cyanobacteria biomass in phycocyanin probes. The more commonly applied units in recent assessments are given as total concentration of chlorophyll *a* expressed in µg L^{-1} and Ratio Fluorescence Units (RFU) as separate measures of phycocyanin concentration for cyanobacteria. Units of cell mL^{-1} are not recommended because of the large variability of cell size that can lead to the misinterpretation of probe results (Ahn et al., 2007; McQuaid et al., 2011).

The application of phycocyanin probes raise questions about the precision and accuracy of results as various water matrix interferences can occur such as turbidity and the presence of green algae (Zamyadi et al., 2012 c). In turbid waters, a lower accuracy results in false positives in waters with turbidity greater than 50 NTU (Symes & Ogtrop, 2016). Furthermore, phycocyanin pigments are strongly dependent on growth rate and the cell densities of different cyanobacteria (Korak et al., 2015).

2.2 Cyanobacteria passage through bank filtration

Most of the monitoring frameworks have been developed for surface waters. However, little information is available for monitoring cyanobacteria in bank-filtered waters. Cyanobacteria have rarely been observed in bank-filtered water. Table 2-3 presents a summary of studies that have explored bank filtration for the removal of cyanobacteria and cyanotoxins. An expanded version of Table 2-3 is presented in Chapter 6 that includes bank filtration for the removal of bacterial indicators that was submitted as supplementary materials with the manuscript.

Table 2-3: Selected studies on the removal of microorganisms and toxins relevant for bank filtration.

Scale of study	Parameter studied	Comments	References
Field study	*Synechococcus sp.*	Most removal of cyanobacteria occurs in the colmation layer of natural bank filtration (82% in top 3 cm of layer).	**(Harvey et al., 2015)**
Field study	Benthic microalgal and meiofaunal assemblages	The colmation layer in bank filtration is a highly active and dynamic biological zone. Clogging of the layer is reversible.	**(Hoffmann & Gunkel, 2011)**
Field and column study	Bloom dominated by *Cylindrospermopsis raciborskii*	No cyanobacterial cells or cell fragments were observed in bank filtered well water, cyanotoxins were below detection limits in well water. Cell removal was 95% in top 2 cm of infiltration.	**(Romero et al., 2014)**
Field study	*Prochlorococcus sp., Synechococcus sp., Cyanobacteria, and Pico/ nanoplankton*	Bank filtration prior to desalination showed that wells with larger distances from the sea did not have cyanobacterial breakthrough but breakthrough occurred in a horizontal well system with potential hydraulic connections to the source.	**(Rachman et al., 2014)**
Field study	*Limnothrix redekei, Oscillatoria subbrevis, Pseudanabaena catenata, Aphanizomenon sp. Spirulina sp*.	A bank filtration production well located 65 m from a river in Brazil did not demonstrate passage of cyanobacterial cells.	**(Freitas et al., 2012)**
Column studies	Microcystin	Physical filtration of cells was important for microcystins removal in slow sand filtration. Little retardation of microcystins from sorption; biodegradation was the more important process as compared to sorption.	**(Grützmacher et al., 2009)**
Column study	Microcystin-LR	A lab cultured strain of *Sphingomonas sp*. was capable of degrading microcystin LR in slow sand filtration	**(Bourne et al., 2006)**
Column study	Cylindrospermopsin	Cylindrospermopsin degradation only occurred in the sediment, not in the water body. Degradation is more important than retention for the sediments tested.	**(Klitzke et al., 2010)**
Field study	Microcystins	Cyanobacterial cells were generally absent from bank filtered water although some coccoid cells were found at one plant with filamentous cyanobacterial cell fragments. Microcystins were detected in bank filtered water.	**(Lahti et al., 2001)**

2.3 History of cyanobacterial blooms in Lake Erie

The acceleration of nutrient loading (1920-1970) and pollution from sewage converted Lake Erie into a dead zone (Makarewicz, 1993) and a strategy was adopted by water authorities to avoid further catastrophe. As a result of the implementation of a phosphorus reduction program and regulated nutrient inputs, severe cyanobacterial blooms disappeared in middle of the 1980s (Conroy et al., 2005). The improved water quality in Lake Erie did not hold out for long, as the cyanobacteria blooms reappeared in the western basin of Lake Erie with the arrival of the mussels of the genus *Dreissena* (zebra mussels). Increasing agricultural nonpoint sources of soluble reactive phosphorus (SRP), the intense spring rains and runoff and other environmental factors such as increasing temperature are considered the main reasons of increasing cyanobacterial bloom frequency in recent years. Table 2-4 summarizes relevant water quality studies for Lake Erie from 1900 to the present day.

Table 2-4: History of cyanobacterial blooms in Lake Erie

Period	Basin location	Trends	Drivers	References
1900-1950	Western	-) Bloom of Diatoms in mid-winter under the ice in west basin -) Near-shore blue-green and green algae blooms -) with a late summer or early fall dominance of blue-green algae (largely Anabaena) and the Chlorophyta Oocytes	-) acceleration of nutrient loading (1920-70) -) use of phosphate-based detergents	**(Chandler, 1940)** **(Allinger & Reavie, 2013)**
	Central	-) central Lake Erie was mesotrophic to eutrophic since about 1850		**(Allinger & Reavie, 2013)**
1950-1980	Western, Central	-) seasonal algal blooms, most reported blue-green algal blooms of *Anabaena*, *Aphanizomenon* and *Microcystis* -) Eutrophication of the western driven -) summer phytoplankton biomass was greatest in the 1970–78 period -) the implementation of a phosphorus reduction program	-) years of unregulated inputs of nutrients	**(Allinger & Reavie, 2013)** **(Matisoff & Ciborowski, 2005)** **(Conroy et al., 2005)** **(Matisoff & Ciborowski, 2005)**
	Central	-) Eutrophication of the central basin driven by years of unregulated inputs of nutrients		
Mid 1980	Western	-) Lake Erie water quality was indeed improving -) The arrival of Dreissena in the late 1980s associated with further decreases of phytoplankton biomass in near shore areas and the shallow west basin -) Zebra mussel density increased dramatically from 1989 to 1990	-) annual phosphorus loads to Lake Erie decreased	**(Makarewicz et al., 1999)** **(Ghadouani & Smith, 2005)**
	Western, central and eastern	-) decreased total phytoplankton Biomass -) reduction in the abundance of eutrophic indicator species (e.g., *Aphanizomenon flos-aquae*)		**(Makarewicz, 1993), (McGucken, 2000)**

Table 2-4: History of cyanobacterial blooms in Lake Erie (continued)

Period	Basin location	Trends	Drivers	References
	Central	-) frequency and duration of central basin hypoxia/anoxia		**(McGucken, 2000)**
1990-2000	Western	-) Total phytoplankton biomass, measured through enumeration and size-frequency distributions, has increased -) increased summer cyanobacteria biomass is due to a resurgence throughout Lake Erie of *Microcystis* spp., a non-nitrogen-fixing cyanobacterium in all basins after the dreissenid invasion -) appearance of *Microcystis* spp., a non-nitrogen-fixing cyanobacterium consistently each summer -) Lake Erie was returning to more eutrophic condition -) increasing of frequency of cyanobacterial blooms		**(Conroy et al., 2005)** **(Makarewicz et al., 1999)** **(Rinta-Kanto et al., 2005)** **(Rockwell et al., 2005)**
	Central	-) still dreissenid mussels were abundant -) decreases in phytoplankton biomass (measured as chlorophyll *a* concentration in in spring and increasing during summer) -) increasing the hypo limnetic oxygen depletion rates -) spring TP concentrations increased but summer TP concentrations declined	-) increasing total phosphorus concentrations	**(Matisoff & Ciborowski, 2005)** **(Burns et al., 2005)** **(Rockwell et al., 2005)**

Table 2-4: History of cyanobacterial blooms in Lake Erie (continued)

Period	Basin location	Trends	Drivers	References
2001-2011	Western	-) using the satellite observation on bloom monitoring -) Chlorophytes, Bacillariophytes and Cyanobacteria were dominant in 2003, 2004 and 2005 respectively -) Peak blooms occurred in August or September in all years except 2011, which peaked in early October -) 2011 the largest harmful algal bloom in its recorded history, with a peak intensity over three times greater than any previously observed bloom -) winter blooms of diatoms occur annually prior to the onset of ice cover -) western Lake Erie may be again moving towards a more eutrophic state -) occurrence of potentially toxic cyanobacteria *Microcystis spp.* in form of bloom -) microcystin toxicity exceeding 1 μg L^{-1} (microcystin-LR activity equivalents) -) remaining of bloom for two month on summer 2008 -) The Maumee River is the major source of nutrients and suspended sediments to western Lake Erie -) 80% probability of dominancy of the phytoplankton community as TP greater than 3.23 μM -) Microcystis is a significant component of the cyanobacterial community in Lake Erie during summer seasons species, specifically Dreissena type -) in 2011 bloom, surface toxin concentrations could have reached over 4,500 μg/L and water-column integrated concentrations ranging from 0.1 μg/L to 8.7 μg/L in early August	-) increases in agricultural nonpoint sources of bioavailable phosphorus, the presence of invasive mussel -) Mixing of blooms in water columns by high wind stress	**(Rinta-Kanto et al., 2005)** **(Wynne et al., 2010)** **(Chaffin et al., 2011)** **(Bridgeman et al., 2012)** **(Downing et al., 2005)** **(Rinta-Kanto et al., 2009)** **(Michalak et al., 2013)** **(Stumpf et al., 2012)** **(Millie et al., 2009)** **(Twiss et al., 2010)**

Table 2-4: History of cyanobacterial blooms in Lake Erie (continued)

Period	Basin location	Trends	Drivers	References
	Central	-) hypolimnial oxygen depletion continued to occur	-) increase of internal phosphorus loading to Lake Erie's central basin	**(Matisoff & Ciborowski, 2005)** **(Cao et al., 2008)**
	Eastern	-) the biomass of phytoplankton, measured as chlorophyll *a* appeared to be at historically low levels		**(Matisoff & Ciborowski, 2005)**
2012-2014	Western	-) Large blooms of toxic or potentially toxic cyanobacterial HABs and the nuisance green alga -) Overall bloom will be smaller than in 2013 with varying impacts across lake's western basin -) cyanobacteria bloom in 2014 plagued northwest Ohio's water(Toledo)		**(Kane et al., 2014)** **(Michalak et al., 2013)** **(NOAA, 2014)**
	Central	dissolved oxygen depletion and hypoxia/anoxia in the central basin continue to be problematic		**(Kane et al., 2014)** **(Obenour et al., 2014)**
	Eastern	-		-

2.4 Diversity of cyanobacteria in Lake Erie

At the beginning of the early twentieth century, the proliferation of undesirable phytoplankton overwhelmed Lake Erie. Even though diatoms were the most abundant at first, the lake continued to experience harmful algal blooms and severe oxygen-depleted "dead zones" for years. Blooms comprised of *Microcystis* and *Anabaena* in the western basin appeared with peak cyanobacteria blooms occurring in September and October. Other types of potentially toxic cyanobacteria such as *Anabaena* spp., *Aphanizomenon* spp., *Lyngbya* spp., and planktonic *Oscillatoria* spp. were also observed. Severe blooms disappeared following phosphorus abatement strategies for Lake Erie. However, since then, diffuse sources of pollution have continued to increase bioavailable nutrients. Recently, *Microcystis aeruginosa* has become a dominant species in blooms. Table 2-5 presents a brief history of the diversity of phytoplankton in Lake Erie.

Table 2-5: A summary of the diversity of phytoplankton in Lake Erie

Period of study	Dominancy status of the cyanobacterial Community and cyanotoxins in Lake Erie	References
1900s	overall largely comprised of diatoms and cyanobacteria contained genera Microcystis and Anabaena in western basin	**(Davis, 1958)**
1950s	A peak in cyanobacterial biomass during the months of September and October, referred to as the "autumnal maximum." (*Anabaena spp., Aphanizomenon spp., Lyngbya spp., and planktonic Oscillatoria spp.* — now reclassified as *Planktothrix spp.*) The most abundant microcystin during autumn was Aphanizomenon and later with another filamentous *Oscillatoria* (Planktothrix) *spp*.	**(Davis, 1954)**

Table 2-5: A summary of the diversity of phytoplankton in Lake Erie (continued)

1980	biomass of phytoplankton, especially the dominant cyanobacterium *Aphanizomenon flos-aquae*	**(Makarewicz, 1993)**
1990s	The identification of toxic cyanobacteria in summer blooms, now *Microcystis aeruginosa* dominates and accounts for 95% of the blooms in western Lake Erie.	**(Conroy et al., 2005; Millie et al., 2009)**
2000s	*Microcystis flos-aquae, botrys, novacekii, viridis, and wesenbergii*, but generally dominated by *Microcystis aeruginosa*. The most extensive in Lake Erie's recent history blooms are typically dominated by *Microcystis aeruginosa* and to a lesser extent Anabaena	**(Michalak et al., 2013)**
2010s	*Microcystis aeruginosa* frequently abundant	**(Bridgeman et al., 2013)**

2.5 Application of remote sensing and fluorescence for phytoplankton monitoring in Lake Erie

Anthropogenic eutrophication in the Western Basin of Lake Erie challenges the water treatment authorities to implement the remote-sensing tools to improve predictions for better management (Ortiz et al., 2013). In 2005, *in situ* fluorescence tools were applied on a lake-wide scale to determine the patterns of phytoplankton abundance and cyanobacteria dynamics. Chlorophyll *a* fluorescence was used to measure spatial and temporal variations of phytoplankton composition and cyanobacterial biomass as a function of depth. Results were then compared with microscopic analyses (Ghadouani & Smith, 2005). Remote sensing has been used with ground-truth data to explain the appearance of blooms in relation to a variety of environmental factors, including lake circulation and residence times (Michalak et al., 2013). Most importantly, NOAA provides bloom

forecasts to be used by water managers and drinking water treatment plants on Lake Erie (Wynne et al., 2013), although these were not able to prevent the do-not-drink advisory in Toledo, Ohio in 2014 resulting from a possible breakthrough of cyanotoxins into drinking water.

2.6 Summary of research needs

A few studies have focused on establishing a source water alert level framework based on online phycocyanin and chlorophyll *a* probes and cyanobacterial cell densities. However, there is a need to validate these suggested frameworks in other geographical regions with other cyanobacterial communities. The distribution of cyanobacterial cell densities related to alert levels should be determined for local areas to determine the accuracy of local alert levels. Probes have not been tested on Lake Erie drinking water sources that have experienced severe cyanobacterial blooms in recent years.

Given that probes work best when there are few interferences from turbidity and green algae, it is also important to determine whether probes will be useful tools for bank-filtered source water monitoring, even if concentrations of cyanobacteria would be expected to be low and closer to detection limits in bank-filtered water as compared to surface water blooms.

In this Master's thesis, previously published alert levels will be validated for two additional water sources: 1) Lake Erie and 2) a bank-filtered drinking water supply.

CHAPTER 3 OBJECTIVES AND HYPOTHESES

This research project aimed to increase our understanding of the linkages between conventional monitoring methods from previous studies and a new rapid monitoring method for a drinking water intake in the Western Basin of Lake Erie and a bank-filtered water supply. Plant managers are planning to implement online monitoring to minimize the risk of cyanobacteria breakthrough. This study involved assessing the performance of a submersible phycocyanin probe as a tool for surveillance and management.

The main objectives of the present study are as below:

1) Validate *in situ* phycocyanin probes reading for cyanobacteria in two previously untested drinking water sources.

2) Determine whether previous thresholds for an Alert Level framework are applicable at the two new field sites

Additional specific objectives related to the bank-filtration were the following: 1) to estimate the efficiency of bank filtration for removing phytoplankton and bacterial indicators, 2) to determine whether there was preferential removal of certain species of phytoplankton including cyanobacteria, and 3) provide recommendations on the use of phycocyanin probes for monitoring the fate of cyanobacteria in bank filtration (Table 3-1).

Table 3-1: Specific objectives and hypothesis of the research of this project

Objectives	Hypotheses	Experimental approach
Evaluate the accuracy of a phycocyanin probe for monitoring cyanobacteria in two drinking water supplies	Correlations exist between taxonomic cell counts and probe fluorescence are independent of source water	-) Comparing the probe readings data on phycocyanin pigments in surface waters by comparing it to taxonomic cell counts results conducted in laboratory -) microscopic cell counts including cyanobacteria density, cyanobacteria biovolume, phytoplankton density and phytoplankton biovolume and microcystin concentration Using statistical software to calculate the regression and p-value for all measured data in probe reading and lab test results Estimate the environmental conditions are suitable for blue-green algae and can increase to excessive levels and form visible 'blooms' which can adversely affect water quality. Environmental variables such as turbidity, Temperature, pH, and chlorophyll fluorescence.
Establish local alert level thresholds using phycocyanin probe measurements	Thresholds determined by McQuaid et al. (2011) are applicable to other source waters	Based on the regression found between two methods, creating the new alert level system as RFU equivalent to biovolume mm^3/l.
Assess the efficiency of a bank filtration system for cyanobacteria and microbial indicators	Travel time is the most important factor for bank filtration efficiency	Tracing the accumulation of cyanobacteria in depth of lakes and sediment samples by applying the phycocyanin probe in different water levels in Lakes. Comparing species composition in both lakes and well water.

CHAPTER 4 MATERIALS AND METHODS

4.1 Site locations

The first study site was a drinking water treatment plant located in the Western Basin of Lake Erie in Ontario, Canada. Opened in 1960, the plant takes water from Lake Erie via two intake pipes located 500 meters offshore and supplies potable water to approximately 56 000 residents in Southwestern Ontario (Figure 4-1).

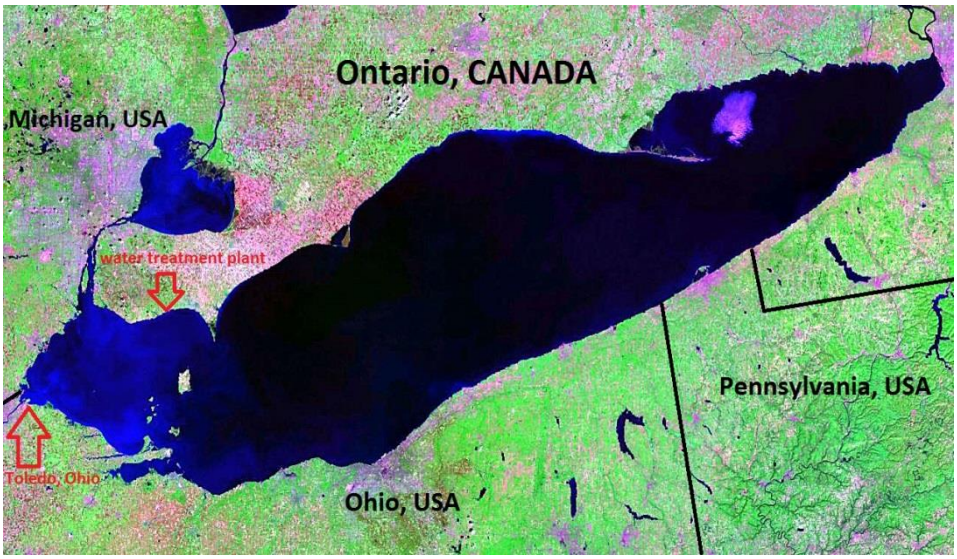

Figure 4.1: Lake Erie, the western basin is indicated by red arrows.

All details with regards to materials and methods for the second study site are provided in Chapter 6.

4.2 Sampling methods

Table 4-1 provides the cyanobacteria monitoring protocol at the drinking water treatment plant. Based on this protocol, the conventional monitoring by grab sampling proceeded with online fluorescence monitoring by using an *in situ* phycocyanin probe (YSI 6600 V2-4 water-quality multi-probe (YSI, Yellow Springs, Ohio, USA)). The water grab samples were collected from the pumping station raw water from the water pipe prior to any treatment. Collected samples were conserved in an appropriate condition (Lahti et al., 2001). For taxonomic analyzes of

phytoplankton, as the densities of cyanobacterium exceeded 2 RFU as measured by the probe to phycocyanin (PC), water samples were taken for microscopic analysis while preserved using Lugol's iodine and were sent to University of Quebec in Montreal's (UQAM) in Biological Sciences Department for species identification, biomass and biovolume calculations. The microcystin quantification was conducted with freezing the grab raw water samples at -25°C and samples were sent to the local accredited laboratory. All water samples were taken in parallel with PC probe readings.

Table 4-1: proposed cyanobacteria monitoring protocol in Lake Erie

Monitoring	**Threshold (Following YSI cyanobacteria probe readings)**	**Cell counts and speciation**	**Cyanotoxin analysis**
continuous	Probe automatic reading: every 30 minutes	-	-
Regular sampling	probe reading < 2 RFU	Sampling once a week from the raw water	Sampling once a week from the raw water.
Intensive sampling	2 RFU < probe reading < 5 RFU	Sampling 2 times per week from raw water, sludge of the clarifier, surface of the clarification basin, filtered water, and treated water	Sampling 2 times per week from raw water, sludge of the clarifier, surface of the clarification basin, filtered water, and treated water
Bloom event sampling	5 RFU < probe reading	Daily sampling from raw water, sludge of the clarifier, surface of the clarification basin, filtered water, and treated water	Daily sampling from raw water, sludge of the clarifier, surface of the clarification basin, filtered water, and treated water

4.3 YSI instrument, specification, calibration

The following physical parameters were measured using an *in situ* YSI multi-parameter probes model YSI 6600 V2-4 water-quality multi-probe (YSI, Yellow Springs, Ohio, USA) with the self-cleaning "wiped": PC (cell/ml), PC RFU (Relative Fluorescence Unit)Chl-a (µg/l), chlorophyll *a* (RFU), temperature (°C), specific electrical conductivity (mS), turbidity (NTU), DO (mg/l), and pH (C. C. Davis, 1954). To quantify the fluorescence, the system detector is a photodiode of high sensitivity that is screened by an optical filter that restricts the detected light. The YSI probe excites

an orange light emitting diode (LED) at 590 nm for the excitation process. To quantify the fluorescence of the phycocyanin pigment in blue-green algae, a high sensitive system detector in probe screens the emitted light at 660 nm. For reading the chlorophyll *a* pigment, the same procedure is conducted with a different wavelength of 470 nm of excited light and the emission of pigment light at 680 nm. The probe has lower and upper detection limits of reading PC pigments under 0.1 RFU and over 100 RFU, respectively (McQuaid et al., 2011). The detected wavelength from the output of the sensor is expressed in either generic fluorescence units (RFU) or cell/mL of PC-containing blue-green algae. The turbidity, pH and conductivity were calibrated with the calibration liquids from the manufacturer and for the chlorophyll *a*, PC sensor and dissolved oxygen a de-ionized water was used as a one-point calibration.

CHAPTER 5 RESULTS AND DISCUSSION – LAKE ERIE

5.1 Environmental variables (temperature, pH, turbidity, chlorophyll *a*)

During the study period from September 2012 to November 2014, the mean water temperature as measured by the probe was 20.9 °C (Appendix C). The pH varied from a mean low of 7.9 to a high of 9.0 In general, the pH was above 8.6. Previous studies of turbidity in Lake Erie have shown that wave action induced by winds and river discharges in the western end of the lake are predominant factors affecting turbidity (Van Oosten, 1948). Turbidity also plays a role in affecting conditions for the growth of phytoplankton. Higher turbidity can be advantageous to cyanobacteria species such as *Microcystis* as to prevent the damage of exposure to intense sunlight (Chaffin et al., 2011). During the study period, turbidity went as high as 81.4 NTU on November 14 2013. The high turbidity in November occurred during the period with the highest chlorophyll *a* concentrations (3 µg/L and 0.8 RFU) suggesting that phytoplankton cells were an important component of the turbidity. During the summer, the turbidity was generally low (mean 8.2 NTU) (Appendix C).

5.2 Temporal dynamics of phytoplankton

5.2.1 Phytoplankton distribution

The results of phytoplankton monitoring conducted from August 2012 to October 2014 are discussed in this section with the general distribution of phytoplankton groups shown in Figures 5-1 and 5-2. As previously mentioned before, two major counting units are used to express the density of cyanobacteria: (1) biovolume (mm^3L^{-1}) and (2) abundance (cell mL^{-1}), with Figures 5-1 and 5-2 demonstrating how different units can affect the interpretation of results. The wide range of phytoplankton biovolumes expressed in $mm^3 L^{-1}$ consists of 52.6 % diatoms as the dominant group and 22.3 % of cyanobacteria. On three days (25 September 2012, 7 and 15 October 2013), the RFU exceeded 1.0 and the presence of cyanobacteria was noted as dominant phytoplankton group with 41.7% (0.66 mm^3L^{-1}), 77.6% (2.8 mm^3L^{-1}) and 86.6 % (1.2 mm^3L^{-1}) of total phytoplankton biovolume, respectively.

Six types of phytoplankton were detected in raw water intake samples. Cyanophyceae were most abundant in terms of mean cell counts (45589 cell/mL) followed by green algae (2425 cell/mL), Diatomophyceae (1011 cell/mL), Chrysophyceae (436 cell/mL), Cryptophyceae (300 cell/mL), and Dinophyceae (20 cell/mL). Diatomophyceae were less numerous, making up approximately 2.0 % of all cell counts while cyanobacteria made up 92 % of all cell counts (Figure 5-2). The relative proportion of phytoplankton changed when considering units of biovolume, as diatoms comprised 52.4 % (mean 0.99 mm^3L^{-1}) of all phytoplankton while the cyanobacteria made up 22.2 % (0.4 mm^3 L^{-1}) of the total biovolume from intake samples (Figure 5-1).

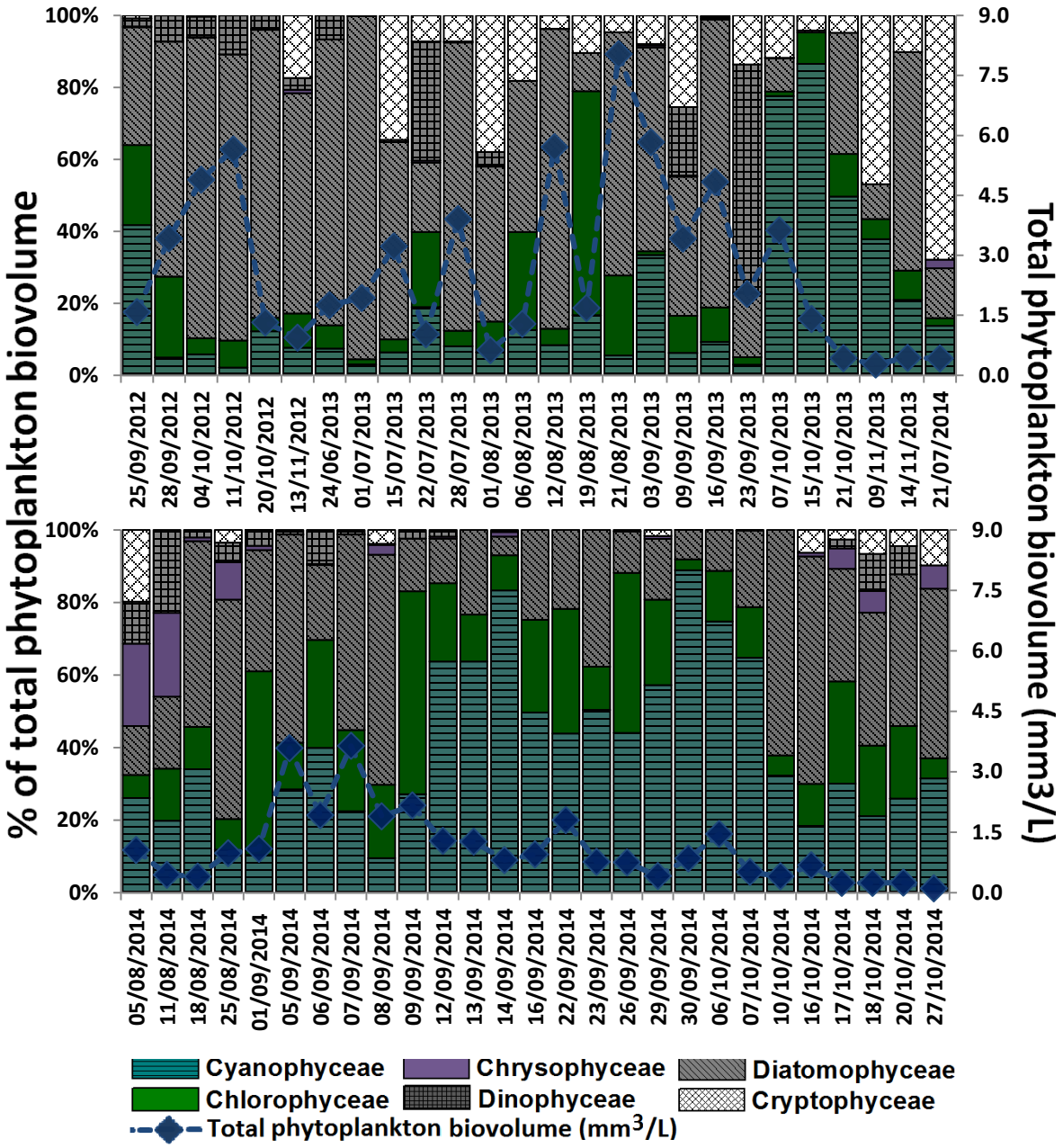

Figure 5.1: Temporal variation of phytoplankton total biovolume from raw water intake grabs samples.

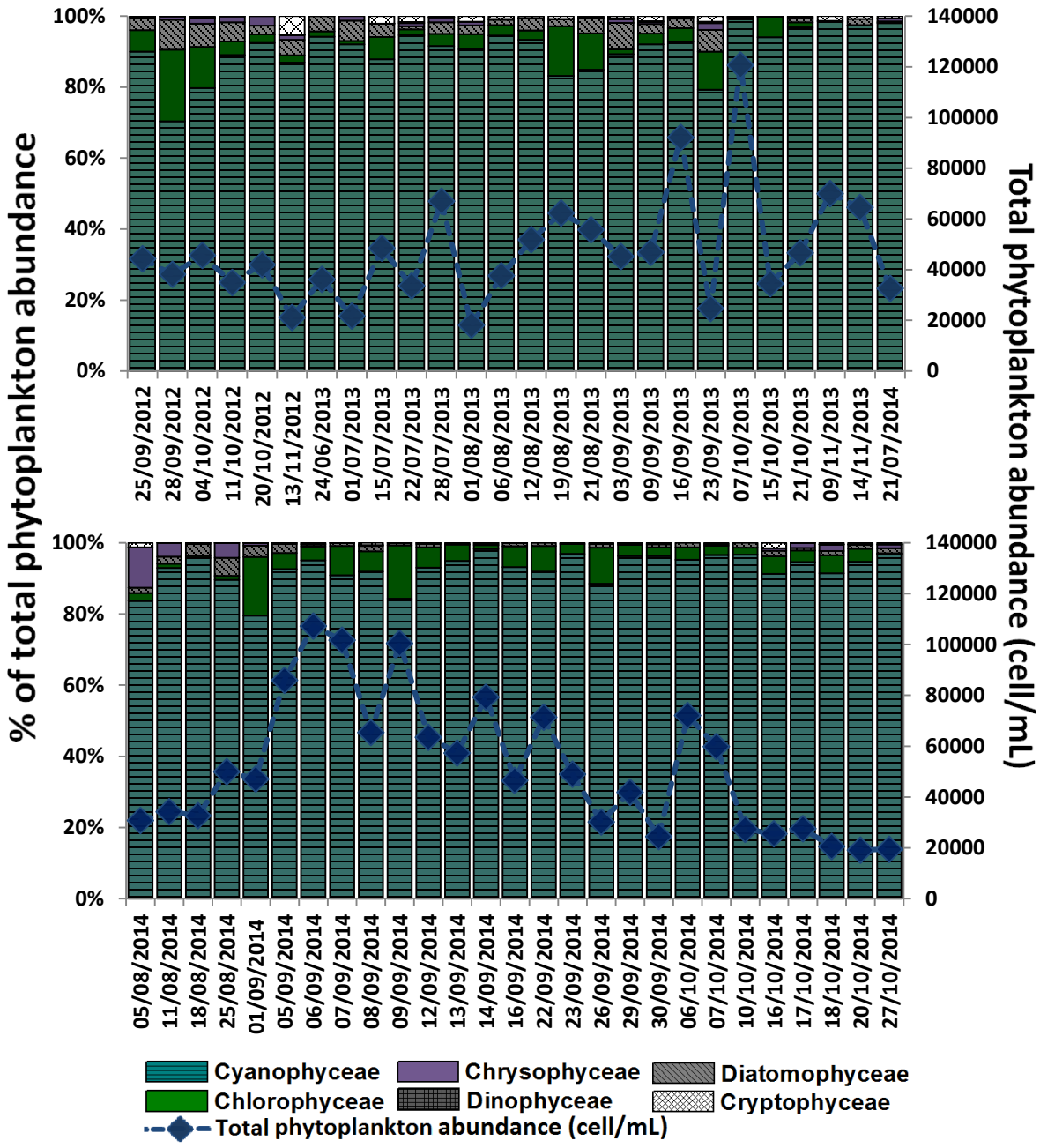

Figure 5.2: Temporal variation of phytoplankton abundance from raw water intake grabs samples.

5.2.2 Temporal distribution of cyanobacteria

Figures 5-3 and 5-4 show the temporal variation of cyanobacterial species in the raw water of the drinking water treatment plant in Western Lake Erie. Eighteen species of cyanobacteria were identified by taxonomic cell counts during the study period. *Microcystis aeruginosa* was the dominant species (mean 0.26 mm^3L^{-1}) in terms of biovolume, consisting of 58.7 % of the total biovolume of cyanobacteria species. *Pseudanabaena mucicola* was the next most important cyanobacterial species with regards to biovolume (5.8 %) (Figure 5-3). Cyanobacteria abundance presented different patterns according to the quantification unit in cells/mL - *Aphanothece clathrata brevis* was the dominant species 48.1 % in cell/mL (22051 cell/mL) (Figure 5-4). Potentially toxic cyanobacterial species were observed in almost 69 % of all raw water samples and frequently consisted of *Microcystis aeruginosa* in terms of biovolume (and 11 % in term of abundance).

The most abundant potentially toxic species observed in our study was *Microcystis aeruginosa* making up 58.7% of the total cyanobacteria biovolume (Figure 5-3). Table 5-1 summarizes the means of cyanobacteria cell volume from the taxonomic analyses of all grab samples from Lake Erie raw water. The mean of cell size volume shows the importance of biovolume rather than the number of cells in term of abundance (Kahlert & McKie, 2014; McQuaid et al., 2011). The cell volume of cyanobacteria species varied from 0.3 μm^3 (*Merismopedia minima*) to 170.6 μm^3 (*Anabaena spiroides*).

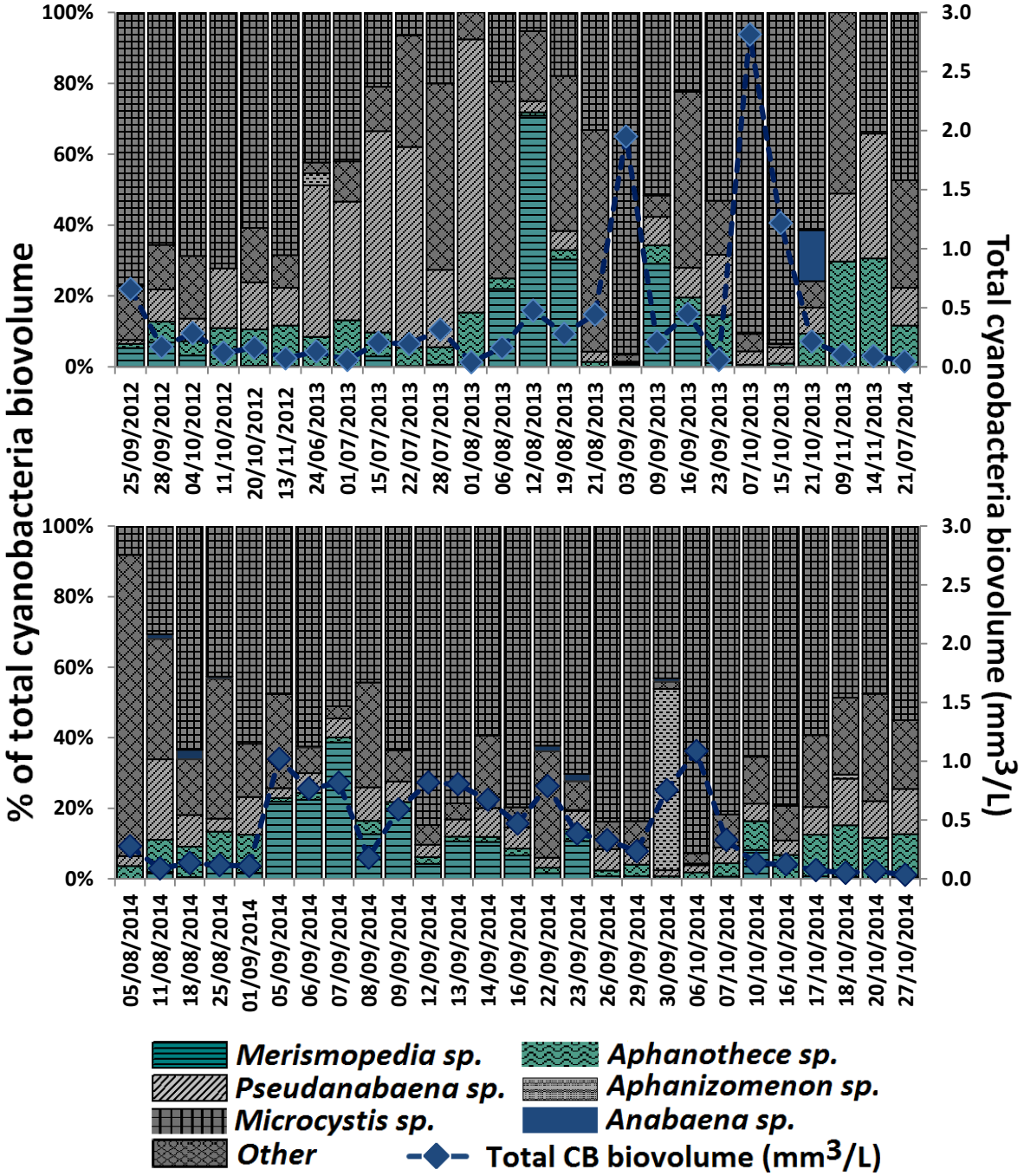

Figure 5.3: The distribution of cyanobacteria biovolumes from grab samples from the raw water intake on Lake Erie (2012 – 2014).

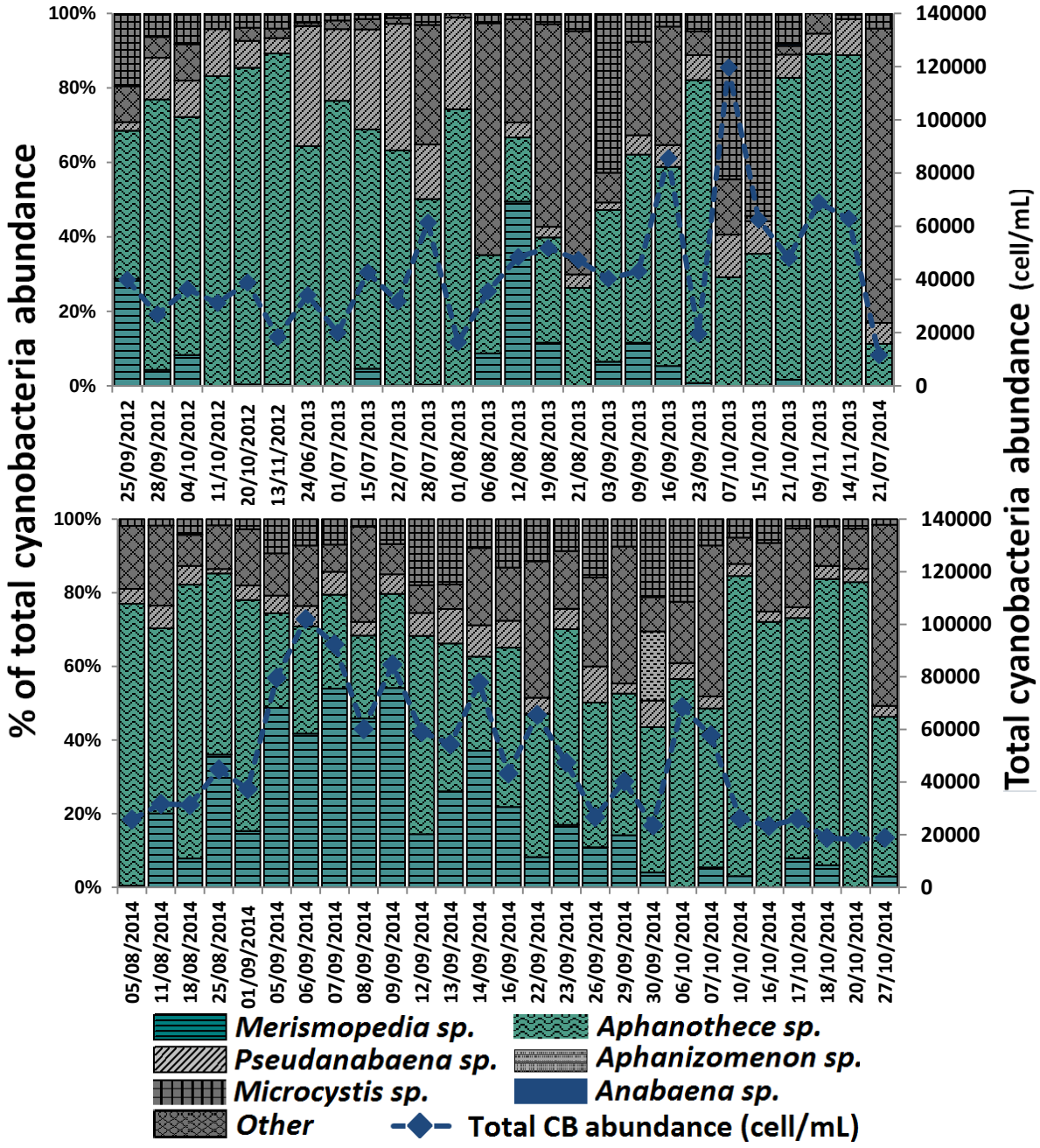

Figure 5.4: The distribution of cyanobacterial abundance (cell/mL) from grab samples from the raw water intake on Lake Erie (2012 – 2014).

Table 5-1 Cellular volume of different cyanobacterial species observed in Lake Erie

CB genus	CB species	Mean cell volume (μm³)	% of total CB biovolume (n= 53)
Anabaena	*flos-aquae*	137.8	0.9
	Spiroides	170.6	1.4
Microcystis	*Aeruginosa*	57.2	40.0
	Wesenbergii	113.1	5.3
Aphanizomenon	*Skujae*	75.4	12.5
Aphanothece	*clathrata brevis*	0.5	1.8
Aphanocapsa	*Delicatissima*	0.3	0.3
	Planctonica	10.4	5.3
Chroococcus	*Minimus*	4.2	2.2
	Prescottii	165.1	5.2
	disperses	14.1	2.4
Planktolyngbya	*Limnetica*	10.2	0.9
Pseudanabaena	*Mucicola*	7.9	4.2
	Limnetica	9.2	1.0
Coelosphaerium	*Kuetzingianum*	9.4	4.9
Merismopedia	*Minima*	0.3	0.3
	punctate	14.1	9.4
	Tenuissima	3.3	2.0

5.2.3 *In vivo* monitoring and comparison to taxonomic counts

As previously mentioned, *in vivo* probes can be reliable to rapidly detect and determine the spatial and temporal distribution of cyanobacteria blooms in a drinking water plant (Zamyadi et al., 2014). Figure 5-5 shows all the observed PC probe readings in Lake Erie corresponding to the collection of samples for taxonomic counts.

The mean concentrations of PC ranged from 0 to 3.9 RFU based on exact times of probe reading; however the PC concentration ranged from 0.02 to 0.9 RFU using daily averaged PC probe readings. The difference in trends shows the importance of the method for calculating means (to choose the exact time or compute daily average results). Overall, the phycocyanin probe values were low and mostly approached the detection limit of the PC probe (0.2 RFU). The maximum intensity of phycocyanin fluorescence (3.9 RFU measured on the October 7 2013) showed that cyanobacteria entered the drinking water treatment plant in higher numbers once during the study period. The low RFU values suggest that accumulation of cyanobacteria did not occur in this local area of Lake Erie despite causing severe problems in other regions of Lake Erie, most notably in Toledo, Ohio (Figure 5-5).

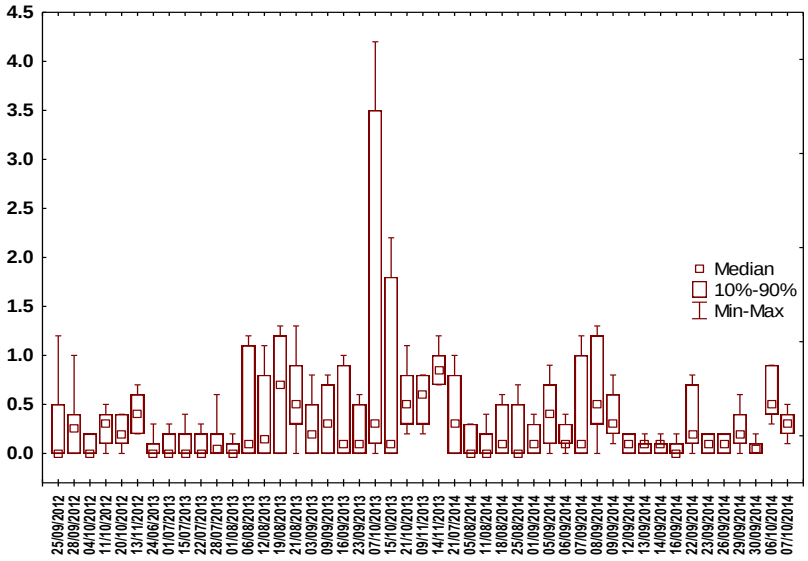

Figure 5.5: Evolution of phycocyanin measured by PC probe during the study period.

The PC probe was evaluated with regards to its ability to accurately measure the density of cyanobacteria. The focus of this validation was to extend the results from (McQuaid et al., 2011). The submersible probe demonstrated a good correlation with observed cyanobacteria in terms of biovolume (Figure 5-6). In order to compare PC probe readings and observed cyanobacterial densities, two approaches were considered. The exact time of probe reading following each thirty minutes of probe instrument setting has been averaged for three time of reading based on the time of collecting the raw water samples for taxonomic cell counts and the other is the daily average of PC probe readings.

Table 5-2: Correlations between PC RFU probe readings and taxonomic cell counts from grab samples (n=53) expressed in biovolume (mm^3L^{-1}).

Biovolume	*Total CB* ($mm^3 L^{-1}$)	*Potentially toxin-producing CB* ($mm^3 L^{-1}$)	*Diatomophyceae* [1] ($mm^3 L^{-1}$)	Microcystis aeruginosa [2] ($mm^3 L^{-1}$)
PC RFU* (day)	$R^2 = 0.12$, p= 0.0034	$R^2 = 0.11$, p = 0.0045	$R^2 = 0.0000$, p = 0.99	$R^2 = 0.18$, p = 0.0005
PC RFU ** (exact time)	$R^2 = 0.41$, p = 0.0000	$R^2 = 0.43$, p = 0.0000	$R^2 = 0.0003$, p = 0.91	$R^2 = 0.55$, p = 0.0000

[*] Probe reading for mean daily Phycocyanin RFU

[**] Probe reading for Phycocyanin RFU at the same time of grab sampling

[1] The Phytoplankton dominant species in biovolume unit is Diatomophyceae.

[2] The Cyanobacteria dominant species in biovolume unit is *Microcystis aeruginosa*.

The submersible PC probe demonstrated good correlations with taxonomic counts. Varieties of correlations were assessed and are presented in Table 5-2. Given the high temporal variability of probe readings, the readings most closely associated with the exact time a sample was collected showed much higher correlations than daily averages. These significant correlations between *in situ* PC probe and cyanobacteria demonstrate that the probes are sensitive tools for water management, especially in the case of drinking water resources (Zamyadi et al., 2014).

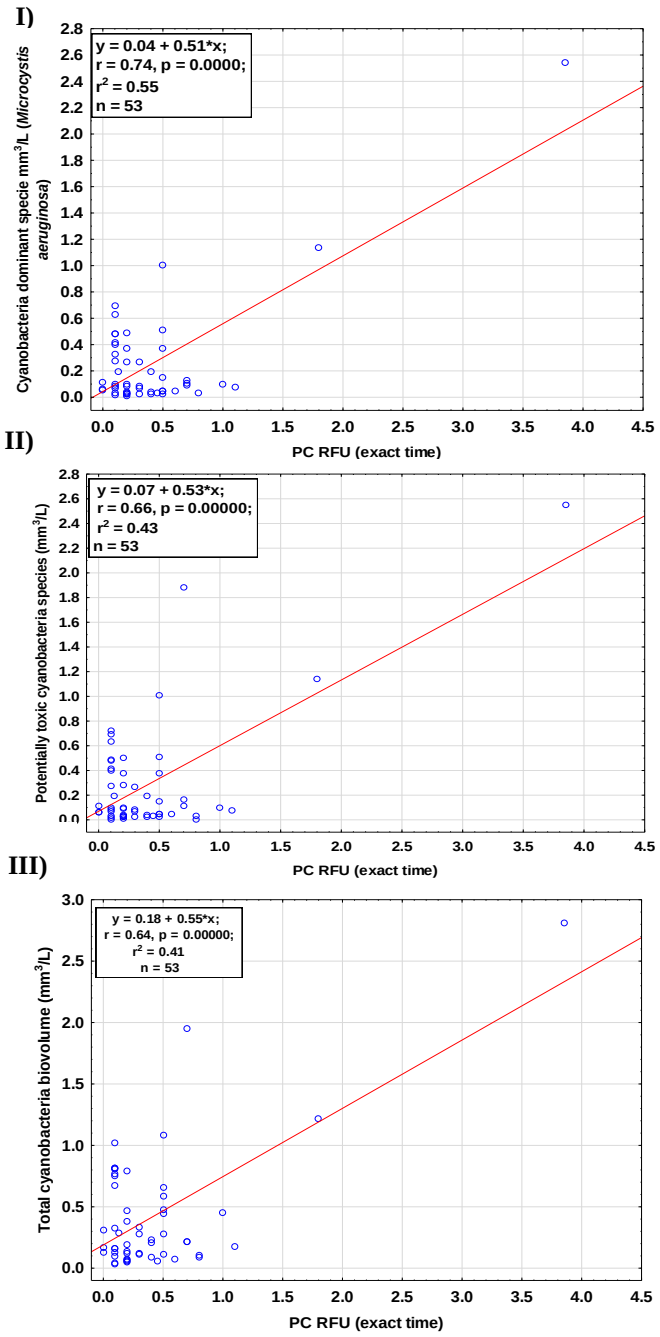

Figure 5.6: Correlation between online phycocyanin RFU measured by in situ submersible PC probe and I) *Microcystis aeruginosa* as dominant CB specie expressed in mm^3L^{-1}, II) potentially toxic cyanobacteria biovolume, III) Total cyanobacteria biovolume.

5.2.4 Efficiency of treatment process to remove the cyanobacteria

During the study period, the treatment plant experienced cyanobacteria breakthrough only on October 7 2013, the day the probe reading measured 3.9 RFU in the raw water. The high probe reading led to an additional sampling protocol whereby samples were collected from the raw water, clarified, filter and treated water at the same time. The composition of cyanobacteria species in term of abundance (cell/mL) was 44.5 % *Microcystis aeruginosa* as the most abundant species (53

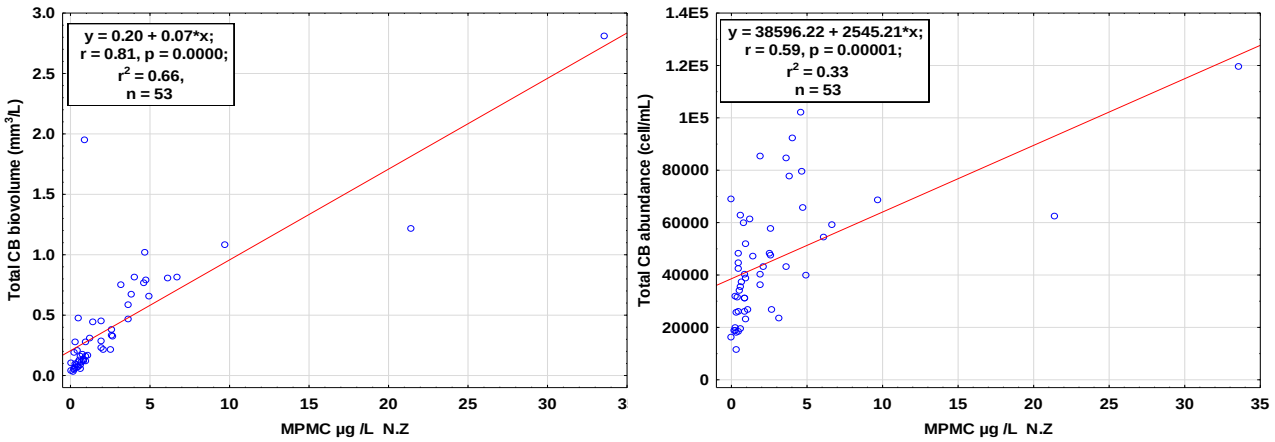

Figure 5.7: Maximum potential microcystin concentrations given concentrations of cyanobacteria, left; total cyanobacteria biovolume (mm^3/L), right; total cyanobacteria biovolume (cell/mL) and right: *Microcystis aeruginosa* abundance (cell/mL).

264 cell mL^{-1}) and the rest made up of other cyanobacterial species. In terms of biovolumes, *Microcystis aeruginosa* remained the dominant cyanobacteria species consisting of 90.3% all cyanobacterial biovolume (2.54 mm^3 L^{-1}) and in the treated water was reduced to 0.03 mm^3/L. The overall efficiency of the drinking water treatment plant to remove the cyanobacteria was about 98.6 % (0.7 log) considering units of cell/mL and 99% (2.0 log) in units of total cyanobacterial biovolume.

5.2.5 Microcystin LR

are expected given the generally low concentrations of cyanobacteria occurring at the drinking water intake. There is also a possibility that a greater abundance of non-toxic *Microcystis* existed among all *Microcystis* genotypes (Joung et al., 2011). Although toxic and non-toxic strains usually co-exist in a water body, the distinction of toxic from non-toxic genotypes is not possible using the conventional cell counts under a microscope. PCR is one of the best methods to quantify the proportion of toxic genotypes (Rinta-Kanto et al., 2005).

Previous studies have explored the effects of various environmental factors such as temperature, nitrogen and total phosphorus, and light intensity on the proportion of toxic, non-toxic *Microcystis* genotypes (Rinta-Kanto et al., 2009; Yoshida et al., 2007). In this study, the MC-LR had a moderate correlation ($R^2 \approx 0.33$) to the density of phycocyanin of *in situ* PC probe expressed in cell mL^{-1} ($r = 0.58$, $p < 0.05$) and RFU ($r = 0.57$, $p < 0.05$) (Figure 5-7). Further the concentration of MC-LR was weakly correlated to the environmental variables such as temperature, pH, turbidity and dissolved oxygen ($R^2 < 0.05$).

The cyanotoxin production in drinking water depends on environmental variations and the precise identification of cyanobacteria genotypes (Codd et al., 2005). Since the estimated toxin content is unpredictable, WHO suggested a maximum microcystin cell quota of an average toxin content per

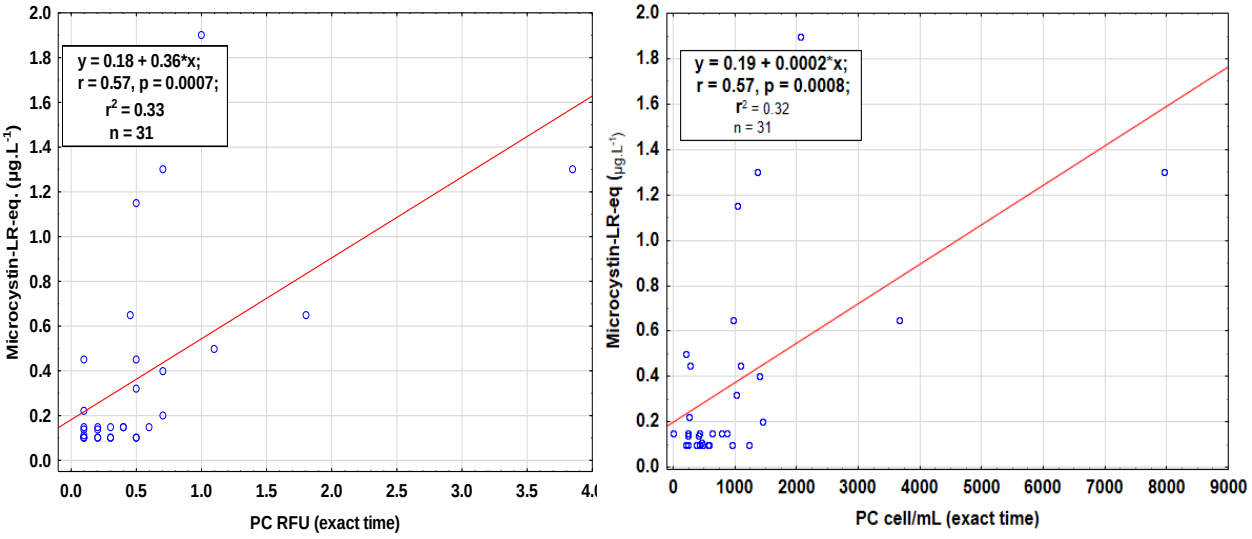

Figure 5.8: Linear regressions between MC-LR eq. concentration (µg/L) and left: *in situ* PC fluorescence RFU at the exact time of sampling and right: *in situ* PC fluorescence cell mL^{-1}.

cell of 0.2 pg in total microcystins (if the bloom consists of *Microcystis*) per *Microcystis* cell (WHO, 1999); although a 0.63 pg of microcystin cell quota has been established by New Zealand ministry of the environment (New Zealand Ministry for the Environment and the Ministry of Health, 2009). According to the WHO, the guideline levels to 0.2 pg of microcystin per cell corresponds to 100,000 cells of potentially toxic *Microcystis* species per litre (Alert level 1). In our results, *Microcystis aeruginosa* has the largest percentage of total cyanobacteria biovolume and the cyanobacterial bloom was dominated by this species.

To calculate the maximum potential microcystin content, the average biovolume of *Microcystis aeruginosa* was considered to be 57.2 μm^3. Based on these assumptions, the maximum potential values corresponding to the toxins using the World Health Organization (WHO, 1999), ranged from 0.06 to 10.7 µg /L microcystin. Note that the potential concentrations using New Zealand values environment (New Zealand Ministry for the Environment and the Ministry of Health, 2009) are significantly higher ranging from 0.18 to 33.6 µg /L of microcystins. This shows the importance of the choice of the reference value for estimating maximum concentrations in raw water in Lake Erie. Appendix B provides a summary of toxin estimates for the probe readings. Figure 5-8 shows the maximum potential microcystin concentration (MPMC) in the water supply in terms of WHO and New Zealand toxin estimates per cell mass.

CHAPTER 6 ARTICLE 1 : BREAKTHROUGH OF CYANOBACTERIA IN BANK FILTRATION

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Word Count: Total (including main text, tables, figures and references) **= 6978**

Abstract

The increasing frequency of cyanobacterial bloom occurrences worldwide has led to greater concern of the presence of cyanobacteria in drinking water sources and factors affecting their passage through drinking water treatment. The removal of cyanobacteria cells in well water following bank filtration was investigated from a source water consisting of two artificial lakes (A and B). Phycocyanin probes used to monitor cyanobacteria in the source and in filtered well water showed an increase of fluorescence values demonstrating a progressive seasonal growth of cyanobacteria in the source water that were correlated with cyanobacterial biovolumes from taxonomic counts ($r= 0.59$, $p<0.00001$). A strong correlation was observed between the cyanobacterial concentrations in the lake water and in the well water as measured by the phycocyanin probe ($p<0.001$, $0.73 \leq r^2 \leq 0.94$). Concentrations of microcystin-LR were generally under the detection limit except for three samples from a visible bloom in Lake B (1.2 µg L^{-1} to 7.9 µg L^{-1} MCLR eq.). No microcystin-LR was detected in the well water or in scum samples of Lake A. Log removals from bank filtration estimated from taxonomic counts ranged from 0.96 ± (0.5) and varied according to the species of cyanobacteria. Of cyanobacteria that passed through bank filtration, smaller cells were significantly more frequent in well water samples ($p < 0.05$) than larger cells. Cyanobacteria species in the wells were compared with cyanobacteria observed in the lakes and lake sediments and were shown to be most closely related to species found in Lake B.

Given the shorter travel time and distance from Lake B to the well as compared to Lake A, time and distance from source to well are important factors influencing well water quality for cyanobacterial transport through bank filtration in addition to the cyanobacterial species present.

KEYWORDS

Cyanobacteria, bank filtration, microcystins, phytoplankton, phycocyanin, log removal

6.1 Introduction

Cyanobacteria are prokaryote photosynthetic microorganisms that are of concern because of their ability to produce toxins and taste and odour compounds as well as disrupt drinking water treatment (Scott & Marcarelli, 2012; Zamyadi et al., 2013 c). The increasing proliferation of cyanobacteria is linked to the eutrophication of water bodies, notably from nitrogen and phosphorus concentrations (Taranu et al., 2015; Whitton & Potts, 2012). Cyanotoxins are most likely to occur following the accumulation of high densities of cyanobacteria in the form of blooms (Rastogi et al., 2014), with the upregulation of toxin production genes occurring when cells reach high numbers (Wood et al., 2011). Several species of cyanobacteria produce a wide range of toxic compounds with many reviews available on cyanotoxin occurrences worldwide (Gkelis & Zaoutsos, 2014; Rastogi et al., 2014). Given the increasing frequency of cyanobacteria blooms in fresh waters, there is a need for risk management strategies for drinking water suppliers to meet drinking water guidelines (e.g. (Ingrid Chorus, 2012) for the protection of public health (Ibelings et al., 2014; Otten & Paerl, 2015).

In regions with surficial geology appropriate for bank filtration, this technique can be an effective means of improving water quality and controlling a variety of contaminants through natural physical, chemical, and biological processes that occur during ground passage (Tufenkji et al., 2002). Table 6.4 provides an overview of selected studies on the removal of algae, microbial indicators and cyanotoxins by bank filtration. Although cyanotoxins have been measured in bank filtered water with coccoid cells and filamentous cyanobacteria cell fragments (Lahti et al., 2001), other studies have not reported any cyanobacteria cells in bank filtered water with the exception of (Rachman et al., 2014) in one well system with potential direct hydraulic connections to the water source. Previous research on the effectiveness of bank filtration for cyanobacterial removal focused

on the physiochemical parameters involved in filtration, including sorption (Romero et al., 2014) or the importance of the colmation layer in the removal of cells (Harvey et al., 2015). The removal of cyanobacteria through drinking water treatment processes has shown that some species of cyanobacteria are more likely than others to pass through treatment processes (Zamyadi et al., 2013

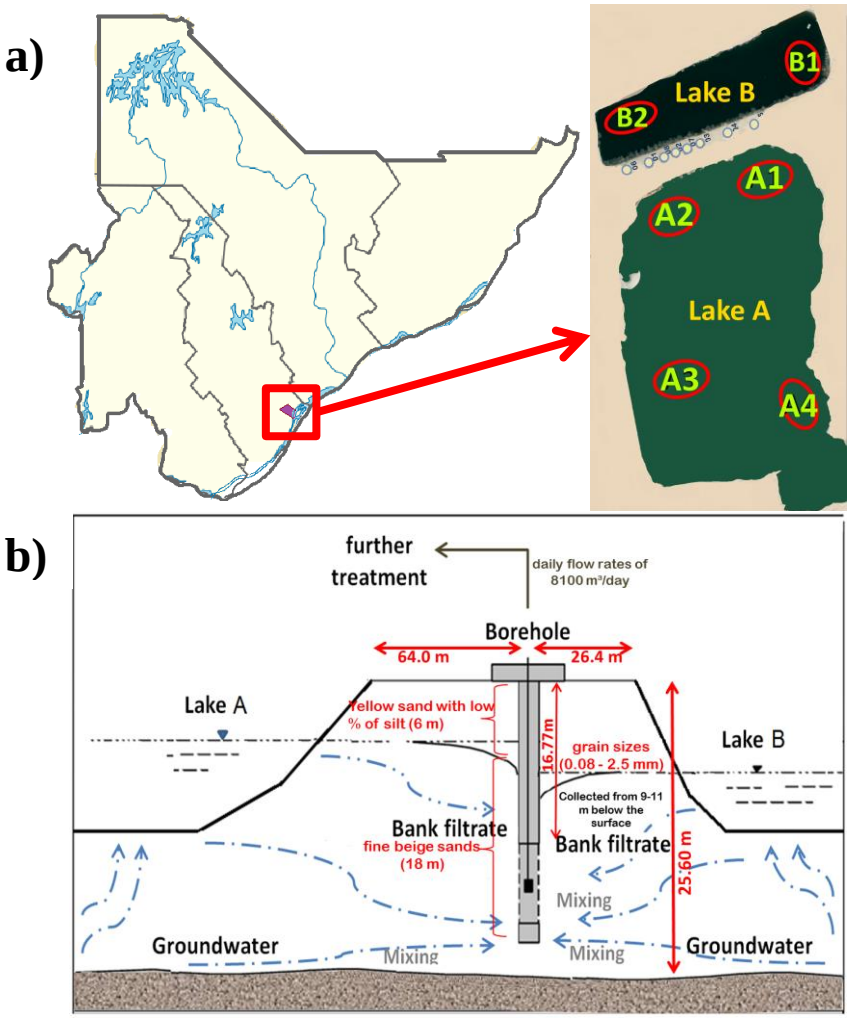


Figure 6.1: a) Location of the study site, Lake A and Lake B situated in southern Quebec, Canada; b) Schematic of bank filtration system.

c). It is unknown whether similar patterns of removal as a function of cyanobacterial species would occur in full-scale bank filtration as no studies have consistently observed the passage of cells. Furthermore, in stratified lakes, different species of cyanobacteria can be present at different depths. It is unknown whether cyanobacteria passing through bank filtration are typically benthic or planktonic species, or whether some species are more effectively removed than others. An

important short-term strategy for drinking water supplies is related to monitoring activities in support of operational decision-making. Conventional monitoring of water samples includes laboratory methods such as taxonomic analyses with cell counts and biovolume measurements and cyanotoxin analysis, triggered by the appearance of blooms in source waters or other chemical signals (Izydorczyk et al., 2009; Newcombe et al., 2010; Preez & Van Baalen, 2006). A more recent approach for monitoring cyanobacteria in source waters is based on *in situ* measurement of phycocyanin-specific fluorescence that can be used with real-time operational decision making to prevent cyanobacterial breakthrough to treated drinking water in surface water sources (Srivastava et al., 2013; Zamyadi et al., 2014; Zamyadi et al., 2012 b). However, there is a need to determine appropriate monitoring protocols for bank filtration during cyanobacterial blooms. The specific objectives were the following: 1) to estimate the efficiency of bank filtration for removing phytoplankton and bacterial indicators, 2) to determine whether there was preferential removal of certain species of phytoplankton including cyanobacteria, and 3) to provide recommendations on the use of phycocyanin probes for monitoring the fate of cyanobacteria through bank filtration.

6.2 Materials and methods

6.2.1 Study Site

The study site is a bank filtration system consisting of eight wells that pump water through the bank from two artificial lakes, A and B (Figure 6.1). Lake B was created as a result of many years of sand quarrying, and Lake A was created immediately after Lake B and remains an active sand quarry. An approximately 85 meter wide bank separates the two lakes within which are located 8 wet wells. In order to supply the population with drinking water, seven of the eight wells (with the 8^{th} as a stand-by pump) produce a mean daily flow rates of 8 100 m^3/day. Both lakes have a maximum depth of greater than 10 m. The distance of Lake A to the well field is approximately 64 m and 26 m for Lake B. Approximately 80% of the water supply comes from the southwest (Lake A) and 20% from the northeast (Lake B), as per technical information provided by municipality. The sand bank filter materials vary from grain sizes of 0.08 to 2.5 mm from samples collected near the wells. Three classes of sands are present around the wells: a) a yellow sand with a low percent of silt is present in the first 6 m layer, b) a middle layer consisting of 18 m of fine beige sands, and

c) the bottom layer (< 2 m) consisting of fine sand and silt, as per technical information provided by the municipality.

6.2.2 Water and sediment sampling and analysis

Lake and well water monitoring consisted of measurements conducted with: 1) an *in situ* YSI multi-parameter probe model YSI 6600 V2-4 (YSI, Yellow Spring, Ohio, USA) and 2) grab samples for phytoplankton taxonomic counts, cyanotoxin and nutrient concentrations. The *in situ* multi-probe measured phycocyanin (PC) (Relative Fluorescence Units (RFU)), chlorophyll *a* (µg/l or RFU), temperature (°C), specific electrical conductivity (mS), turbidity (NTU), DO (mg/l), and pH. A description of the probe and its use including calibration is provided in McQuaid et al. (2011). The phosphorus and nitrogen concentrations (total phosphorus, orthophosphate and Total Kjeldahl Nitrogen (TKN) from two measurement points in Lake A (A2 and A4), one measurement point in Lake B (B1) and well water were performed twice during the sampling period. Orthophosphates, total phosphorus, and Total Kjeldahl Nitrogen (TKN) were analysed as per Standard Methods (American Public Health Association (APHA), 2005).

Six primary measurements points were selected in the lakes, including four in Lake A: A1, A2, A3, A4 and two in Lake B: B1 and B2 (Figure 6.1). Sampling points A1 and A2 were located close to the bank; A3 was located near a stream discharging into Lake A, and A4 was a discharge from Lake A. The sampling locations B1 and B2 were at points where cyanobacteria blooms were detected in 2012 (Hydrophila, 2012). Samples were collected or measured *in situ* in both Lakes A and B in August, September and October 2013. The later sampling dates included additional sampling locations to include samples of surface scums collected from both lakes during blooms on the 30th of September and the 1st, 3rd and 15th of October 2013 at 0.5 m depth below the surface or from the surface scum. At each sampling point, the probe measured the profiles at depths of <0.5 m, 1.0 m, 5 m and 10 m of the water column and in the well water following bank filtration but prior to treatment. Grab samples were collected from near the surface (0.5 m) and in the well water. Analyses of microcystin-LR and taxonomic counts of phytoplankton were performed on all selected water samples. The samples were divided into two sub-samples for 1) taxonomic counts

and preserved with Lugol's iodine solution directly after sampling and stored at 4°C; and 2) toxin analyses and frozen prior to analysis for microcystin-LR within 7 days. Following the late summer-early fall bloom period, sediment samples were collected twice in the winter and early spring to measure the accumulation of cyanobacteria in lake sediments. Sediment samples were collected from the surface to a depth of 30 cm using a Wildco® Hand Core Sediment Sampler with 30 cm Liner Core Tube. Lake sediment core samples were taken at 10 m below the surface of both lakes on the 23rd of January and 13th of March 2014 in the presence of a stable ice cover. The samples were taken from the same point for both days on both lakes near the banks.

Microcystin-LR measurements were performed using ELISA kits (an enzyme-linked immunosorbent assay - Abraxis LLC, Pennsylvania, USA) with a detection limit of 0.15 micrograms L^{-1} MCLR eq. Cyanobacterial cells were lysed by three freeze-thaw cycles as described by (McQuaid et al., 2011) in order to measure both intra and extra cellular toxins. Taxonomic analyses were conducted in Limnology and Aquatic Environment Laboratory (GRIL) at the University of Quebec at Montreal (UQAM) using an inverted microscope as described in (McQuaid et al., 2011). Microbial indicators (total coliforms, enterococci, *E. coli* and aerobic spores) were analysed from samples collected over the course of 9 weeks from both lakes and in bank-filtered well water using Standard Methods (American Public Health Association (APHA), 2005). Water samples were collected for microbial indicators as per cyanobacterial samples.

6.2.3 Statistical analyses and log removal calculations

Probe and cyanobacteria biovolume data were lognormally distributed and were thus log transformed prior to statistical analyses. Statistical analyses (correlation analyses, linear regressions, Mann-Whitney U Tests) were conducted using the Statistica 12 software package (Statsoft, Tulsa, Oklahoma, USA). Origin6.0 (OriginLab, Northampton, England) and Microsoft Excel (Microsoft, Redmond, WA) were used as graphing and data analysis software packages. Depth profiles using interpolated phycocyanin RFU data (from the PC multi-probe) from both lakes and well water were used to estimate mean phycocyanin concentrations for each lake and were compared to RFU data from well water. A principal component analysis (PCA) was used to

compare mean biovolumes of cyanobacteria species among sites using the R program, version 3.1.3 (Borcard et al., 2011). A Hellinger transformation was applied on data for PCA analyses to reduce the asymmetry of the species distributions since the distribution was composed of heavily skewed abundance data (Legendre & Legendre, 2012). The length of a PCA vector is proportional to the importance of the descriptor to the sites.

The temporal and spatial heterogeneity of cells within the water column and throughout the lakes presents a challenge for estimating log removals. Therefore, several methods were used to calculate log removals to provide ranges of estimates. From a log reduction perspective, the efficiency of a filtration process to remove microorganisms are estimated by taking the logarithm of the ratio of influent concentration of microorganism to the effluent concentration of filtered water as shown in Equation 1.

$$Log\ removal = Log_{10}\left[\frac{influent\ \ concentration\ (from\ lakes)}{effluent\ \ concentration\ (bank\ filtered\ well\ water)}\right] \quad (1)$$

In the first method, taxonomic counts from lake and well water samples were used to estimate the phytoplankton log removal through bank filtration. The mean biovolume for each lake was calculated using the two sampling locations from Lake B and the two sampling locations closest to the bank for Lake A. Scum samples were not included in the calculation of mean Lake Biovolumes or log removals. The total biovolume from both lakes was estimated as a weighted mean based on the proportion of the flow coming from each lake (80% of flow pumped from Lake A and 20% from Lake B). Log removal for microbiological indicators used the flow weighted mean concentrations for each lake calculated from 4 sampling points (A1, A2, A3, A4) from Lake A and two sampling points (B1, B2) from Lake B. For phytoplankton, log removals were only calculated for groups detected in bank filtered water. Hence Chlorophyceae, Diatomophyceae and Dinophyceae that were not detected in filtered water were all considered as > 3 Log removal. For microbiological indicators and other regularly occurring phytoplankton groups, concentrations below detection limits in bank filtered water were assigned the value of the detection limit or the biovolume equivalent of 1 cell. A second method for calculating cyanobacterial log removal used interpolated depth-averaged probe readings in the Lakes with probe readings from the well water.

A third method considered probe readings at 10 m depth in Lake B with well water probe readings. Log removals were also compared with removals modified to include the effects of travel time (7 days for Lake A, no lag for Lake B since Lake B's travel time is less than 1 week which was generally the frequency of sampling).

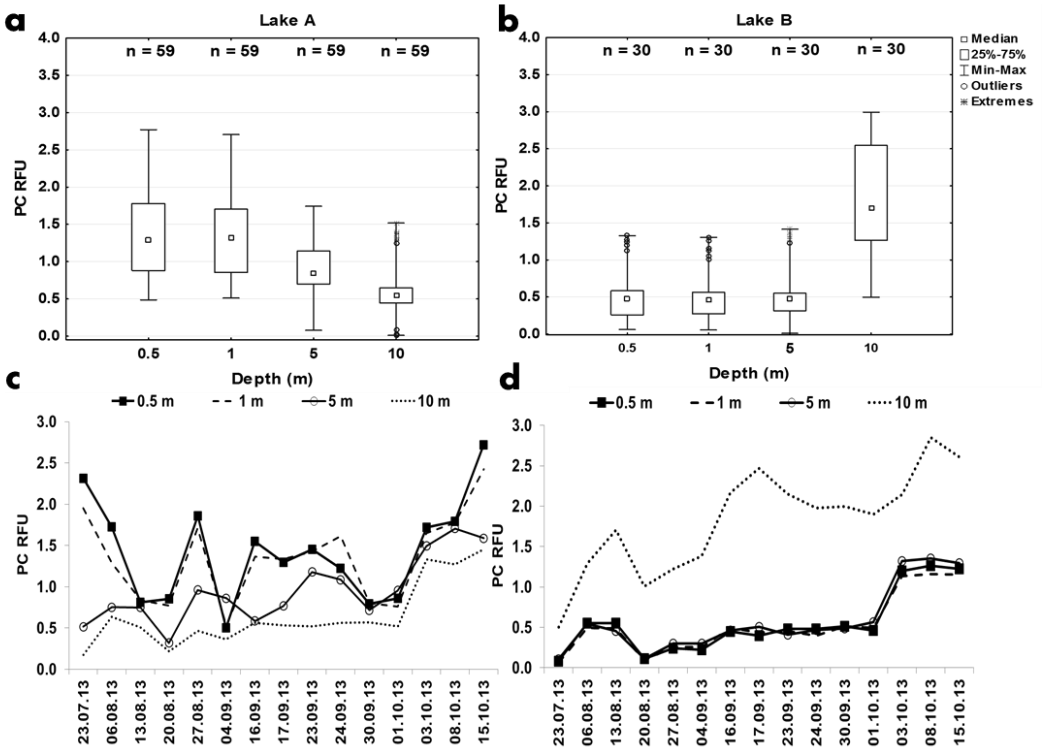
6.3 Results and discussion

6.3.1 Lake water characterization and *in situ* monitoring

Table 6.1 summarizes the results of environmental monitoring at the surface of the two lakes and environmental variable profiles measured by the probe are presented in the Supplementary Information section (Figure 6.8). From all six samples for total phosphorus in both lakes, five were under limit detection (0.01 mg L^{-1}) and only the sample collected from A4 in Lake A was above the detection limit (0.03 mg L^{-1}). The Kjeldahl nitrogen concentration was above the detection limit at points A2 (0.70 mg L^{-1}) and A4 (0.73 mg L^{-1}) in Lake A and the remaining samples were below the detection limit (< 0.4 mg L^{-1}). The higher pH in Lake A is consistent with a greater nutrient enrichment of Lake A (i.e. more photosynthesis and removal of CO_2 from the water column); however, nutrient samples were not collected throughout the period of study. *In situ* monitoring revealed similar temperature profiles in both lakes (Supplementary Information Figure 6.8). Dissolved oxygen concentrations and pH followed similar trends, but with lower overall pH in Lake B. Stratification is a key factor influencing the growth rate of cyanobacteria in many water bodies (Paerl & Otten, 2013).

Figure 6.2 summarizes cyanobacteria densities estimated by the *in situ* probes (RFU) in the two lakes sampled weekly at different depths for the period of July 27th to October 15th. Sampling was carried out during the morning (between 9 and 11 am) on Lake A and in the early afternoon (between 11 and 1 pm) in Lake B. *In situ* readings show a reverse gradient of phycocyanin RFU in the two lakes, with almost the same range of RFU value from 0.5 to 2.8 RFU in Lake A and 0.5 to 3 RFU in Lake B, with Lake B showing the highest RFU values at a depth of 10 m. The time of day of sampling could affect results (morning for Lake A versus afternoon for Lake B) as cyanobacteria can move through the water column according to light exposure. Cyanobacteria use light as a source of energy through photosynthesis. Conceptual models of cyanobacteria buoyancy

suggest that cells increase their density following light exposure and move down towards the sediments where nutrients are available (Ingrid Chorus, 2012; Kromkamp & Walsby, 1990; Newcombe et al., 2010). However, these conceptual models are overly simplistic for full-scale systems and the relationship between light and cell density is affected by other factors that have not been fully elucidated (Ndong et al., 2014). Little variation in the water column was observed in Lake B over the course of 1 day (data not shown).



6.2: PC fluorescence (RFU) at various depths in (a) Lake A and (b) Lake B for all samples combined; Time series of PC fluorescence as a mean of (c) points A1 and A2 in Lake A between July and October 2013, and (d) and of B1 and B2 in Lake B.

Figures 6.2c and 6.2d show an increase in RFU values from the beginning to the end of the monitoring period, suggesting a progressive accumulation of cyanobacteria. Dense accumulations of cyanobacteria appeared as scums and were present towards the end of the study. *In situ* PC fluorescence demonstrated statistically significant correlations between concentrations in Lakes A or B and in well water (Figure 6.3, $R > 0.86$ and $p < 0.0003$) as well as between Lake A and Lake B ($R = 0.66$, $p<0.008$). Thus, as expected, the appearance of cyanobacterial cells in well water is associated with the source water quality of both lakes and environmental factors influencing cyanobacterial growth and accumulation. The correlation was stronger between well water and

Lake B, suggesting a larger influence of Lake B water quality on well water potentially related to the shorter travel time. Even without including the scum samples, probe readings from both lakes were significantly correlated with measured cyanobacterial biovolumes ($r= 0.59$, $p<0.00001$) (Supplementary Information, Figure 6.9).

Table 6-1: General water quality characteristics of well water and Lakes A and B from the mean values of grab samples collected at 0.5 m below the surface from July to October 2013 (n=15).

Station	T (°C)	pH	DO (mg L^{-1})	Chl a (mg L^{-1})	Turbidity (NTU)	PC RFU
B1	19.4	7.8	9.9	2.84	0.7	0.6
B2	19.5	7.8	9.9	2.35	0.4	0.5
A1	18.9	8.5	11.0	8.15	5.3	1.5
A2	18.9	8.5	11.1	8.60	5.2	1.4
A3	18.9	8.5	11.0	8.50	5.3	1.4
A4	18.5	8.4	10.5	8.65	5.3	1.3
Well water	17.8	7.3	2.1	1.80	0.2	0.5

The uncertainty of probe readings as percentage errors are higher when the cyanobacterial biomass is less 1 mm^3 L^{-1} (McQuaid et al., 2011), which was the case for about 60% of the analyzed water samples during the campaign sampling. Below 1 mm^3 L^{-1}, the PC probe may underestimate the cyanobacterial biovolume by about 20%. However, the probe was able to detect the breakthrough of cyanobacteria into the wells. A useful threshold of the probe for decision-making purposes with regards to cyanobacteria breakthrough in the well was 0.6 RFU that was related to a total cyanobacteria biovolume of 0.2 mm^3/L, corresponding to Alert Level 1 as proposed by (Bartram et al., 1999). Thus, a value of 0.2 mm^3/L could be considered as the lower cyanobacteria biovolume monitoring threshold for this system. Lake A had a trend of slightly higher PC RFU values in surface layers especially at 0.5 and 1 meters (Figure 6.2a). Cyanobacteria blooms in Lake A were more dispersed throughout the lake; however, cyanobacteria blooms in Lake B were more concentrated at specific locations. The prevailing winds move from west to east, and thus tend to push blooms from Lake A towards the filter bank. Wind is an important factor influencing

cyanobacteria accumulation in Lake A. Accumulation at the water's surface near the filter bank in Lake A is of concern because Lake A is the main source of pumped water consisting of 80% of the flow (Hydrophila, 2013). Although Lake A contributes a greater proportion of flow towards the wells, travel time is also an important factor as are the specific phytoplankton characteristics that could influence cell passage through bank filtration that are discussed in Section 3.2.

6.3.2 Phytoplankton species and toxins in lakes, wells and sediments

Excluding samples from visible bloom days, the highest cyanobacteria biovolume (7.3 mm^3 L^{-1} was measured at the beginning of sampling campaign on August 20th in Lake A (Figure 6.4). The observed cyanobacteria biovolumes varied from 0.01 and 8.5 $mm^3 L^{-1}$ in Lake A and from 0.003 to 1.7 $mm^3 L^{-1}$ in Lake B (Figure 6.4). As expected, the lowest biovolume values were observed during the winter sampling on March 13 2014. A Mann-Whitney U Test comparing biovolumes with Lake A and Lake B showed that measured biovolumes from samples collected at a depth of 0.5 m were slightly higher in Lake A ($p<0.022$), which is consistent with the higher pH values (Zamyadi et al., 2012 b) from the water observed in Lake A (Table 6.1). The mean cyanobacteria biovolume during non-bloom periods of study (8 days out of 12 days of sampling) varied from 0.1 (Lake B) to 0.4 $mm^3 L^{-1}$ (Lake A). All well water samples were positive for total cyanobacteria, but had the lowest biovolume concentrations (0.02 to 0.4 $mm^3 L^{-1}$).

The cyanobacteria scum samples collected from surface during visible blooms are shown in Figure 6.5. The succession of cyanobacteria species over the course of the study is also shown in Figures 6.4 and 6.5 with overall phytoplankton succession provided in the Supplementary Information section Figures S3 and S4. Table 6-5 provides a list of the 21 separate cyanobacterial species including their mean cell volumes from all samples from Lakes A and B, well water, sediments and scums. The PCA analysis comparing cyanobacteria from all sites revealed that mean biovolumes of species found in well water were most similar to species found in Lake B as compared to Lake A or to winter sediments (Figure 6.6). The passage of cyanobacteria to well water occurred year round demonstrating that cyanobacteria were always present in the bank filter. It is possible that cells accumulate within the bank filter during blooms and continue to be released

throughout the year. No relationship was observed between well water concentrations and sediment concentrations from the lakes, but no samples were collected from deeper bank filter materials. It has been demonstrated that drinking water treatment plants with only low densities in the raw water may be vulnerable to an accumulation of cells within various treatment processes (Zamyadi et al., 2013 c). Thus, it is conceivable that bank filter materials may also serve as media for cell

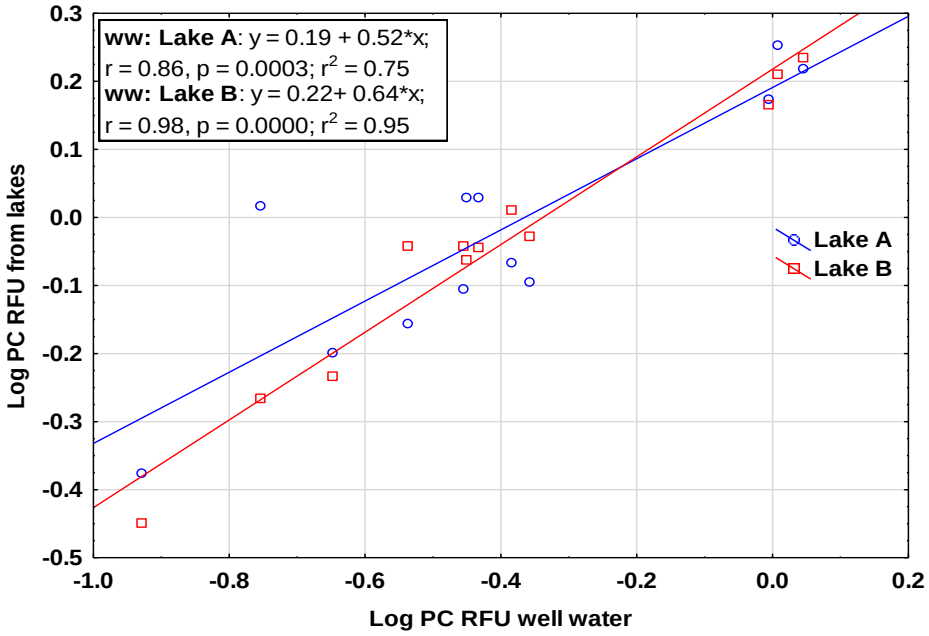


Figure 6.3: Correlations between PC RFU estimated by probes in Lakes A and B with well water. The RFU in Lake B is the log transformed mean value of interpolated probe readings from all measured depths (0.5m, 1m, 5m, and 10m) from stations B1 and B2. RFUs in Lake A are the log transformed mean of interpolated probe readings from all measured depths (0.5m, 1m, 5m, 10m) from the two points closest to wells (A1 and A2).

accumulation. Lake A is considered to contribute a greater proportion of the flow, but is located at a greater distance from the wells. These results demonstrate the importance that distance and therefore travel time plays in the removal of cyanobacterial cells. Although it is known that travel time and morphotype of cyanobacteria influence bank filtration removal, few studies have been able to suggest a distance below which cell breakthrough will occur as they have not documented the breakthrough of cells (e.g. (Romero et al., 2014). Our results show that travel times less than a week can lead to the breakthrough of cyanobacterial cells. An important aspect of this research

is the paired lake system that allows a direct comparison of cyanobacterial species from both lakes. Travel time is shorter for Lake B and the distance from the lake to the well is less than half that of Lake A. Given the greater similarity of species from Lake B and well water, the travel distance or time is more influential than relative proportion of flows or even the Lake cyanobacterial cell concentrations (as Lake A had higher concentrations and growth of cyanobacteria).

Sediment samples collected in winter and spring demonstrated that diatoms were the most common type of phytoplankton with a mean biovolume value of 0.9 $mm^3 L^{-1}$ in Lake A and 5.7 $mm^3 L^{-1}$in Lake B (Supplementary Information Figure 6.11). The presence of cyanobacteria in sediment samples (mean of 0.07 $mm^3 L^{-1}$ from Lake A and 2.02 $mm^3 L^{-1}$ from Lake B $mm^3 L^{-1}$) shows a greater accumulation of cyanobacteria at a greater depth and in sediments in Lake B (Figure 6.11). In winter well water samples, 91% of observed phytoplankton consisted of cyanobacteria (0.18 $mm^3 L^{-1}$) and diatoms were not detected (Figure 6.11), which is similar to summer and early fall samples that show the selective passage of some cyanobacterial species and not other types of phytoplankton (Figure 6.5).

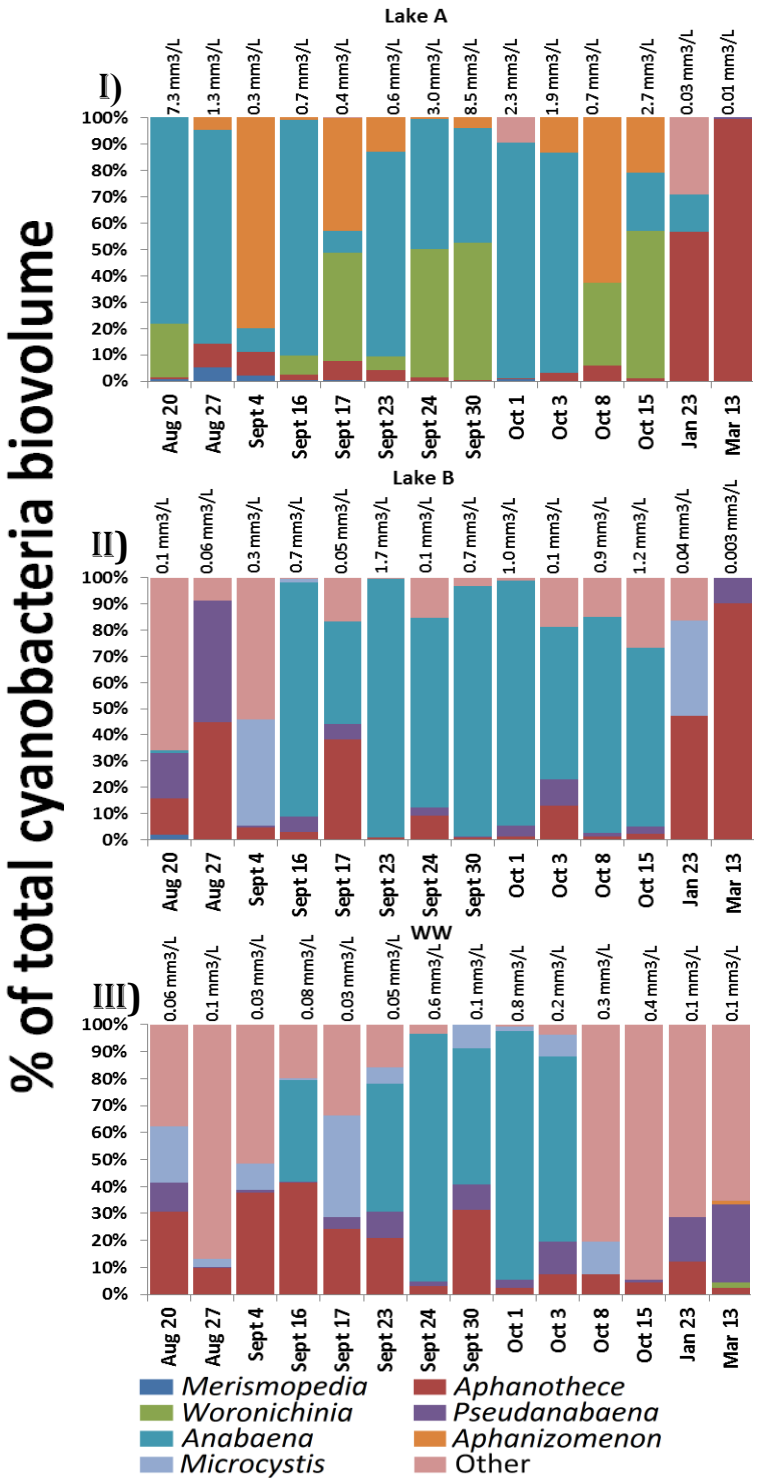


Figure 6.4: Distribution of the biovolume of cyanobacterial genera in I) Lake A, II) Lake B, III) well water samples from August 2013 to March 2014. The total cyanobacterial biovolume in mm^3/L is shown above each bar in the graph. Other genera include: *Chroococcus sp.*, *Planktolyngbya sp.*, *Coelosphaerium sp.*, *Cyanodictyon sp.*, *Cryptomonas sp.*

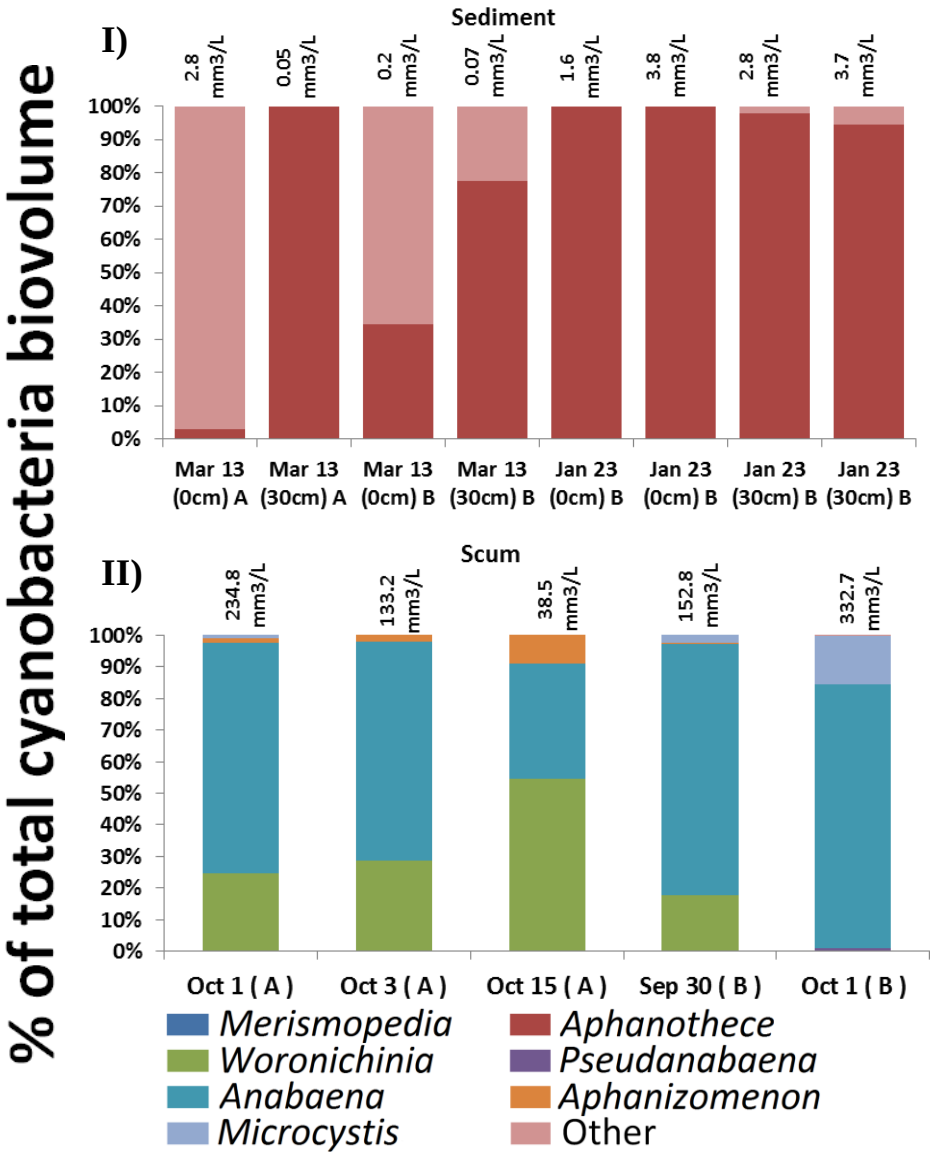


Figure 6.5: Distribution of the biovolume of cyanobacterial genera in I) sediments, II) scum. The total cyanobacterial biovolume in mm^3/L is shown above each bar in the graph. Other genera include: *Chroococcus sp.*, *Planktolyngbya sp.*, *Coelosphaerium sp.*, *Cyanodictyon sp.*, *Cryptomonas sp.*

Several of the cyanobacterial species that were present throughout the sampling period were potential toxin producers. A total of 58 water samples taken from both lakes (n=44) and well water (n=14) were analyzed for microcystin LR-eq (MCLR-eq). The total also includes all samples collected during visible blooms. The limit of detection was 0.15 µg L^{-1} and only three samples out of 58 had a MCLR-eq concentration above the detection limit. The 3 samples with MCLR-eq above

the detection limit were from scum samples collected from visible bloom areas on Lake B on the 1st (1.6 µg L^{-1} of MC LR-eq), 3rd (7.02 µg L^{-1}) and 15th (1.2 µg L^{-1} MC LR- eq) of October. *Microcystis spp.* and *Anabena spp.* were the dominant species in samples collected during the blooms on those dates. Several factors could have influenced the production of toxins by cyanobacteria, including: 1) a succession of non-toxic genotypes to toxic genotypes (Kardinaal et al., 2007), and 2) toxin production only began in toxic genotypes once sufficient

cell density was reached (Wood et al., 2011). Although no well water samples were above detection limits for microcystins, the greatest concern would be the passage of cells containing

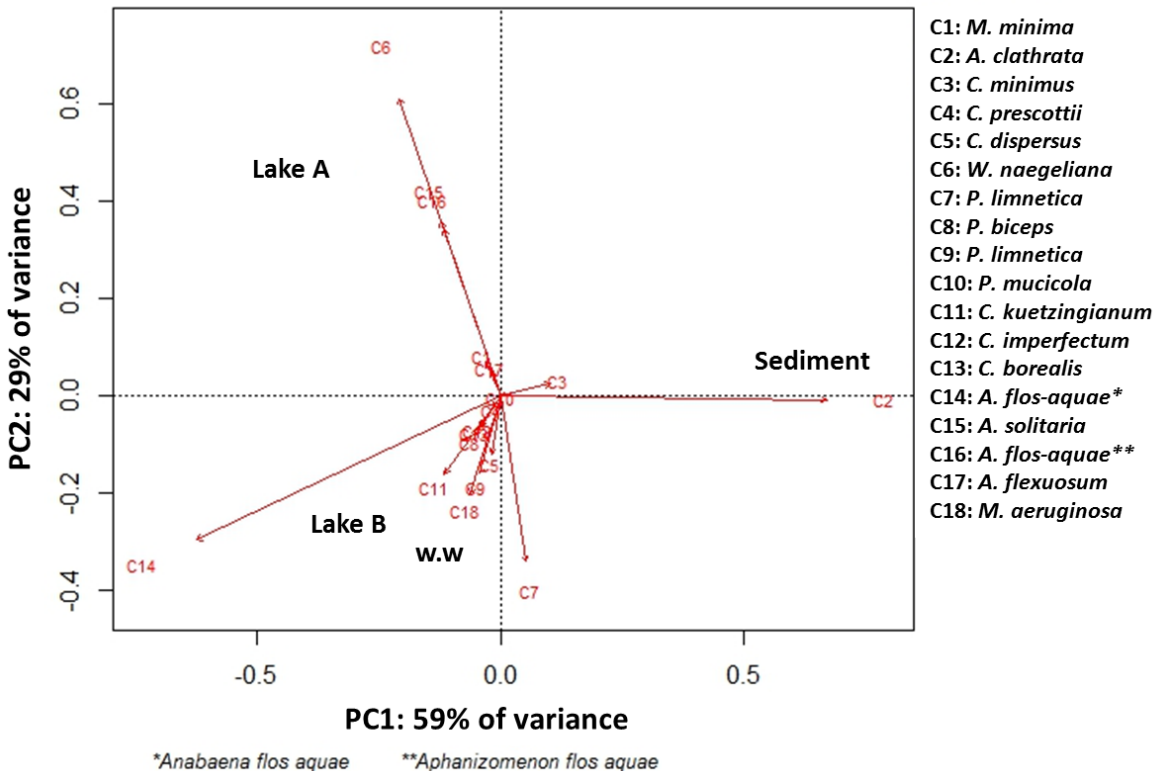


Figure 6.6: Principle Component Analysis of mean biovolumes of cyanobacteria species (mm³/L) at each site.

intracellular toxins, as most toxins are intracellular (e.g. (Zamyadi et al., 2013 c). Extracellular toxins have been shown to be removed in the first few meters of underground passage (I Chorus et al., 2004) and many cyanotoxins have been shown to sorb readily to lake sediments (Maghsoudi et al., 2015). However, (Klitzke et al., 2010) found that for cylindrospermopsin, a toxin frequently

found in extracellular form, biodegradation was a more important process than retention in filter materials.

Although no toxins were measured in well water samples, it is possible to estimate the maximum potential microcystin concentration as a worst-case scenario of toxin production using measured cell biovolumes in the wells and values of microcystin per cell from the literature. Two values for the maximum production of microcystins per cell of *Microcystis* sp. were used: 0.2 pg from the World Health Organization (WHO) (WHO, 1999) and 0.63 pg from New Zealand's Ministry for the Environment and Ministry of Health (New Zealand Ministry for the Environment and the Ministry of Health, 2009). The highest calculated maximum potential microcystin concentration in well water samples occurred on October 8 when the highest density of *Microcystis* sp. was found in well water samples (0.59 µg/L, Table 6-7) and remains below drinking water standards in Québec. The main treatment post bank filtration is chlorination. Given the presence of cyanobacteria species that potentially produce anatoxin-a, a concern is that chlorination does not effectively oxidize anatoxin-a (Zamyadi et al., 2013 c). However, no samples were collected for anatoxin-a analysis.

6.4 Log removal during bank filtration

Log removals based on observed phytoplankton biovolumes in the Lakes and in well water are presented in Table 6.2. Chlorophyceae, Diatomophyceae and Dinophyceae had ≥ 3 log removal (i.e. none were detected in well water). Other types of phytoplankton were also largely removed with Cryptophyceae and Chrysophyceae having mean log removals of 2.9 ± 0.8 and 3.53 ± 0.9, respectively (considering travel time, Table 6.2). Cyanobacteria showed the lowest removal (0.89 ± 0.5) among the various types of phytoplankton (considering travel time, Table 6.2). The variable results of cyanobacteria log removal could be explained by the heterogeneity of size and shapes of cyanobacteria species ranging in size from 0.3 μm^3 to 1570.8 μm^3 (Table 6-5). Cyanobacteria species occupy a variety of ecological niches and some are capable of regulating buoyancy, whereas others are predominantly benthic. However, even cyanobacteria that are typically found in surface scums (such as *Microcystis* sp.) were found in well water. Although cell morphotype has been suggested to be an important factor affecting cell passage through filtration (Romero et al.,

2014), we observed a significant relationship between cell size and the frequency a given cyanobacterial species was observed in well water (Spearman rank correlation, $p<0.05$, Table 6-6). Thus, of well water samples that were positive for given cyanobacterial species, the smallest cells were most frequently observed. For example, the filamentous cyanobacterium, *Anabaena*

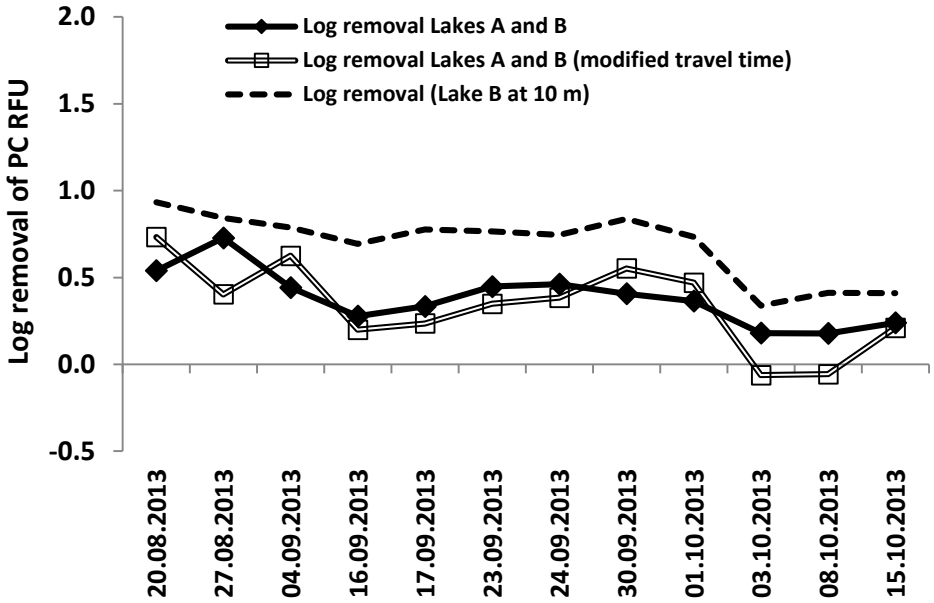


Figure 6.7: Log removal based on phycocyanin RFU passing through bank filtration from Lakes A and B with and without considering travel time using flow weighted mean interpolated probe readings from B1 and B2 for Lake B and A1 and A2 for Lake A. The dotted black line is the Log removal of mean RFU from probe readings at 10 m depth in Lake B (B1 and B2) without considering flow weighted means.

flos-aquae, was observed half as frequently in well water samples as compared to the much smaller *Aphanothece clathrata brevis* that was observed in all well water samples.

The majority of previous studies have not observed the full-scale passage of phytoplankton through bank filtration, with the exception of Rachman et al. (2014), where passage was observed for 1 of 4 well systems tested in a system with the potential for direct hydraulic connections to the seawater used for a desalination plant. Although few studies show passage of cyanobacterial cells, the passage of low concentrations of microcystins has been documented (Lahti et al., 2001), but was not the case in our study despite the passage of cells.

Table 6-2: Average log removal (± standard deviation) for cyanobacteria and other phytoplankton estimated from taxonomic counts in Lakes A and B and in well water. Concentrations of Chlorophyceae, Diatomophyceae and Dinophyceae were below detection limits in well water, thus the log removal was considered > 3 Log and are not included in the table.

	Cyanobacteria		Chrysophyceae *		Cryptophyceae **	
Date	**Est. 1**	**Est. 2**	**Est. 1**	**Est. 2**	**Est. 1**	**Est. 2**
20.08.13	2.02	***	2.82	***	3.97	***
27.08.13	1.15	1.79	3.89	3.81	2.97	2.72
04.09.13	0.96	1.66	2.52	2.10	2.05	2.79
16.09.13	0.95	0.70	3.56	3.02	3.07	2.32
17.09.13	1.16	1.00	4.10	3.83	4.27	3.88
23.09.13	1.19	1.24	4.63	4.76	3.44	3.79
24.09.13	1.62	0.90	4.95	4.57	2.87	3.01
30.09.13	1.07	-0.01	2.67	2.36	2.00	2.02
01.10.13	1.17	1.18	4.90	4.94	3.61	3.35
03.10.13	0.28	0.37	1.84	1.92	2.01	1.99
08.10.15	0.32	0.66	4.59	4.87	4.58	4.06
15.10.13	0.43	0.35	2.42	2.70	1.96	1.93
Overall mean ± SD	1.03 ± (0.5)	0.89 ± (0.5)	3.57 ± (1.0)	3.53 ± (1.1)	3.07 ± (0.9)	2.90 ± (0.8)
Cell size (μm^3)	150.7		675.5		2000.7	

Estimation 1: Log removal using flow weighted mean concentrations
Estimation 2: Modified log removal based on flow weighted mean concentrations considering travel time
*** The biovolume for well water samples values under the detection limit were calculated using the biovolume of the smallest Chrysophyceae (3.10456E-05 mm^3/L).**
**** The biovolume for well water samples values under the detection limit were calculated using the biovolume of the smallest Cryptophyceae (3.93E-04 mm^3/L).**
***** For these dates, there was no sampling campaign prior to the date thus a log removal based on travel time could not be calculated.**

Figure 6.7 presents the evolution of log removal throughout the study using the probe measurements in RFU. The use of the probe enables a more representative characterization of mean concentrations of cyanobacteria in the lakes. In addition, it was possible to determine the log removal for the system considering only the RFU values at a depth of 10 m from Lake B as being more representative of concentrations entering the bank. RFU-based log removal calculations were comparable to calculations using taxonomic counts. However, when considering only RFU concentrations at a depth of 10 m in Lake B, the log removal was slightly higher, but generally remained below 1.0.

Table 6-3: Mean log removal of microbial indicators by bank filtration computed from flow weighted mean values from Lakes A and B and concentrations in well water.

Microbial indicator	**Mean log removal (min-max) N=8**	**Mean log removal (min-max) modified by travel time* N=8**
Aerobic bacterial spores	**2.8 (2.0- 3.5)**	**2.7 (1.6 - 3.5)**
Total coliform	**3.0 (2.4- 3.4)**	**3.0 (2.8 – 3.1)**
Fecal enterococci	**2.3 (1.7 – 2.8)**	**2.1 (1.6 – 2.8)**
E. coli	**1.6 (1.1 – 1.9)**	**1.8 (1.3 – 2.1)**

***Travel time of approximately 7 days from Lake A and same day from Lake B**

Table 6.3 summarizes the performance of bank removal of all microbiological indicators. Relatively low concentrations of microbial indicators were present in the raw water and consequently their concentrations in well water were below the limit of detection with the exception of the aerobic spore-formed bacteria. Log removals of microbial indicators were higher than log removals of cyanobacteria. Given that *E. coli* were below detection limits in well water, it was useful to monitor a series of bacterial indicators since aerobic bacterial spores and total coliforms have also been shown to be appropriate indicators in filtration studies (Bauer et al., 2011; Betancourt et al., 2014; Jenkins et al., 2011).

6.5 Conclusions

Our study emphasizes the importance of having an intensive phytoplankton monitoring program to detect the potential for breakthrough of cyanobacterial bloom events through bank filtration. Previous studies have not demonstrated the passage of cells and yet this can occur even for filamentous cyanobacterial species. Results of our investigation have shown:

- Distance and therefore travel time are important factors for the removal of cyanobacteria through bank filtration. A greater similarity was observed between cyanobacteria species in Lake B with the shorter travel time and well water.
- Removal of cyanobacterial cells in bank filters with travel times between a few days to a week were frequently below 1 log.
- Removal of microbial indicators was higher than removal of cyanobacteria
- Cyanobacteria preferentially passed through bank filtration as compared to other larger types of phytoplankton.
- Smaller cyanobacterial cells were more frequently observed in wells as compared to larger cyanobacterial cells.
- The application of an online PC fluorescence probe is a useful tool for rapid monitoring of the spatial and temporal distribution of cyanobacteria.
- A threshold of 0.6 RFU can be used for identifying cyanobacteria breakthrough into well water and was associated with a total cyanobacteria biovolume of 0.2 mm^3/L, corresponding to Alert Level 1 as proposed by Bartram et al. (1999).

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SUPPLEMENTARY INFORMATION

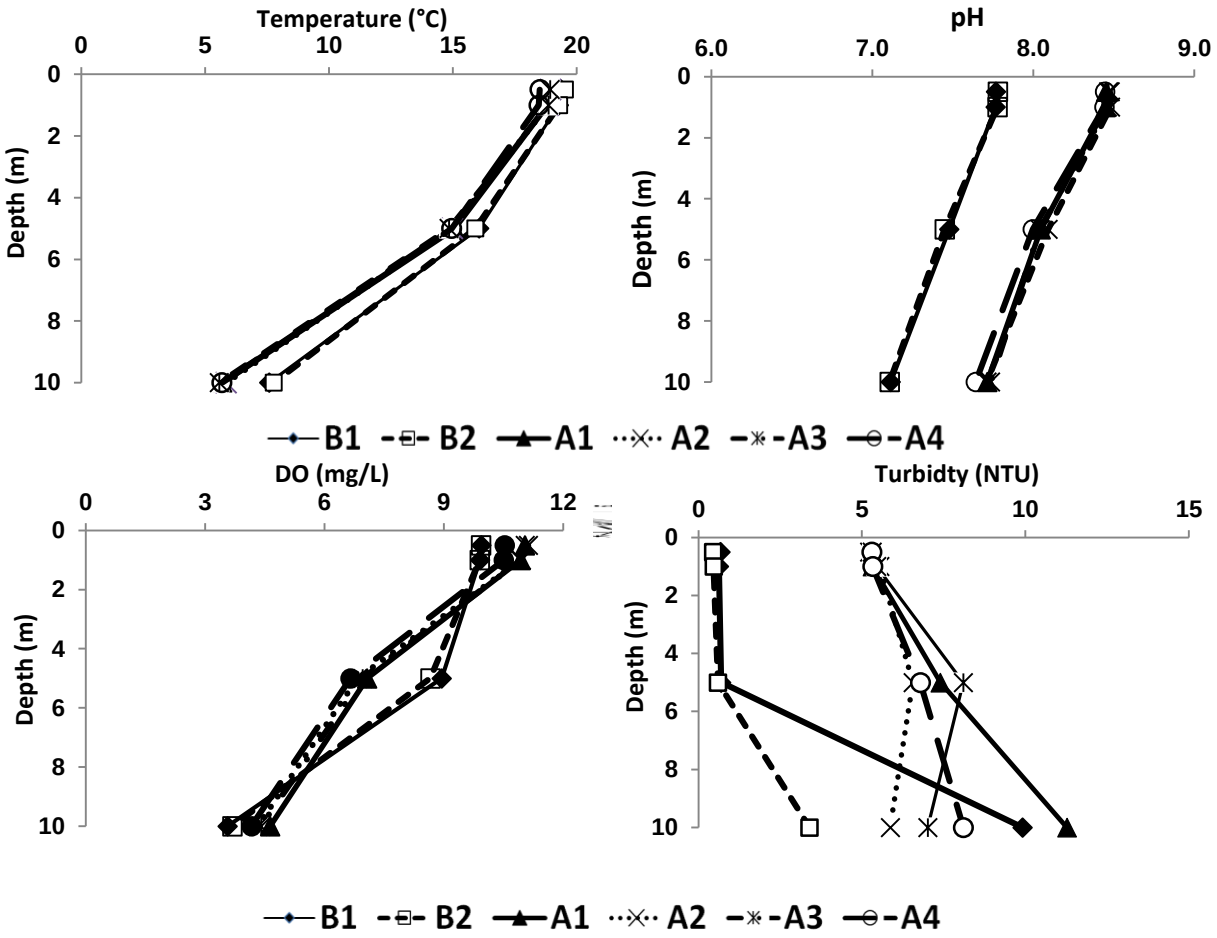

Figure 6.8: Environmental variable profiles as measured by in situ PC multi-probe from all measurement points in Lake A (A1, A2, A3 and A4) and Lake B (B1 and B2).

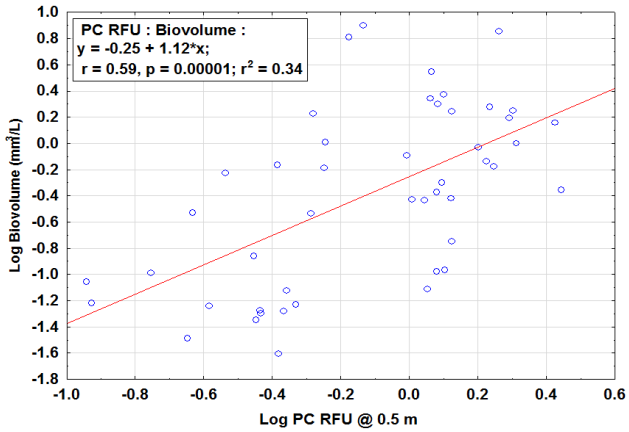

Figure 6.9: Correlation between PC RFU estimated by probes in Lakes A and B and corresponding cyanobacteria biovolume (mm3/L) from Lakes A and B.

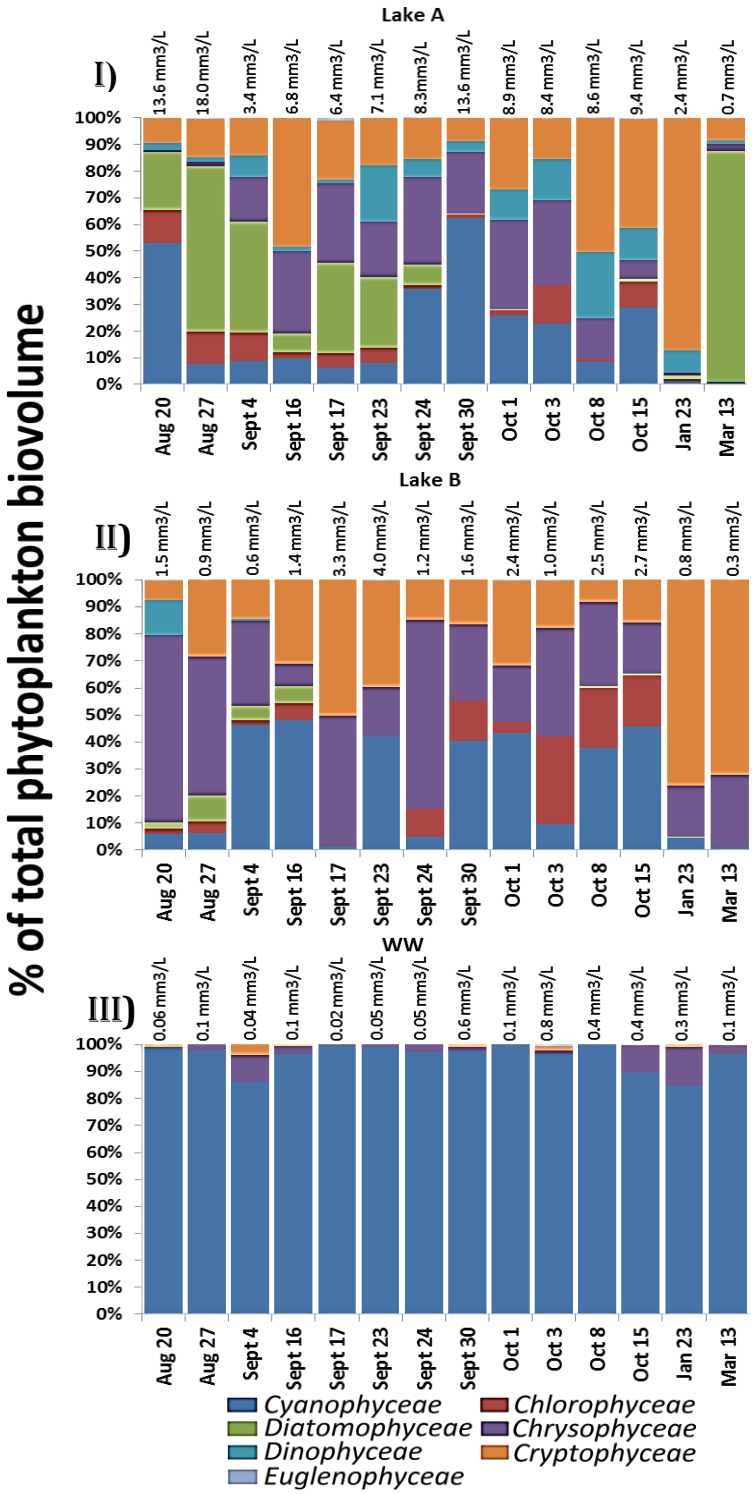

Figure 6.10: Distribution of the biovolume of plankton species in I) Lake A, II) Lake B, III) well water samples from August 2013 to March 2014. The total biovolume in mm3/L is shown above each bar in the graph.

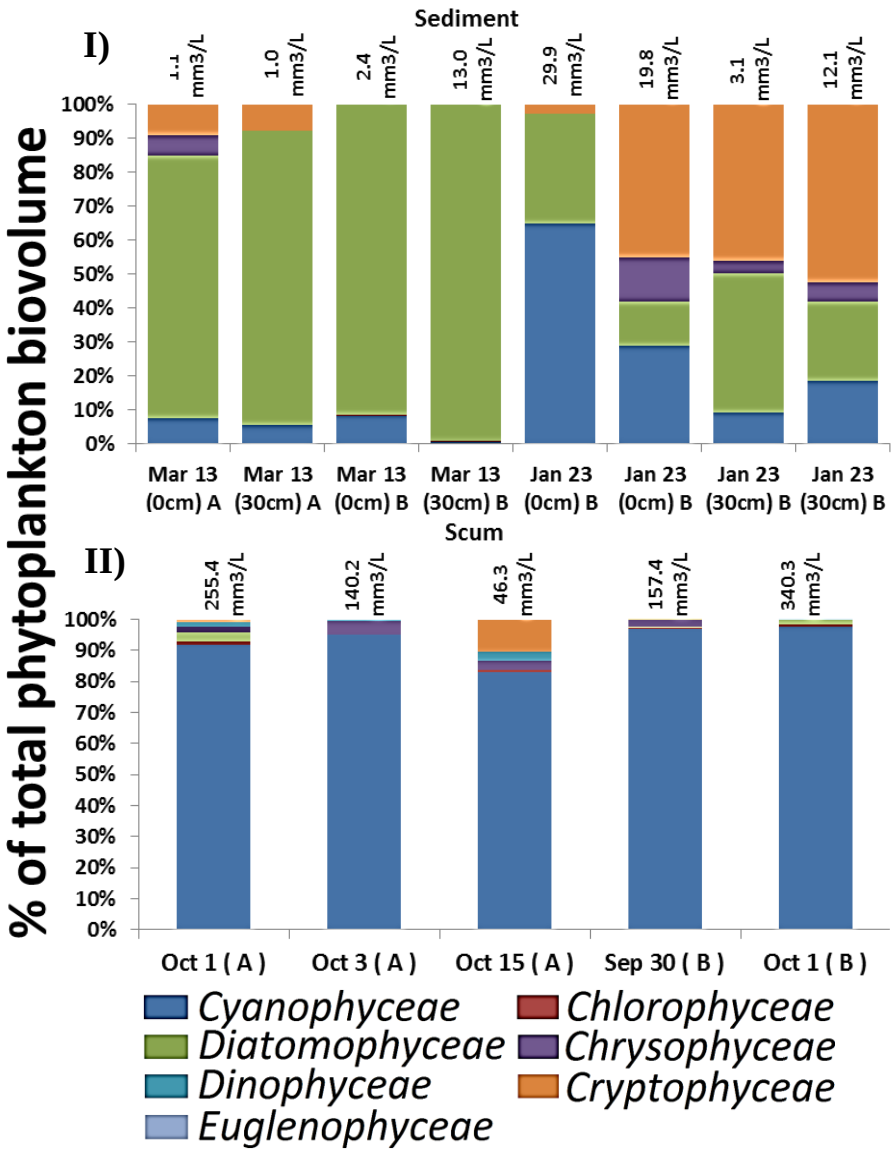

Figure 6.11: Distribution of the biovolume of plankton species in I) sediments, II) scum. The total biovolume in mm3/L is shown above each bar in the graph.

Table 6-4: Selected studies on the removal of microorganisms and toxins relevant for bank filtration.

Scale of study	Parameter studied	Comments	References
Field study	*Synechococcus sp.*	Most removal of cyanobacteria occurs in the colmation layer of natural bank filtration (82% in top 3 cm of layer).	**(Harvey et al., 2015)**
Field study	Benthic microalgal and meiofaunal assemblages	The colmation layer in bank filtration is a highly active and dynamic biological zone. Clogging of the layer is reversible.	**(Hoffmann & Gunkel, 2011)**
Field and column study	Bloom dominated by *Cylindrospermopsis raciborskii*	No cyanobacterial cells or cell fragments were observed in bank filtered well water, cyanotoxins were below detection limits in well water. Cell removal was 95% in top 2 cm of infiltration.	**(Romero et al., 2014)**
Field study	*Prochlorococcus sp., Synechococcus sp., Cyanobacteria, and Pico/ nanoplankton*	Bank filtration prior to desalination showed that wells with larger distances from the sea did not have cyanobacterial breakthrough but breakthrough occurred in a horizontal well system with potential hydraulic connections to the source.	**(Rachman et al., 2014)**
Field study	*Limnothrix redekei, Oscillatoria subbrevis, Pseudanabaena catenata, Aphanizomenon sp. Spirulina sp.*	A bank filtration production well located 65 m from a river in Brazil did not demonstrate passage of cyanobacterial cells.	**(Freitas et al., 2012)**
Column studies	Microcystin	Physical filtration of cells was important for microcystins removal in slow sand filtration. Little retardation of microcystins from sorption; biodegradation was the more important process as compared to sorption.	**(Grützmacher et al., 2009)**

Table 6.4: Selected studies on the removal of microorganisms and toxins relevant for bank filtration. (continued)

Scale of study	Parameter studied	Comments	References
Column study	Microcystin-LR	A lab cultured strain of *Sphingomonas sp*. was capable of degrading microcystin LR in slow sand filtration	**(Bourne et al., 2006)**
Column study	Cylindrospermopsin	Cylindrospermopsin degradation only occurred in the sediment, not in the water body. Degradation is more important than retention for the sediments tested.	**(Klitzke et al., 2010)**
Field study	Microcystins	Cyanobacterial cells were generally absent from bank filtered water although some coccoid cells were found at one plant with filamentous cyanobacterial cell fragments. Microcystins were detected in bank filtered water.	**(Lahti et al., 2001)**
Field study	F-specific RNA bacteriophages Somatic coliphages Enteric viruses Total coliforms Spores of sulphite reducing clostridia Fecal streptococci	A literature review that includes the log removals of microbiological indictors from several sites including travel times and distances. All microbiological indictor removals were > 3 log. The largest log removals were for a site with the greatest travel distance. The shorter travel distances and times had lower log removals.	**(Tufenkji et al., 2002)**
Field study	Total and fecal coliforms	Overall log removals of coliforms were > 4 log for travel times of as low as 1-2 days for some of the wells.	**(Dash et al., 2008)**
Field study	Total and fecal coliforms	Bank filtration led to a 2 to 4 log reduction of coliforms for a travel time of 28 to 35 days.	**(Thakur et al., 2013)**
Field study	Aerobic and anaerobic spore forming bacteria Male-specific and somatic bacteriophage Total coliforms	Spore forming bacteria were reduced by 0.4 to > 4.9 logs and bacteriophages by more than 2.1 logs. Total coliforms were rarely detected in well waters with mean log reductions of > 5 logs.	**(Weiss et al., 2005)**

Table 6-5: Mean cyanobacteria cell volumes from all sites during the period of study.

Cyanobacterial genus	Cyanobacteria species	Mean cell volume (µm^3)
Anabaena	*A. flos-aquae*	251.4
	A. solitaria planctonica	
	A. solitaria solitaria	
	A. flos-aquae treleasii	
Aphanizomenon	*A. flos-aquae*	65.2
	A. flexuosum	
Microcystis	*M. aeruginosa*	49.5
Aphanothece	*A. clathrata brevis*	2.9
	A. clathrata	
Chroococcus	*C. prescottii*	45.4
	C. dispersus	
	C. minimus	
Cyanodictyon	*C. imperfectum*	0.5
Merismopedia	*M. minima*	0.3
Woronichinia	*W. naegeliana*	67.9
Coelosphaerium	*C. kuetzingianum*	9.4
Pseudanabaena	*P. biceps*	13.7
	P. limnetica	
	P. mucicola	
Cryptomonas	*C. borealis*	1570.8
Planktolyngbya	*P. limnetica*	11.0

Table 6-6: Frequency of positive detection of cyanobacteria species in well water samples as a function of cell size.

Species observed in well water	**Cell size (μm^3)**	**Frequency**
Aphanothece clathrata brevis	0.5	1.0
Aphanothece clathrata	5.3	1.0
Pseudanabaena limnetica	8.5	0.92
Planktolyngbya limnetica	11.1	1.0
Chroococcus dispersus	14.1	0.58
Pseudanabaena biceps	23.8	0.08
Microcystis aeruginosa	42.4	0.83
Anabaena flos-aquae	183.3	0.50

Table 6-7: Maximum potential concentration of microcystin (µg/L) in well water calculated based on two scenarios of New Zealand MPMC **(New Zealand Ministry for the Environment and the Ministry of Health, 2009)** and WHO MPMC **(WHO, 1999)** and measured *M. aeruginosa* abundance (cell/mL) in well water.

Date	*M. aeruginosa* (cell/mL)	Maximum potential production of microcystin µg/L **(New Zealand Ministry for the Environment and the Ministry of Health, 2009)**	Maximum potential production of microcystin µg/L **(WHO, 1999)**
20.08.2013	375.10	0.24	0.08
27.08.2013	93.77	0.06	0.02
04.09.2013	93.77	0.06	0.02
12.09.2013	93.77	0.06	0.02
16.09.2013	18.12	0.01	0.00
17.09.2013	281.32	0.18	0.06
23.09.2013	93.77	0.06	0.02
24.09.2013	0.00	0.00	0.00
30.09.2013	375.10	0.24	0.08
01.10.2013	468.87	0.30	0.09
03.10.2013	375.10	0.24	0.08
08.10.2013	937.75	0.59	0.19
15.10.2013	0.00	0.00	0.00

CHAPTER 7 GENERAL DISCUSSION - PERFORMANCE OF ONLINE PHYCOCYANIN MONITORING TO ESTABLISH LOCAL ALERT LEVELS FOR CYANOBACTERIA

7.1 Local alert level thresholds

Phycocyanin showed a strong correlation with a variety of bloom-related factors, particularly the most abundant cyanobacteria species' biovolume and total cyanobacteria biovolume (Figure 5-6). This enables an adaptation of the current alert system criteria based on phycocyanin concentrations and cyanobacteria cell counts to local conditions (McQuaid et al., 2011).

Based on the WHO guideline value for microcystins in the drinking source waters, the maximum potential microcystin concentrations (MPMC) were calculated and are presented in Table 6-1. In Lake Erie the estimated MCMP for biovolumes of 0.4 to 1 $mm^3 L^{-1}$ are equivalent of 2.2 to 36 µg L^{-1} of microcystins. In Table 7-1, the new alert level threshold has been calculated based on the linear regression from Figure 5-6 (regression between cyanobacteria true density biovolume and the phycocyanin measured by *in situ* PC probe). The new threshold alert levels were obtained at a cyanobacteria biovolume of 0.4 to 1 $mm^3 L^{-1}$ as the lower biovolume threshold. The new threshold is comparable to the McQuaid et al. (2011) alert level but demonstrates that the probe readings and taxonomic cell counts for Lake Erie were generally lower than at drinking water intake on Missisquoi Bay where the threshold was initially developed.

The new threshold is equivalent to 0.5 to 1.5 RFU as biovolumes ranged from 0.4 to 1 $mm^3 L^{-1}$ respectively (Table 7-1). Furthermore, the equivalent PC RFU for the two main global alert levels established by the WHO ($0.2 < CB < 10\ mm^3 L^{-1}$) and Newcombe et al (2010) ($0.2 < CB < 0.6\ mm^3 L^{-1}$) was calculated based on the regression between cyanobacteria biovolume and PC RFU from probe readings ($y = 0.069+0.6131*x$). The threshold of alert level 2 are CB >10 $mm^3 L^{-1}$ (WHO, 1999) and CB > 6 $mm^3 L^{-1}$ (Newcombe et al., 2010) which are equivalent to 16.2 RFU and 9.7 RFU respectively. It is important to note that all these alert level thresholds for cyanobacteria biovolumes were developed on the basis of the assumption that the principal bloom-formers are cyanobacteria,

and that most abundant genre are content of cyanobacteria potentially toxic species (Ahn et al., 2007).

Table 7-1 Estimated RFU equivalent values and maximum potential microcystin concentrations (MPMC) linked to cyanobacterial biovolume thresholds

Guideline references	**Alert level content ($mm^3 L^{-1}$)**	**Cyanobacteria biovolume threshold values ($mm^3 L^{-1}$)**	**Calculated RFU values based on R^2 : 0.41 ($y = 0.1892+0.5562*x$)[1]**	**MPMC ($\mu g L^{-1}$)**
(WHO, 1999)	Alert level 1 biovolume	0.2 < CB < 10	0.02 < RFU < 17.6	2.2 < MPMC < 110.1
	Alert level 2 biovolume	CB >10	RFU > 17.6	MPMC > 110.1
(Newcombe et al., 2010)	Alert level 1 biovolume	0.2 < CB < 0.6	0.02 < RFU < 0.74	2.2 < MPMC < 6.6
	Alert level 2 biovolume	0.6 < CB < 6	0.74 < RFU < 10.4	6.6 < MPMC < 66.1
	Alert level 3 biovolume	CB > 6	RFU > 10.4	MPMC > 66.1
(McQuaid et al., 2011)	Lower biovolume	1	1.5	11.0
Lake Erie local threshold	Selected biovolume threshold [2]	0.4 < CB < 1	0.4 < RFU < 1.5	4.4 < MPMC < 11.0

[1] The formula was derived from the regression graph in Figure 5-6.
[2] The lower biovolume for Lake Erie derived from McQuaid lower biovolume (1 $mm^3 L^{-1}$) and 0.4 $mm^3 L^{-1}$ as lower biovolume for Lake Erie selected based on mean biovolume from total cyanobacteria cell counts in Lake Erie.

7.2 Applying the local Alert level threshold to Lake Erie

This study shows the feasibility of using fluorescence probes as a rapid assessment tool for determining cyanobacterial density in the drinking water systems despite concentrations near lower detection limits for the probe. Figure 7-1 shows local alert level thresholds values similar to those of Newcombe et al., 2010.

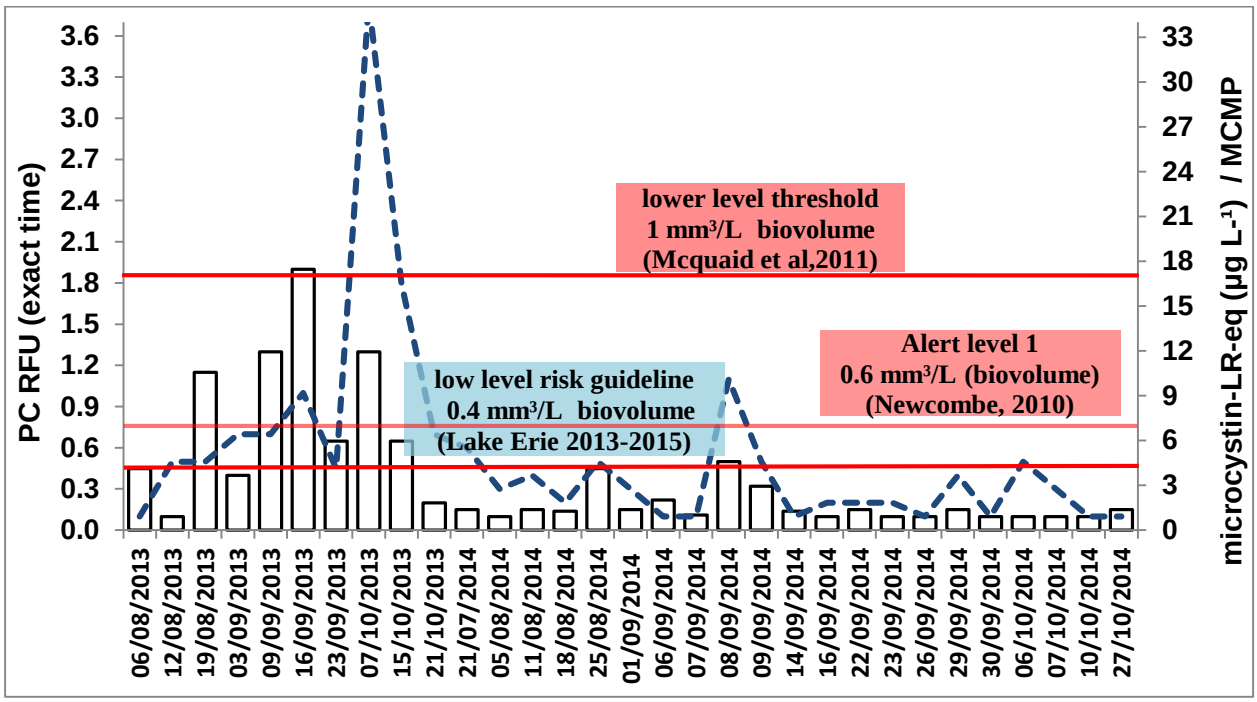

Figure 7.1: Application of the local RFU threshold and comparison with other alert levels. The dashed lines represent PC RFU concentration measured by in situ PC probe during the study period. The box plots indicate the concentration of Microcystin-LR eq. µg.L^{-1} as measured by ELISA kit. The second Y axis represents the maximum potential microcystin concentrationµg.L^{-1} calculated based on cyanobacteria biovolume thresholds.

CHAPTER 8 CONCLUSIONS AND RECOMMENDATIONS

This study investigated the feasibility of using a PC probe as a rapid assessment tool to monitor the dynamic cyanobacteria conditions in two drinking water systems. The first system was on Lake Erie, Ontario and the second was in Southern Québec. In contrast to conventional laboratory monitoring methods, which are costly and time consuming, the online PC fluorescence was shown to be a useful tool for rapid monitoring of cyanobacteria in water bodies. Satisfactory correlations existed between cell biovolumes and PC probe measurements in Lake Erie and the two source water lakes in Quebec ($r \geq 0.6$, $p<0.00001$).

The Alert Level threshold initially proposed by McQuaid et al. (2011) is applicable for both Lake Erie and for the bank-filtered water system. However, the lower concentrations and significant regressions enabled a lowering of the threshold for the Lake Erie drinking water supply that would facilitate action to be taken in the plant as soon as the threshold is passed. Examples of actions to be taken can include modifying doses of chemicals used in drinking water treatment. The threshold approach was also useful for the bank filtration system and could be used to ensure that additional monitoring such as toxin analyses of treated water be conducted as soon as the threshold is passed.

The large number of measurement points using the probe allowed for a more detailed assessment of the efficiency of bank filtration. Given the large spatial heterogeneity, the probe enabled more robust calculations of lake concentrations for log removal estimates. Finally, the taxonomic counts in well water demonstrated that the lake with the shortest travel time was the more important source of cyanobacteria in the well. Thus, travel time was an important factor for the log removal of cyanobacteria.

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APPENDICES

APPENDIX A – MOST COMMON CYANOBACTERIA GENERA

Most common toxigenic species of cyanobacteria	Toxin groups	Toxins and other metabolites	Organ affected	Health issue
Microcystis, Anabaena, Planktothrix *Anabaenopsis, Aphanizomenon, Nostoc, Hapalosiphon*	hepatotoxins Microcystins	Microcystins Nodularin	Liver	Abdominal pain Vomiting and diarrhea Liver inflammation and hemorrhage Acute pneumonia Acute dermatitis Kidney damage Potential tumor growth promotion
Nodularia, Anabaena, Planktothri, (Oscillatoria), Aphanizomenon	hepatotoxins Nodularin			
Cylindrospermopsis, Aphanizomenon, Anabaena, Lyngbya, Rhaphidiopsis, Umezakia	Alkaloids	Cylindrospermopsin		
Anabaena, Planktothrix, Aphanizomenon, Cylindrospermopsis, Oscillator		Anatoxin-a	Nervous System	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death
Anabaena Aphanizomenon, Lyngbya, Cylindrospermopsis		Saxitoxins	Nerve axons	
Lyngbya, Schizothrix, Planktothrix (Oscillatoria)		Aplysiatoxins	Skin	possible tumour promoter

APPENDIX B – MAXIMUM POTENTIAL OF MICROCYSTIN CONCENTRATION

The estimation of maximum potential concentration of microcystin (MPMC) produced by equivalent *Microcystis aeruginosa* based on maximum microcystin content established by New Zealand environmental ministry and WHO guidelines.

Date	Total CB biovolume (mm^3/L)	Number of cells of *Microcystis aeruginosa* (cell/mL)	MPMC µg/L **(New Zealand Ministry for the Environment and the Ministry of Health, 2009)**	MPMC µg/L **(WHO, 1999)**
25/09/2012	0.66	7783.3	4.9	1.6
28-09-2012	0.17	1687.9	1.1	0.3
04/10/2012	0.29	3000.8	1.9	0.6
11/10/2012	0.12	1312.8	0.8	0.3
20/10/2012	0.16	1500.4	0.9	0.3
13/11/2012	0.07	750.2	0.5	0.2
24-06-2013	0.13	844.0	0.5	0.2
01-07-2013	0.06	375.1	0.2	0.1
15-07-2013	0.20	656.4	0.4	0.1
22-07-2013	0.19	375.1	0.2	0.1
28-07-2013	0.31	1875.5	1.2	0.4
01-08-2013	0.04		0.0	0.0
06-08-2013	0.16	937.7	0.6	0.2
12-08-2013	0.48	750.2	0.5	0.2
19-08-2013	0.28	1500.4	0.9	0.3
21-08-2013	0.44	2250.6	1.4	0.5
03-09-2013	1.95	1406.6	0.9	0.3
09-09-2013	0.21	3282.1	2.1	0.7
16-09-2013	0.45	3000.8	1.9	0.6
23-09-2013	0.06	937.7	0.6	0.2
07-10-2013	2.81	53264.1	33.6	10.7
15-10-2013	1.22	33946.5	21.4	6.8
21-10-2013	0.21	3938.5	2.5	0.8
09-11-2013	0.10		0.0	0.0
14-11-2013	0.09	937.7	0.6	0.2
21-07-2014	0.13	468.9	0.3	0.1
05-08-2014	0.28	468.9	0.3	0.1
11-08-2014	0.09	562.6	0.4	0.1
18-08-2014	0.14	1312.8	0.8	0.3
25-08-2014	0.12	750.2	0.5	0.2
01/09/2014	0.11	1031.5	0.6	0.2
05-09-2014	1.02	7408.2	4.7	1.5
06-09-2014	0.77	7314.4	4.6	1.5
07-09-2014	0.82	6376.7	4.0	1.3
08-09-2014	0.18	1219.1	0.8	0.2
09-09-2014	0.59	5720.3	3.6	1.1
12-09-2014	0.82	10596.6	6.7	2.1
13-09-2014	0.80	9658.8	6.1	1.9

Date	Total CB biovolume (mm^3/L)	Number of cells of *Microcystis aeruginosa* (cell/mL)	MPMC µg/L **(New Zealand Ministry for the Environment and the Ministry of Health, 2009)**	MPMC µg/L **(WHO, 1999)**
14-09-2014	0.67	6095.4	3.8	1.2
16-09-2014	0.47	5720.3	3.6	1.1
22-09-2014	0.79	7502.0	4.7	1.5
23-09-2014	0.38	4126.1	2.6	0.8
26-09-2014	0.33	4219.9	2.7	0.8
29-09-2014	0.23	3000.8	1.9	0.6
30-09-2014	0.75	4970.1	3.1	1.0
06-10-2014	1.09	15379.1	9.7	3.1
07-10-2014	0.33	4126.1	2.6	0.8
10-10-2014	0.13	1312.8	0.8	0.3
16-10-2014	0.12	1500.4	0.9	0.3
17-10-2014	0.07	656.4	0.4	0.1
18-10-2014	0.05	375.1	0.2	0.1
20-10-2014	0.06	468.9	0.3	0.1
27-10-2014	0.03	281.3	0.2	0.1

APPENDIX C – ENVIRONMENTAL VARIABLES MEASURED BY PC PROBE