

Titre: Real-Time Cyanobacteria Monitoring in Water Supply Systems to
Manage Risks to Drinking Water Quality

Auteur: Liya Ma
Author:

Date: 2023

Type: Mémoire ou thèse / Dissertation or Thesis

Référence: Ma, L. (2023). Real-Time Cyanobacteria Monitoring in Water Supply Systems to
Manage Risks to Drinking Water Quality [Thèse de doctorat, Polytechnique
Montréal]. PolyPublie. <https://publications.polymtl.ca/55223/>

 **Document en libre accès dans PolyPublie**
Open Access document in PolyPublie

URL de PolyPublie: <https://publications.polymtl.ca/55223/>
PolyPublie URL:

**Directeurs de
recherche:** Michèle Prévost, Sarah Dorner, & Arash Zamyadi
Advisors:

Programme: Génie civil
Program:

POLYTECHNIQUE MONTRÉAL

affiliée à l'Université de Montréal

**Real-time cyanobacteria monitoring in water supply systems to manage risks
to drinking water quality**

LIYA MA

Département des génies civil, géologique et des mines

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

Génie civil

Juin 2023

POLYTECHNIQUE MONTRÉAL

affiliée à l'Université de Montréal

Cette thèse intitulée :

Real-time cyanobacteria monitoring in water supply systems to manage risks to drinking water quality

présentée par **Liya MA**

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

a été dûment acceptée par le jury d'examen constitué de :

Benoit BARBEAU, président

Michèle PRÉVOST, membre et directrice de recherche

Sarah DORNER, membre et codirectrice de recherche

Arash ZAMYADI, membre et codirecteur de recherche

Émilie BÉDARD, membre

Dolores PLANAS, membre externe

DEDICATION

To my parents for their endless love and support throughout my education

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my supervisor Prof. Michèle Prévost for offering me the opportunity to work in her team. This thesis would not have been possible without her constructive feedback, insightful guidance, and warm encouragement. Her gentle words and smiles consistently encouraged me throughout my study.

I also appreciate Dr. Arash Zamyadi, who guided me at the early stage of my project. Thanks for the tremendous help. Also, I would like to thank my co-supervisor Prof. Sarah Dorner for the priceless advice and continuous support throughout my Ph.D.

I acknowledge the members of my committee, Prof. Benoit Barbeau, Prof. Dolores Planas, and Prof. Émilie Bédard for examining my thesis.

Special thanks to Juan Francisco Guerra Maldonado for his kind support on data analysis and graph plotting.

I am deeply thankful to the Chair staff, for their valuable professional support. Particularly, Tetiana Elyart and Yves Fontaine for maintaining the probe system. I enjoyed all those hours of sampling with them. I would like to thank, Jacinthe Mailly and Julie Philibert, for all their lab analysis and great advice. I am thankful the secretary of CGM, Laura Razafinjanahary.

I would like to thank Génome Québec and Genome Canada for their financial support, which enabled me to conduct this research.

Without all my colleagues and friends, all the hours spent in the field, lab and office would not have been enjoyable. Saber, Farhad, Thien-Kim, Pegah, Luan, and Léonie. I was lucky to have you beside me throughout this journey.

Most of all, I am grateful to my boyfriend Amir for his unconditional support. I also thank my beloved family for their support during my graduate studies.

RÉSUMÉ

Dans le monde entier, il y a eu une augmentation de la présence de proliférations des cyanobactéries (CB) potentiellement toxiques dans les sources d'eau potable et dans les usines de traitement de l'eau potable (UTEP). Les méthodes conventionnelles de surveillance de l'abondance de CB sont fastidieuses, chronophage et nécessitent des opérateurs hautement qualifiés et expérimentés. De plus, ces méthodes ne sont pas toujours efficaces à capturer les fluctuations de la densité de CB. Cela pourrait entraîner un délai entre l'échantillonnage ponctuel et les ajustements de traitement correspondants. Par conséquent, ce retard entrave la capacité des opérateurs à réagir à la formation et à l'atténuation des proliférations de CB.

Des sondes de fluorescence *in situ* en temps quasi réel pour la détection rapide du phycocyanine (PC), un pigment spécifique aux cyanobactéries, ont été efficacement déployées comme moyen pour déterminer l'abondance de CB dans les sources d'eau potable. L'apparition d'efflorescences peut affecter l'efficacité à éliminer les CB, entraînant une accumulation des CB et des cyanotoxines dans divers processus de UTEP. Jusqu'à présent l'adoption des sondes est relativement moins appliquée à l'intérieur des stations d'épuration à grande échelle.

Cette recherche a étudié l'application de la sonde de fluorescence PC *in situ* comme outil de surveillance en temps réel du CB à la prise d'eau et dans les stations d'épuration. La recherche contient trois thèmes principaux: (1) les interférences associées aux lectures de la sonde; (2) l'interprétation du risque CB via l'utilisation des sondes *in situ* au sein des UTEP; et (3) la gestion des efflorescences à la source et dans UTEP.

La première partie de l'étude consistait à étudier les facteurs affectant l'interprétation des lectures de fluorescence PC *in situ*. L'interférence de la Suwannee River NOM (SRNOM) et de la température de l'eau a été réalisée avec (1) des espèces de CB cultivées en laboratoire et/ou (2) des pigments de PC extraits. Les résultats ont montré qu'une teneur élevée en SRNOM dans l'eau pouvait entraîner une sous-estimation des lectures des PC liées aux cellules des CB. La matrice d'excitation-émission de fluorescence (EEM) a confirmé la diminution de l'intensité de fluorescence cellulaire causée par l'ajout de SRNOM. Nous avons également constaté que les lectures des sondes PC peuvent être considérablement affectées par la température dans la plage de leur application typique (6 à 33 ° C), et ces interférences pourraient être réduites en appliquant les algorithmes de correction. De plus, des échantillons de terrain provenant d'une prise d'eau d'une

UTEP ont également été évalués. Nous avons constaté que la corrélation entre les lectures de la sonde et le nombre des cellules taxonomiques des échantillons de terrain n'est pas fiable, et seules les estimations basées sur le biovolume pourraient être pertinentes à des fins de gestion. Une mesure simultanée des autres paramètres physico-chimiques par la sonde peut aider à augmenter la précision des lectures du PC.

La deuxième partie de cette étude visait à valider l'utilisation de sondes *in situ* dans les UTEP à grande échelle. Des sondes PC *in situ* ont été conçues pour le contrôle à distance et l'enregistrement des données afin de surveiller le niveau de CB dans l'eau brute, clarifiée, filtrée et traitée dans trois stations d'épuration à grande échelle (comprenant à la fois un risque CB élevé et faible). Une sonde complémentaire a été implémentée pour mesurer le surnageant des boues dans le bac de stockage des boues de l'UTEP à risque. Les lectures de PC dans l'eau brute de l'usine à risque élevé de CB ont montré une fluctuation de 93 fois en 24 heures (de 0,6 RFU à 55,8 RFU), avec des niveaux de PC plus élevés pendant la période de l'après-midi. Ces données indiquent que sans l'entrée d'une mesure dynamique des cyanobactéries entrantes à l'UTEP, un événement de charge maximale de CB n'a pas été identifié, entraînant la percée de cellules CB et de toxines, car les opérateurs n'ont pas pu mettre en œuvre en temps opportun les réponses de traitement. Une moyenne mobile sur 2 heures des lectures de la sonde PC a été proposée pour améliorer l'interprétation des tendances du signal PC et guider la réponse au traitement, en évitant de répondre aux pics de très courte durée. La sonde *in situ* a détecté avec succès une forte densité et des biovolumes élevés de CB entrantes dans le processus de clarification dans l'usine à haut risque. Un biovolume élevé de CB dans les boues pour les stations d'épuration à haut et à faible risque a été aussi observé.

La dernière partie du travail consiste en l'interprétation des lectures de PC *in situ* pour l'évaluation du risque CB. Les performances d'élimination du CB à travers les processus de traitement, telles que mesurées par un échantillonnage ponctuel du biovolume total des cellules CB, ont été comparées aux flux de masse intégrés estimés à partir de lectures de PC *in situ* à sonde multi-paramètres multi-flux. De fortes corrélations ont pu être obtenues entre les efficacités d'élimination évaluées par le biovolume cellulaire et par les lectures de sonde pour le processus de clarification, ce qui permet de se fier à l'indication d'élimination des CB par les lectures de PC. Et cela pourrait servir d'évaluation plus utile du risque de percée de CB dans l'eau traitée. De plus, à partir de l'analyse du biovolume, *Aphanothece clathrata brevis* s'est avéré moins facilement éliminée par clarification que les autres espèces de CB à partir de l'observation de trois UTEP. De plus, en

évaluant les paramètres physico-chimiques mesurés par la sonde, nous avons constaté que les processus de traitement ont révélé que le potentiel de turbidité ou de chlorophylle-a ne peuvent pas servir de proxy pour la surveillance du biovolume de CB en raison d'un regroupement clair entre le brut et le clarifié/eaux filtrées/traitées.

Enfin, des recommandations pour l'utilisation de la surveillance multi-flux *in situ* des PC ont été proposé pour gérer les risques de percée des cellules CB dans l'eau traitée. Des estimations des PC moyennes mobiles sur 2h sont proposées pour fournir une alerte à court terme dans l'eau brute et établir des périodes de vulnérabilité ou de dysfonctionnement du traitement, tandis que les valeurs PC moyennes quotidiennes peuvent être utilisées pour estimer le total et traiter les abattements en logs spécifiques pour justifier les ajustements de traitement. Les avantages et les limites des sondes *in situ* testées pour l'application des niveaux de vigilance et d'alerte sont discutés et les principales lacunes dans les connaissances pour les recherches futures et les orientations ont été énoncées.

ABSTRACT

Worldwide, there has been an increase in the presence of potentially toxic cyanobacterial (CB) blooms in drinking water sources and within drinking water treatment plants (DWTPs). Conventional monitoring methods for CB abundance are tedious and time consuming and require highly trained and experienced operators. Moreover, such methods may not capture the fluctuations of CB density. This could result in the delay between discrete sampling and the corresponding treatment adjustments. Consequently, this delay impedes the ability of the plant operators to respond to the formation and mitigation of CB blooms.

Near real time *in situ* fluorescence probes for rapid detection of the cyanobacteria-specific pigment, i.e. phycocyanin (PC), have been effectively deployed as a means to determine CB abundance in drinking water sources. The occurrence of bloom may affect the CB removal efficiency, leading to CB and cyanotoxins breakthrough into various processes of DWTPs. Yet, there is relatively less adoption of the probe applied inside the full-scale DWTPs.

This research investigated the application of *in situ* PC fluorescence probe as a tool for real-time monitoring of CB at the water intake and within the DWTPs. The research contains three main themes: (1) interferences associated with probe readings; (2) CB risk interpretation by *in situ* probes within the DWTPs; and (3) management of blooms at the source and across the drinking water treatment plant.

The first part of the study was to investigate the factors affecting the interpretation of *in situ* PC fluorescence readings. The interference of Suwannee River NOM (SRNOM) and the water temperature was conducted with laboratory-cultivated CB species and/or extracted PC pigment. The results showed that high SRNOM content in the water could lead to an underestimation of cell-bound CB PC readings. The fluorescence excitation-emission matrix (EEM) confirmed the decreased cell fluorescence intensity caused by the addition of SRNOM. We also found PC probe readings can be significantly affected by temperature in the range of their typical application (6–33 °C), and such interference could be reduced by applying the correction algorithms. Additionally, field samples from a water intake of a DWTP were also evaluated. We found the correlation between probe readings and taxonomic cell counts from the field samples is unreliable, and only biovolume-based estimations are a relevant option for management purposes. A simultaneous

measurement of the other physical-chemical parameters by the probe may help increase the accuracy of PC readings.

The second section of this study was to validate the use of *in situ* probes in the full-scale DWTPs. *In situ* PC probes were devised for remote control and data logging to monitor the CB in raw, clarified, filtered, and treated water in three full-scale DWTPs (including both high and low CB risk). An additional probe was measuring the surface of the sludge storage tank in a high-risk DWTP. PC readings in the raw water of the high CB risk plant showed a 93-fold fluctuation within 24 hours (from 0.6 RFU to 55.8 RFU), with higher PC levels during the afternoon period. These data provide that without the input of a dynamic measurement of incoming CB, one event of peak CB loading was not identified leading to the breakthrough of CB cells and toxins as the operators failed to timely implement treatment responses. A 2-hour moving average of the PC probe readings was proposed to improve the interpretation of PC signal trends and guide treatment response, avoiding responding to peaks of very short duration. The *in situ* probe successfully detected high CB density entering the clarification process in the high-risk plant. High CB biovolume in the sludge for both high and low-risk DWTPs was observed.

The next part of the work focuses on the interpretation of *in situ* PC readings for CB risk assessment. CB removal performance across the treatment processes, as measured by grab sampling total CB cell biovolume, was compared with integrated mass fluxes estimated from PC readings from multi-stream multi-parameter probe. Strong correlations could be found between the removal efficiencies evaluated by cell biovolume and by probe readings for clarification process, providing the reliance on the indication CB removal by PC readings. And it could serve as a more useful assessment of CB breakthrough risk in treated water. Additionally, from the biovolume analysis, *Aphanothece clathrata brevis* was found to be less easily removed by clarification than other CB species from the observation of three DWTPs. Furthermore, by evaluating the physio-chemical parameters measured by the probe, we found that the treatment processes did not show the potential for turbidity or chlorophyll-a to serve as a proxy for CB biovolume monitoring because of clear clustering between raw and the clarified/filtered/treated waters.

Finally, a framework to use *in situ* multi-stream PC monitoring is proposed to manage risks of breakthrough of CB cells in treated water. Estimates of 2-hour moving average PC readings are proposed to provide short term alert in raw water and establish periods of treatment vulnerability

or dysfunction, while daily mean PC values can be used to estimate total and process specific log removals to justify treatment adjustments. Benefits and limitations of the tested *in situ* probes for the application of vigilance and alert levels are discussed and key knowledge gaps for future research and guidance identified.

TABLE OF CONTENTS

DEDICATION	III
ACKNOWLEDGEMENTS	IV
RÉSUMÉ.....	V
ABSTRACT.....	VIII
TABLE OF CONTENTS	XI
LIST OF TABLES	XV
LIST OF FIGURES.....	XVII
LIST OF SYMBOLS AND ABBREVIATIONS.....	XXIII
LIST OF APPENDICES	XXV
CHAPTER 1 INTRODUCTION.....	1
CHAPTER 2 LITERATURE REVIEW.....	4
2.1 Occurrence of cyanobacteria, and their associated cyanotoxins	4
2.2 Cyanobacteria and cyanotoxins monitoring guidelines and regulations	7
2.3 Methods to measure cyanobacteria and cyanotoxins	7
2.3.1 Cyanobacteria measurement methods	7
2.3.2 Cyanotoxin measurement methods	14
2.4 <i>In situ</i> fluorescence detection of cyanobacteria	16
2.4.1 Principle of <i>in situ</i> fluorescence detection of cyanobacteria	16
2.4.2 Sources of interference.....	21
2.5 Application of <i>in situ</i> fluorescence probes for the management of the drinking water	24
2.5.1 Source monitoring at drinking water intakes or reservoirs	24
2.5.2 Drinking water treatment plant monitoring.....	24
CHAPTER 3 RESEARCH OBJECTIVES, HYPOTHESES AND METHODOLOGY	26

3.1	Critical review and problem statement.....	26
3.2	Objectives and hypotheses	26
3.2.1	General objective.....	26
3.2.2	Specific objectives, and hypotheses	27
3.3	Research strategy and methodology	27
CHAPTER 4 ARTICLE 1 - FACTORS AFFECTING THE INTERPRETATION OF ONLINE PHYCOCYANIN FLUORESCENCE TO MANAGE CYANOBACTERIA IN DRINKING WATER SOURCES.....		31
4.1	Introduction	33
4.2	Materials and methods	35
4.2.1	Cell culture	35
4.2.2	Microscopic taxonomic cell counts	36
4.2.3	Fluorometer measurements	36
4.2.4	NOM addition	37
4.2.5	Water temperature effect.....	37
4.2.6	Fluorescence excitation-emission matrix spectra analysis.....	38
4.2.7	Field sampling	38
4.2.8	Data analysis	38
4.3	Results and discussion.....	39
4.3.1	Addition of NOM.....	39
4.3.2	Varying water matrix temperature	45
4.3.3	Validation of probes with field samples.....	51
4.4	Conclusions	60

CHAPTER 5	ARTICLE 2 - MONITORING OF CYANOBACTERIA BREAKTHROUGH AND ACCUMULATION BY <i>IN SITU</i> PHYCOCYANIN PROBE SYSTEM WITHIN FULL-SCALE TREATMENT PLANTS	62
5.1	Introduction	64
5.2	Materials and methods	66
5.2.1	Water source and site description.....	66
5.2.2	<i>In situ</i> phycocyanin monitoring.....	67
5.2.3	Sampling procedure and analysis of water quality parameters	68
5.2.4	Data analysis	69
5.3	Results	69
5.3.1	Time series analysis of the intake water of a high-risk DWTP	69
5.3.2	Applying the probe readings to estimate the risk of cyanobacteria within a high-risk DWTP	74
5.3.3	Applying the probe readings to estimate the risk of cyanobacteria within two low-risk DWTPs.....	77
5.4	Discussion	82
5.4.1	Daily and hourly variations affect the ability to assess risk	82
5.4.2	Relationships between phycocyanin RFU and total cyanobacterial biovolumes...84	
5.4.3	Mapping the location of cyanobacterial cells in the high and low-risk DWTPs....86	
5.5	Conclusions	87
CHAPTER 6	ARTICLE 3 - A FRAMEWORK TO USE <i>IN VIVO</i> PHYCOCYANIN READINGS FOR CYANOBACTERIAL RISK ASSESSMENT WITHIN DRINKING WATER TREATMENT PLANTS.....	89
6.1	Introduction	91
6.2	Materials and Methods	92
6.2.1	Water source and site description.....	92

6.2.2	<i>In situ</i> phycocyanin monitoring.....	93
6.2.3	Grab sampling procedure and taxonomic analysis.....	93
6.2.4	Data analysis	94
6.3	Results and discussion.....	94
6.3.1	Treatment efficiencies evaluated from grab sampling	94
6.3.2	Treatment efficacies evaluated by <i>in situ</i> phycocyanin probes and biovolumes .	104
6.3.3	Implications for regulations and operational guidance	106
6.3.4	Trends of online water quality parameters across DWTP processes	111
6.4	Conclusion.....	113
CHAPTER 7	GENERAL DISCUSSION.....	114
7.1	Overview of the research's scope	114
7.2	The design and operation of multi-flow remote control data collection system	115
7.3	Probe precision and interference	117
7.4	Risk interpretation of <i>in situ</i> probe monitoring.....	118
7.5	Selective removal of cyanobacterial species by treatment.....	119
7.6	Management implications	120
7.7	The novelty of the research	121
CHAPTER 8	CONCLUSIONS AND RECOMMENDATIONS.....	122
8.1	Conclusions	122
8.2	Recommendations	123
REFERENCES.....		124
APPENDICES.....		144

LIST OF TABLES

Table 2.1 The main type of cyanotoxin in fresh water, their producer organism and the examples of occurrence (adapted from (Svircev et al., 2019).....	6
Table 2.2 Monitoring tools for detecting CB (Almuhtaram et al., 2021; Liu et al., 2021; Lou et al., 2017; Te et al., 2015; Zamyadi, 2011; Zamyadi et al., 2016a)	11
Table 2.3 Monitoring methods for freshwater cyanotoxins detection (Pacheco et al., 2016; Sanseverino et al., 2017)	15
Table 2.4 Commercial characteristics of commonly applied <i>in situ</i> PC fluorescence probes	20
Table 3.1 Research hypotheses and the novelties	28
Table 3.2 Experimental approach to validate the hypothesis and related chapter in thesis	29
Table 4.1 Physico-chemical variables for the raw water of Bedford DWTP during July and August of 2018.....	57
Table A.1 Correlations between the <i>in situ</i> PC fluorescence probe readings and microscopic cell counts, biovolume estimation, and extracted pigment concentration	145
Table A.2 Main sources of interference associated with the <i>in situ</i> PC fluorescence probe	147
Table B.1 Details of treatment process characteristics of the studied three DWTPs	158
Table C.1 Log removal of the most abundant cyanobacterial species (cell counts and biovolumes) by clarification, filtration, and post-chlorination of DWTP A	168
Table C.2 Log removal of the most abundant cyanobacterial species (cell counts and biovolumes) by clarification, filtration, and post-chlorination of DWTP B	169
Table C.3 Log removal of the most abundant cyanobacterial species (cell counts and biovolumes) by clarification, filtration, and post-chlorination of DWTP C	170
Table C.4 Physicochemical variables for the raw water, clarified water, filtered water, and treated water of the studied three DWTPs	171
Table C.5 Alert level frames, standards and guideline values of countries and regions for cyanobacteria and cyanotoxin in drinking water supplies	172

Table C.6 Comparison of equivalent PC RFU to WHO alert levels (Chorus et al., 2021b)	174
--	-----

LIST OF FIGURES

Figure 2.1 Jablonski energy diagram (adapted from (So et al., 2001)).....	16
Figure 2.2 Excitation and emission spectra of chlorophyll-a (Chl-a), phycocyanin (PC) and phycoerythrin (PE) (adapted from (Pires, 2010)).....	18
Figure 2.3 The overlap of the absorption spectra of PC and Chl-a (adapted from (Cohen et al., 2014))	23
Figure 3.1 Cyanobacterial cultures at CREDEAU, Polytechnique Montréal.....	30
Figure 3.2 Grab sampling at UWSS DWTP	30
Figure 4.1 Phycocyanin readings (RFU) by using the YSI EXO2 probe of 0 (no cells added), 2000, 20,000, and 100,000 cells/mL of cyanobacterial species with spike 0, 0.7, 1.7, 11 mg/L dissolved organic carbon (DOC) of Suwannee River NOM (SRNOM): (a,b) <i>Microcystis aeruginosa</i> strain CPCC 300 suspended in 500 mL ultrapure water and Lake Champlain water; (c,d) <i>Dolichospermum</i> sp. strain CPCC 544 suspended in 500 mL ultrapure water and Lake Champlain water. The probe measured each of the sample at 250 ms interval for 10 min. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$).....	41
Figure 4.2 Excitation-emission matrix for (a) 100,000 cells/L <i>Microcystis aeruginosa</i> CPCC 300 suspension in ultrapure water, (b) 11 mg/L DOC of SRNOM, (c) 11 mg/L DOC of SRNOM added to 100,000 cells/L <i>Microcystis aeruginosa</i> CPCC 300 suspension in ultrapure water. Note the <i>Microcystis aeruginosa</i> intensity is twice as high as that of SRNOM.....	43
Figure 4.3 Phycocyanin readings (RFU) with the YSI EXO2 probe of the 0, 2000, 20,000 and 100,000 cells/mL of <i>Microcystis aeruginosa</i> strain CPCC 300 suspended in 6, 12, 21, 33 °C of ultrapure water. The probe measured each of the samples at a 250 ms interval for 10 min (a). In (b) the temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation 4.3. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box	

corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$)..... 47

Figure 4.4 Phycocyanin readings (RFU) using the YSI EXO2 probe of 1 mg/L extract phycocyanin pigment in 6, 12, 21, 33 °C of ultrapure water. All samples were tested over the period of 10 min. The probe measured each of the sample at a 250 ms interval for 10 min (a). In (b) the temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation 4.3. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$)..... 48

Figure 4.5 Relationship between phycocyanin probe readings and cell biovolume for different cyanobacterial species. Shaded area around line represents 95% confidence interval 53

Figure 4.6 Relationship between phycocyanin probe readings and cell counts for different cyanobacterial species. Shaded area around the line represents the 95% confidence interval 55

Figure 4.7 Correlation plot of physicochemical variables for the raw water of Bedford drinking water treatment plant (DWTP) during July and August of 2018 56

Figure 4.8 Correlations between photosynthetically available radiation (PAR) and phycocyanin probe readings for the raw water of Bedford DWTP during July and August of 2018. Shaded area around the line represents the 95% confidence interval..... 59

Figure 5.1 Schematic of the treatment processes of DWTP A, grab sampling and probe monitoring locations. The water intake is from Missisquoi Bay. Sampling points are indicated by: raw water (RW), flocculated water (FlocW), clarified water (CW), filtered water (FW), treated water (TW), surface of clarifier (Surface C), surface of filter (Surface F), supernatant of sludge storage tank (Sludge Supernatant) and sludge bed of sludge holding tank (Sludge Bed) 67

Figure 5.2 Temporal monitoring of different time-series analysis of *in situ* YSI EXO2 phycocyanin probe readings (RFU) at the raw water intake of the DWTP A during July and August of 2018. (a) exact time, daily, weekly, and 18-h moving averages; (b) exact time, 2, 6, 12-h moving averages 72

Figure 5.3 Phycocyanin (RFU) by hour of the day at DWTP A raw water intake during July (n=7 per hour) and August (n=25 per hour) of 2018. The bottom and top of each box represent the 25–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value..... 73

Figure 5.4 Temporal monitoring of 2-moving average *in situ* phycocyanin fluorescence (RFU) of the (a) raw water (RW), clarified water (CW), filtered water (FW), and treated water (TW), (b) supernatant of sludge storage tank (Sludge Supernatant) and its total cyanobacterial biovolumes (mm^3/L) inside the DWTP A during the bloom season of 2018. Gray vertical lines indicate when grab samplings were collected. Background colors indicate the equivalent phycocyanin RFU alert levels (1.8 and 5.0 RFU) to WHO 2021 alert levels 75

Figure 5.5 Total cyanobacterial cell counts, cell biovolumes and total MCs in water raw water (RW), clarified water (CW), filtered water (FW), treated water (TW), flocculated water (FlocW), apparent accumulation at the surface of clarified water (Surface C), apparent accumulation at the surface of filtered water (Surface F), supernatant of sludge storage tank (Sludge Supernatant), and sludge bed of sludge holding tank (Sludge Bed) of the DWTP A 76

Figure 5.6 Temporal monitoring of 2-h moving average *in situ* phycocyanin fluorescence (RFU) of the raw water (RW), clarified water (CW), filtered water (FW), and treated water (TW) inside the (a) DWTP B, and (b) DWTP C during the bloom season of 2018. Gray vertical lines indicate when grab samplings were collected 79

Figure 5.7 Total cyanobacterial cell counts, cell biovolumes and total MCs at raw water (RW), clarified water (CW), filtered water (FW) treated water (TW), and sludge bed of sludge holding tank (Sludge Bed) of the DWTP B..... 80

Figure 5.8 Total cyanobacterial cell counts, cell biovolumes and total MCs at raw water (RW), clarified water (CW), filtered water (FW) treated water (TW), surface of clarified water (Surface C), surface of filtered water (Surface F), and sludge bed of sludge holding tank (Sludge Bed) of the DWTP C..... 81

Figure 6.1 Log removal of total cyanobacterial biovolumes (mm^3/L) by clarification, filtration, and post-chlorination in studied three DWTPs. Red dots, triangles, and squares indicate the total cyanobacterial cell biovolumes (mm^3/L) coming into the corresponding treatment processes. n = 23, 9, and 4 for DWTP A, B and C 97

Figure 6.2 Log removal of total cyanobacterial biovolumes (mm^3/L) by (a) clarification, (b) filtration, and (c) post-chlorination versus the incoming cell biovolumes (mm^3/L) coming into the corresponding treatment processes. Paired data are from the samples of all three DWTPs ... 98

Figure 6.3 Log removal of turbidity (NTU) by clarification, filtration, and post-chlorination in studied three DWTPs. Red dots, triangles, and squares indicate the turbidity (NTU) coming into the corresponding treatment processes. $n = 23, 9$, and 4 for DWTP A, B and C..... 99

Figure 6.4 Cyanobacteria speciation evaluated as cell counts (cells/mL) in the raw, clarified, filtered, and treated water in DWTP A 102

Figure 6.5 Cyanobacteria speciation evaluated as cell biovolumes (mm^3/L) in the raw, clarified, filtered, and treated water in DWTP A 103

Figure 6.6 Phycocyanin RFU log removal efficacies estimated from (a) 2-hour moving average, (b) daily mean. Boxes represent the 25–75th percentile. The line within each box corresponds to the median value, and the circle corresponds to the mean value. The number of observations is indicated by n 105

Figure 6.7 The clarification and filtration log removal efficacies evaluated by cell biovolumes versus evaluated by (a) 2-hour moving average of the phycocyanin readings, (b) daily mean phycocyanin readings. Paired data are from the samples of all DWTPs 105

Figure 6.8 Proposed framework for the management of cyanobacterial risks at the source and across DWTPs using *in situ* phycocyanin measurements with a multi-stream probe..... 110

Figure 6.9 Principal component analysis (PCA) as *biplot* of the physio-chemical parameters of the raw, clarified, filtered, and treated water in DWTP A. Parameters are (a) PC = phycocyanin, Turb = turbidity, Chl-a = chlorophyll-a, DO = dissolved oxygen, Cond = conductivity, Temp = temperature and pH, (b) PC, Turb and Chl-a 112

Figure 7.1 Summary of the research themes 115

Figure 7.2 YSI EXO2 PC probe remote control and data collection system 116

Figure A.1 PC readings (RFU) with the YSI EXO2 probe of the 0, 2000, 20,000, and 100,000 cells/mL of *Microcystis aeruginosa* strain CPCC 300 suspended in 6, 12, 21, 33 °C of ultrapure water. The probe measured each of the sample at a 250 ms interval for 10 min. The temperature

interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation 4.2. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$)..... 149

Figure A.2 PC readings (RFU) using the YSI EXO2 probe of 1 mg/L of extracted PC pigment in the 6, 12, 21, 33 °C of ultrapure water. All samples were tested over the period of 10 min. The probe measured each of the samples at a 250 ms interval for 10 min. The temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation 4.2. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$ 150

Figure B.1 The location of the studied three DWTPs: A and B are located in southern Quebec, and C is located in southwest Ontario..... 152

Figure B.2 Schematic of the treatment processes of DWTP B, grab sampling and probe monitoring locations. The water intake is from Lemieux reservoir. Sampling points are indicated by: raw water (RW), clarified water (CW), filtered water (FW), treated water (TW), and sludge bed of sludge holding tank (Sludge Bed)..... 153

Figure B.3 Schematic of the treatment processes of DWTP C, grab sampling and probe monitoring locations. The water intake is from Lake Erie. Sampling points are indicated by: raw water (RW), clarified water (CW), filtered water (FW), treated water (TW), surface of clarifier (Surface C), surface of filter (Surface F), and sludge bed of sludge holding tank (Sludge Bed) 154

Figure B.4 Example of YSI EXO2 probe data collection system at the studied three DWTPs. Sampling points are indicated by: raw water (RW), clarified water (CW), filtered water (FW) and treated water (TW) 155

Figure B.5 Post-hoc analysis (Tukey’s HSD) of the phycocyanin RFU readings during July following ANOVA with group variables of 18 h and the other measured times (significance level $\alpha = 0.1$). Numbers in the graph indicate the adjusted p value 156

Figure B.6 Correlations between phycocyanin (RFU) YSI EXO2 probe readings computed by different time series analysis (exact time, 2, 6, 12 and 18-h moving averages, daily moving average and weekly moving average) and total cyanobacterial biovolume (mm^3/L) from grab samples of the raw water of DWTP A.....	157
Figure C.1 Schematic of the treatment processes of the studied three DWTPs, grab sampling and probe monitoring locations.....	160
Figure C.2 Log removal of total cyanobacterial cell biovolume (mm^3/L) versus log removal of turbidity (NTU) by (a) clarification, and (b) filtration	161
Figure C.3 Cyanobacteria speciation evaluated as cell counts (cells/mL) in the raw, clarified, filtered, and treated water in DWTP B.....	162
Figure C.4 Cyanobacteria speciation evaluated as cell biovolumes (mm^3/L) in the raw, clarified, filtered, and treated water in DWTP B.....	163
Figure C.5 Cyanobacteria speciation evaluated as cell counts (cells/mL) in the raw, clarified, filtered, and treated water in DWTP C.....	164
Figure C.6 Cyanobacteria speciation evaluated as cell biovolumes (mm^3/L) in the raw, clarified, filtered, and treated water in DWTP C.....	165
Figure C.7 Principal component analysis (PCA) as <i>biplot</i> of the physio-chemical parameters of the raw, clarified, filtered, and treated water in DWTP B. Parameters are (a) PC = phycocyanin, Turb = turbidity, Chl-a = chlorophyll-a, DO = dissolved oxygen, Cond = conductivity, Temp = temperature and pH, (b) PC, Turb and Chl-a	166
Figure C.8 Principal component analysis (PCA) as <i>biplot</i> of the physio-chemical parameters of the raw, clarified, filtered, and treated water in DWTP C. Parameters are (a) PC = phycocyanin, Turb = turbidity, Chl-a = chlorophyll-a, DO = dissolved oxygen, Cond = conductivity, Temp = temperature and pH, (b) PC, Turb and Chl-a	167
Figure D.1 Cross-correlation between the meteorological parameters and the phycocyanin probe readings at the intake water of DWTP A during July and August of 2018.....	175

LIST OF SYMBOLS AND ABBREVIATIONS

AOM	Algal Organic Matter
ATP	Adenosine Triphosphate
BMAA	β -MethylAmino- l-Alanine
CB	Cyanobacteria
CW	Clarified Water
Chl-a	Chlorophyll-a
CYN	Cylindrospermopsin
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
ddPCR	Droplet Digital Polymerase Chain Reaction
DWTP	Drinking Water Treatment Plant
EEM	Emission Excitation Matrix
ELISA	Enzyme-linked Immunosorbent Assay
FlocW	Flocculated Water
FW	Filtered Water
HPLC	High performance Liquid Chromatography
HPLC-MS/MS	Liquid Chromatography Coupled with Tandem Mass Spectrometry
HTS	High-Throughput Sequencing
HRT	Hydraulic Retention Time
LC- OCD-OND	Liquid Chromatography-Organic Carbon Detection-Organic Nitrogen Detection
LOB	Limit of Blank

LOD	Limit of Detection
MC	Microcystin
MIB	2-methylisoborneol
MS	Mass Spectrometric Detector
NOM	Natural Organic Matter
PAC	Powdered Activated Carbon
PAR	Photosynthetically active radiation
PC	Phycocyanin
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PPIA	Protein Phosphatase Inhibition Assay
RFU	Raw Fluorescence Unit
qPCR	Quantitative Polymerase Chain Reaction
RW	Raw Water
SRNOM	Suwannee River NOM
T&O	Taste and Odor
TDS	Total Dissolved Solid
TCC	Taxonomic Cell Counts
TW	Treated Water
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organization

LIST OF APPENDICES

Appendix A	SUPPLEMENTARY INFORMATION, ARTICLE 1	144
Appendix B	SUPPLEMENTARY INFORMATION, ARTICLE 2	151
Appendix C	SUPPLEMENTARY INFORMATION, ARTICLE 3	159
Appendix D	SUPPLEMENTARY INFORMATION	175

CHAPTER 1 INTRODUCTION

Cyanobacteria (CB), also known as blue-green algae, are prokaryotic photosynthetic, gram-negative bacteria (Rippka et al., 1979). The presence of CB in natural ecosystems dates back to 2 billion years ago, or the Precambrian Era (Wilmotte, 1994). Nowadays, the fast development of urban, agriculture and industry has resulted in an excessive amount of nitrogen and phosphorus which are the main nutrients for the CB into the surface water sources (Paerl et al., 2012). Furthermore, global climate change results in increased water temperature, decreased water level in the reservoir, and increased high-intensity rainfall events which can deliver enhanced nutrient and sediment loads (Elliott, 2012; Paerl et al., 2012; Wells et al., 2015). In combination, such effects have increased the development of CB bloom in water sources (Ahern et al., 2007; Bennett, 2017; Paerl et al., 2012). Reported CB bloom events happened worldwide (O'Neil et al., 2013). In Canada, in recent years, Lake Erie (Almuhtaram et al., 2018; Berry et al., 2017; Chaffin et al., 2019), Lake Winnipeg (Binding et al., 2018; Bishop et al., 2018; Bunting et al., 2016; McKindles et al., 2019), Lake Champlain (Bowling et al., 2015; Fortin et al., 2015; Jalili et al., 2021; Maghsoudi et al., 2016; McQuaid et al., 2011; Moradinejad et al., 2020; Moradinejad et al., 2021; Zamyadi et al., 2015) and Yamaska River (Gagné et al., 2018; Zamyadi et al., 2012b; Zamyadi et al., 2012c) are the examples of the water sources that have been observed with CB bloom.

The harmful effects due to the presence of CB include: 1) production of different types of cyanotoxins which can cause illness or mortality in humans and animals in case of ingestion, and 2) production of nuisance taste and odour (T&O) compounds resulting in the dissatisfaction of the water aesthetic quality and customer complaints (Chorus et al., 2021a; Health Canada, 2021; Zamyadi et al., 2016a). More than 20 CB genera have been documented to express toxins, and the most commonly observed, and problematic genera in Canada are *Microcystis*, *Dolichospermum*, *Aphanizomenon*, *Planktothrix*, *Pseudoanabaena* (Health Canada, 2021; Winter et al., 2011).

Management strategies must be implemented to ensure the water quality in the presence of CB and their associated toxins in the source water, and inside the drinking water treatment plants (DWTPs) (Newcombe et al., 2010; Zamyadi et al., 2016a). Regulatory authorities have issued guidelines, regulations and response frameworks in response to this need (Chorus et al., 2021b; Ellis, 2009; Government of Québec, 2022; Health Canada, 2021; Ministry of Health, 2020; Ministry of Health, 2022; NHMRC, 2022; Ohio EPA, 2022; United States Environmental Protection Agency

(USEPA), 2015). For each alert level, action plans are proposed describing the surveillance and corrective action during and after the CB bloom.

The technologies to monitor CB can be classified into three main categories: microscopic enumeration and identification, molecular-based methods, and phycocyanin (PC) pigment concentration measurements (Almuhtaram et al., 2021; Liu et al., 2021; Lou et al., 2017; Te et al., 2015; Zamyadi et al., 2016a). Most detection methods require sample collection, transportation, preparation, analyzation, and interpretation of the results. Moreover, the analyse process is slow and requires skilled analysts. Such limitations restrict the number of samples that can be analyzed, undermine online assessment, and limit the ability to reflect the CB biomass fluctuation. On the other hand, intensive *in situ* fluorescence monitoring probes can measure the CB specific pigment, i.e. phycocyanin, in real-time (Gregor et al., 2007; Lee et al., 1994; McQuaid et al., 2011; Zamyadi et al., 2012c). The advantage of applying the probes as an online tool for CB monitoring includes operational simplicity, high detection frequency, and low-cost (McQuaid et al., 2011; Zamyadi et al., 2016a; Zamyadi et al., 2014). Such real-time high frequent monitoring tool allows water utilities to: 1) early detection of CB bloom and plan treatment responses, 2) adjust treatment strategies and prevent CB cells and cyanotoxin into drinking water during the critical time, 3) track treatment changes inside the DWTPs (Zamyadi et al., 2014; Zamyadi et al., 2012a).

In situ PC fluorescence probes have been implemented to determine the total CB cell concentration and/or biovolume at DWTP intakes and inside the DWTPs (Almuhtaram et al., 2018; Gregor et al., 2007; Izydorczyk et al., 2009; Izydorczyk et al., 2005; Kong et al., 2014; McQuaid et al., 2011; Pazouki, 2016; Zamyadi et al., 2014; Zamyadi et al., 2013b; Zamyadi et al., 2012b; Zamyadi et al., 2012c). But the interference is still a concern. Various factors can cause the inaccuracy of PC fluorescence analysis, including natural organic matter (NOM) and temperature (Bertone et al., 2018; Zamyadi et al., 2016a). Moreover, rapid changes in CB concentration over hourly temporal time scales are not well-studied. Less is known regarding the frequency, duration, and intensity of probe PC peaks inside the full-scale DWTPs, as well as the consequences on treatment through the plants. Furthermore, the interpretation of *in situ* PC probe readings for CB risk is the primary factor for management purposes, and it is not fully investigated. Answering these questions will help the PC signal trends to better guide the sampling for CB management purposes.

This thesis is divided into 8 Chapters and 1 Appendix. Chapter 1 is the **Introduction**, presenting a general background on CB, their impact and monitoring strategies. Chapter 2 presents a **Literature review** on CB and cyanotoxin management, and the use of *in situ* PC fluorescence probe detection of CB. Chapter 3 includes the **Research objectives**, **Hypotheses** and **Methodology** of this study. Chapter 4 contains the first published paper in the Journal of Water entitled **Factors affecting the interpretation of online phycocyanin fluorescence to manage cyanobacteria in drinking water sources**, followed by Chapter 5 with a second paper entitled **Monitoring of cyanobacterial breakthrough and accumulation by *in situ* phycocyanin probe system within full-scale treatment plants** submitted to Journal of Environmental Monitoring and Assessment. Chapter 6 consists of a manuscript entitled **A framework to use *in situ* phycocyanin readings for cyanobacterial risk assessment within drinking water treatment plants** submitted to Journal of Environmental Management. Chapter 7 presents the **General discussion** followed by **Conclusions** and **Recommendations** in Chapter 8. Supplementary results of this study are presented in the appendix.

CHAPTER 2 LITERATURE REVIEW

The literature review chapter is divided into five sections. The sections address the occurrence of CB and associated cyanotoxin (Section 2.1), CB and cyanotoxins monitoring guidelines (Section 2.2), measurement methods of CB and cyanotoxins (Section 2.3), *in situ* fluorescence detection of CB (Section 2.4), and application of *in situ* fluorescence probes for the management of the drinking water (Section 2.5).

2.1 Occurrence of cyanobacteria, and their associated cyanotoxins

During the Precambrian period, CB were one of the main oxygen producers in the atmosphere. The large amount of oxygen which was produced by the CB played a key role in the appearance of plants and animals on earth (Chorus et al., 2021b; Whitton, 2012). The massive occurrence of CB is known as CB bloom. Nowadays, due to the increased temperature (Paerl et al., 2012), thermal stratification (O'Neil et al., 2013), increased atmospheric CO₂ (Visser et al., 2016), increased extreme weather events (Paerl et al., 2019), urban soil sealing (Sellner et al., 2003), increased growth of invasive species such as *Cylindrospermopsis raciborskii* (Paerl et al., 2012), increased amount of nutrients (P and N) from urban, agriculture and industry activities (Ahern et al., 2007; Carey et al., 2012; Paerl et al., 2012), the risk of CB proliferation in water resources is increased.

CB can biosynthesis cyanotoxins are of growing concern for water utilities (Ho et al., 2012). Toxin-producing CB blooms have been reported since 1970s, and as more cases were published attributing their health concern, they were widely recognized as a potential health hazard since 1980s (Steffen et al., 2017). Cyanotoxins can be divided into three groups based on their chemical structures: 1) cyclic peptides, 2) alkaloids, and 3) lipidic compounds. Such diverse group of compounds with potential toxicological effects allow them to be categorized into hepatotoxins (which mainly act on liver), neurotoxins (which cause injury on the nervous system), and dermatotoxins (detected mainly in marine water, which cause irritant responses on contact) (Sanseverino et al., 2017; Svircev et al., 2019). Table 2.1 shows the major classes of cyanotoxins, their related species, and examples of CB bloom episodes in fresh water from 2000 worldwide. Among them, the hepatotoxic microcystins (MCs) are the most commonly detected cyanotoxins which has an adverse effect on livestock, aquatic animal, as well as human being (Meriluoto et al., 2017; Svrcek et al., 2004), and microcystins-LR is considered as the most common variant across

the world (Graham et al., 2010). Most cyanotoxins are produced by more than one species, and one species produces several kinds of cyanotoxins (Table 2.1). Up to 70% of the freshwater CB bloom are toxic (Pham et al., 2015; Vareli et al., 2012). Recorded human health effects from cyanotoxins ranging from skin disease, gastrointestinal disease, liver failure and cancer, paralysis, motor neurone disease, potential links between Alzheimer, and mortalities (Bishop et al., 2018; Carmichael et al., 2016; Catherine et al., 2013; Chernoff et al., 2017; Zamyadi et al., 2016a).

Human exposure to cyanotoxin can occur through three routes (Merel et al., 2013):

- Exposure to the water containing toxic CB during the recreation activities may cause irritation in eyes, nose, and throat.
- Ingestion of cyanotoxins via consumption of seafood which was grown in contaminated water may cause human illness.
- Ingestion of cyanotoxins via the contaminated drinking water may cause human illness.

In addition, some of the major issues that the excessive CB can cause to aquatic organisms and terrestrial organisms are as follow (Catherine et al., 2013; Merel et al., 2013):

- CB blooming produces a significant amount of biomass over a short period of time, leading to water deoxygenation, thus decrease in diversity of the phytoplankton which live in the water.
- Cyanotoxins can bioaccumulate in aquatic organisms, and harmful for aquatic organisms.
- Terrestrial mammals' illness by ingestion of cyanotoxin contaminated water.

Furthermore, CB blooms have been found to impact DWTPs, the main consequences are as follows (Catherine et al., 2013; Qin et al., 2010; Steffen et al., 2017):

- Large CB blooms overwhelm DWTP, leading to plant shutdown and impacting the residents.
- Large CB in the water lead to the production of undesirable taste and odor (T&O), mainly geosmin and 2-methylisoborneol (MIB), which are difficult to remove by conventional treatment processes and make the water aesthetically unacceptable.
- Accumulation of CB and cyanotoxins in drinking water sources as the by-product of the water treatment processes.

Table 2.1 The main type of cyanotoxin in fresh water, their producer organism and the examples of occurrence (adapted from (Svircev et al., 2019))

Toxin group	Toxin effect	Main target organ in mammals	Producer CB genera	Example of cyanobacteria/cyanotoxin presence in the fresh water and the reference	
Cyclic peptides					
Microcystins (MCs)	Hepatotoxic	Liver (also to kidney, reproductive tissue, colon, brain)	<i>Microcystis</i> , <i>Dolichospermum</i> , <i>Nostoc</i> , <i>Planktothrix</i> , <i>Anabaenopsis</i> , <i>Aphanocapsa</i> , <i>Hapalosiphon</i> , <i>Aphanizomenon</i> , <i>Merismopedia</i> , <i>Phormidium</i> , <i>Synechococcus</i> , <i>Snowella</i> , <i>Arthrospira</i> , <i>Pleurocapsalean</i> , <i>Gloeotrichia</i> , <i>Fischerella</i> , <i>Radiocystis</i> , <i>Woronichinia</i>	Lake Taihu, China, 2007	(Zhang et al., 2010)
Microcystins-LR				Multiple lakes in Nebraska, USA, 2004	(Walker et al., 2008)
Microcystins-RR					
Microcystins-YR				Nile River, Egypt, 2016	(Mohamed, 2016)
Microcystins-LA				Monjolinho River, Brazil, 2004	(Sotero-Santos et al., 2008)
Microcystins-LW					
Microcystins-LF					
Microcystins-LY					
Alkaloids					
Anatoxins-a	Neurotoxic	Nerve synapse	<i>Aphanizomenon</i> , <i>Dolichospermum</i> , <i>Raphidiopsis</i> , <i>Microcystis</i> , <i>Planktothrix</i> , <i>Cylindrospermum</i> , <i>Arthrospira</i> , <i>Tychonema</i>	Lake Aydat, France, 2011–2013	(Sabart et al., 2015)
Anatoxin-a(S)	Neurotoxic	Nerve synapse	<i>Dolichospermum</i> , <i>Planktothrix</i>	Tapacura reservoir, Brazil, 2002	(Molica et al., 2005)
Saxitoxins (STXs)	Neurotoxic	Nerve synapse	<i>Dolichospermum</i> , <i>Cylindrospermopsis</i> , <i>Lyngbya</i> , <i>Aphanizomenon</i>	7 freshwater ecosystems, Portugal, 2017 Champs-sur-Marne, France, 2005–2008	(Moreira et al., 2020); (Ledreux et al., 2010)
Cylindrospermopsins (CYNs)	Cytotoxic, hepatotoxic, neurotoxic, genotoxic	Liver (also to kidney, spleen, lungs, heart, intestine, thymus, skin)	<i>Aphanizomenon</i> , <i>Lyngbya</i> , <i>Dolichospermum</i> , <i>Planktothrix</i> , <i>Phormidium</i> , <i>Scytonema</i> , <i>Geitlerinema</i> ., <i>Raphidiopsis</i> , <i>Cuspidothrix</i> , <i>Cylindrospermopsis</i>	2 lakes in Florida, USA, 2008 16 lakes throughout Quebec and Ontario, Canada, 2018	(Yilmaz et al., 2011) (Roy-Lachapelle et al., 2019)
Debromoaplysiatoxin	Dermatotoxic	Skin	<i>Lyngbya</i> , <i>Schizothrix</i> , <i>Oscillatoria</i>	A lake in Northern California, USA, 2017	(Puschner et al., 2017)
Lipopolysaccharides (LPS)	Endotoxin (irritant)	Any exposed tissue	All cyanobacteria	Lake Chivero, Zimbabwe, 2003–2004	(Mhlanga et al., 2006)
β-N-methylamino-l-alanine (BMAA)	Neurotoxic	Nerve synapse	<i>Aphanizomenon</i> , <i>Cylindrospermopsis</i> , <i>Dolichospermum</i> , <i>Planktothrix</i> , <i>Microcystis</i> , <i>Nodularia</i> , <i>Synechococcus</i> , <i>Synechocystis</i>	Lake Winnipeg, Canada, 2016 12 different reservoirs across Nebraska, USA, 2009–2010 12 environmental samples throughout the UK, 1990–2004	(Bishop et al., 2018) (Al-Sammak et al., 2014) (Metcalf et al., 2008)

2.2 Cyanobacteria and cyanotoxins monitoring guidelines and regulations

Management strategies must be implemented to ensure drinking water quality in the presence of CB and their associated toxins in the source water (Chorus et al., 2021b; Ellis, 2009; Government of Québec, 2022; Health Canada, 2021; Ministry of Health, 2020; Ministry of Health, 2022; NHMRC, 2022; Ohio EPA, 2022; United States Environmental Protection Agency (USEPA), 2015). Regulatory authorities have issued guidelines, regulations and response frameworks in response to this need. They are mainly based on total CB cell counts (cells/mL), total CB cell biovolume (mm^3/L), and chlorophyll-a concentration ($\mu\text{g}/\text{L}$). Each alert level implies the level of CB risk to drinking water, and its corresponding response for water utilities. Detailed action plans are proposed describing the surveillance and corrective action during and after the CB bloom.

Major cyanotoxins guidelines in drinking water were also issued in different countries and public health organizations. The World Health Organization (WHO) (1999) published a provisional guideline value in drinking water of $1 \mu\text{g}/\text{L}$ for the most common cyanotoxin, microcystis-LR (Chorus et al., 1999). Since then, numbers of countries have addressed the cyanotoxin issues and derived their own regulatory approach based on the respective conditions. In Canada, Health Canada first included microcystis-LR in drinking water quality guideline with maximum acceptable concentration (MAC) of $1.5 \mu\text{g}/\text{L}$ in 2002 (Health Canada, 2007); and since 2018, total microcystis, instead of microcystis-LR, in drinking water with MAC of $1.5 \mu\text{g}/\text{L}$ was regulated (Health Canada, 2021). Quebec established total microcystins considering the variants (i.e., MC-LR, MC-LA, MC-RR, MC-YR, and MC-YM) of $1.5 \mu\text{g}/\text{L}$ in drinking water (Government of Québec, 2022). It should be noted, no other cyanotoxins, including cylindrospermopsin and anatoxin-a, are regulated in Canada, as limited health and exposure data are available on these toxins (Health Canada, 2021).

2.3 Methods to measure cyanobacteria and cyanotoxins

2.3.1 Cyanobacteria measurement methods

Microscopic taxonomic cell counts (TCC) provide valuable information about cell numbers and cells identification among the various CB species (Lou et al., 2017). TCC requires collecting samples from the water source, and preserving them with Lugol's iodine solution at room temperature. The samples are then transported to the laboratory, placed in a counting chamber

for counting by a microscope (Chorus et al., 2021b). The advantages of TCC are: it allows for the specification of the sample, and a few equipment other than a microscope is required (Chorus et al., 2021b; Lou et al., 2017). However, the method is very time-consuming and highly dependent on the expertise of the analyst. The time for analyzing one sample can range from a few minutes to several hours depending on the accuracy required and the complexity of the sample, in addition to the time for collection, transportation, and preparation (Chorus et al., 2021b). Because of these delays, the method cannot be used to track the CB biomass in the targeted water source (Zamyadi et al., 2012c). Furthermore, results accuracy depends on the personal experience (Lou et al., 2017). The results are prone to significant error (up to 40%) because of varying cell morphology, aggregation, presence of the preservation solution, analyst bias and experience (America Water Works Association (AWWA), 2010; Chorus et al., 2021b; Hawkins et al., 2005; Newcombe et al., 2010; Sanseverino et al., 2017; Zamyadi et al., 2016a). The errors can increase when the cell counts results are converted to biovolume (Newcombe et al., 2010).

Considering molecular-based methods, conventional polymerase chain reaction (PCR) techniques and high-throughput sequencing (HTS) technologies can greatly improve the ability to identify the microbial community composition, determine the functional genes and trace the activity of the CB cell genes (Chiu et al., 2017; Jalili et al., 2021; John et al., 2018; Lou et al., 2017; Moradinejad et al., 2020; Romanis et al., 2021; Sanseverino et al., 2017; Serrano-Silva et al., 2018; Srivastava et al., 2013; Te et al., 2015; Tromas et al., 2017; Zamyadi et al., 2019; Zhu et al., 2021). Such methods have greatly improved our knowledge on the distribution of toxic genes within the CB cells (Chorus et al., 2021b). Variation in toxin biosynthesis gene content is well documented among major CB strains (Christiansen et al., 2008; Tanabe et al., 2004). PCR allows specific gene fragments to be replicated through amplification by DNA polymerases (Chorus et al., 2021b). A variation technique, quantitative PCR (qPCR), provides the numbers of the actual gene copied in the specific sample, thus allows the quantification of the CB biomass (Lou et al., 2017). Droplet digital PCR (ddPCR) is an alternative method of qPCR which quantifies a target DNA sequence based on PCR of a partitioned DNA sample and facilitates quick multiplex detection (Chorus et al., 2021b). The more recently developed HTS technologies, including targeted 16S rRNA gene sequencing, total metagenome sequencing and total metatranscriptome sequencing, could provide comprehensive and detailed information on CB community structure including potentially toxigenic species (Chorus et al., 2021b; Zhu et al., 2021). The main advantage of the molecular-based methods is

that not only the CB species can be detected and distinguished, toxic and nontoxic strains can also be detected, and the methods are not impacted by the different cell morphologies (Lou et al., 2017; Srivastava et al., 2013). However, as long as molecular-based methods are related, the need for complex processing after sample collection, high cost, and skilled analysts are involved (Lou et al., 2017; Srivastava et al., 2013). Also, the bias could be caused by incomplete gene bank library, primer selection, amplification, and genomic DNA base composition (Bharti et al., 2021; Moradinejad et al., 2020; Tan et al., 2015).

More recently, it has been suggested that adenosine triphosphate (ATP) testing can be used for the early detection and mitigation of CB blooms (Greenstein et al., 2019). ATP is only found in living cells, and can be used to determine cell biomass (Greenstein et al., 2019). In addition to cyanobacteria, other changes in water quality, for instance the *E.coli* from animal faeces and soil due to the rainfall event, may increase ATP content in the source water, misleading the CB biomass results (Bushon et al., 2009). The use of ATP testing as early warning tool of CB bloom remains to be validated, but reports are available to reveal its use in source water monitoring (Greenstein et al., 2019; Huang et al., 2014; Saha et al., 2016).

CB can also be monitored by pigment quantification. PC is a unique phycobilisome pigment of freshwater CB, and it has strong fluorescence signatures (Bryant, 1986). The pigment concentrations can be determined with or without molecular extraction. The method with PC extraction requires sample collection with perseverance, filtration, and pigment extraction through a chemical buffer, then quantified by a spectrophotometer (Sobiechowska-Sasim et al., 2014). The method without extraction, on the other hand, bypasses these procedures and analyzes the samples directly with a fluorometer. There are several techniques of CB monitoring without pigment extraction: 1) epifluorescence microscopy, 2) *in situ* fluorometer, and 3) remote sensing. *In situ* fluorescence is one of the methods of PC pigment measurement without extraction. Compared to the conventional monitoring tools discussed above, the major advantage of *in situ* fluorescence probes is their high detection frequency. There are different types of *in situ* fluorescence probes commercially available, and Zamyadi et al. (2016a) comprehensively summarized their advantages and limitations. It should be noted that PC pigment quantification determines the total PC content, and cannot identify the CB species, cell density or cell toxicity.

For comparison, the advantages and limitations of above-mentioned technologies are listed in Table 2.2.

Table 2.2 Monitoring tools for detecting CB (Almuhtaram et al., 2021; Liu et al., 2021; Lou et al., 2017; Te et al., 2015; Zamyadi, 2011; Zamyadi et al., 2016a)

Measurement method	Description	Advantage	Limitation
Microscopic enumeration			
Cell counting and biovolume methods	CB cells are counted based on morphological characters of cells. The cells of CB can be preserved in Lugol or Glutaraldehyde	- Identifies species and quantifies cell concentrations or biovolumes - Simple to conduct - Allows for direct comparison to biovolume based thresholds (e.g., WHO Alert Level framework)	- Requires considerable time and a high level of experiment - Susceptible to errors from counting and preservation - No indication of metabolite generation
Automated cell imaging	- Identification of CB using a microscope, a camera, and image recognition software - CB can be discriminated from other algal species by their unique fluorescent signatures or by matching sample images to reference databases	- Automatic identification and enumeration of CB - Reduces need for onsite expertise	- Expensive - Accuracy of identification depends on quality of calibration - Only a few CB genera can be adopted in practice
Molecular methods			
Conventional molecular techniques			
PCR and qPCR	Use molecular biology technique to amplify and detect DNA and RNA sequences	- Simple technique to understand and use - Produce results rapidly - Highly sensitive	- Water samples often contain complicated mixtures of organic matter which may inhibit PCR species - Can be used only to identify the presence or absence of a known gene - Incorrect nucleotides can be incorporated into the sequence
Droplet digital PCR (ddPCR)	Quantify a target DNA sequence based on PCR of a partitioned DNA sample	- Less susceptible to PCR inhibition and high background DNA levels - Can give absolute quantification without relying on an external standard reference - More precise than qPCR	- Less sensitive in detecting targets of low concentrations than qPCR
High-throughput sequencing (HTS) technologies			
Amplicon sequencing	Determine the microbial community composition.	- Quick analysis - Low-biomass requirement - Applicable to samples contaminated by host DNA	- PCR and primer biases - Resolution limited to genus level - False positive in low-biomass samples
Metagenomic sequencing	Determine the phylogenetic and functional genes.	- Taxonomic resolution to species or strain level - Functional potential - Uncultured microbial genome	- Expensive - Time-consuming in analysis - Host-derived contamination
Metatranscriptome	Determine the activity of genes	- Can identify live microbes - Can evaluate microbial activity - Transcript-level responses	- Complex sample collection and analysis - Expensive and complex in sequencing - Host mRNA and rRNA contamination

Table 2.2 Monitoring tools for detecting CB (Almuhtaram et al., 2021; Liu et al., 2021; Lou et al., 2017; Te et al., 2015; Zamyadi, 2011; Zamyadi et al., 2016a) (cont'd)

Measurement method	Description	Advantage	Limitation
Estimation of pigments			
With molecular extraction			
Lab-based PC extraction	- Sample collection, filtration and pigment extraction prior to fluorometric analysis - Determination of concentration of PC as the CB specific pigment - Once extracted PC concentration is measured using: (1) Spectrophotometer, (2) Fluorimeter, and (3) HPLC	- Established surrogate for CB biovolume - Less error than cell counting - Highly accurate way to measure pigment concentrations	- Does not provide species identification - Requires numerous purification steps - Time-consuming and expensive - Pigment concentrations per cell are variable - Correlations to cell concentrations are site specific - Cannot provide real-time data
Without molecular extraction			
Epifluorescence microscopy	Cells are counted based on the addition of dyes which highlight the CB possess the PC using special filters	Facilitates the count of low concentrations (0.1 to 10 cells/mL)	Highly sophisticated
Flow cytometry combined with epifluorescence microscopy	Based on the fluorescent properties of PC. The cells, activated one after the other in front of a laser beam, are characterized by their size and their fluorescent properties	- An <i>in situ</i> robust method for the detection of pigments - Allows differentiating phytoplankton subpopulations	- Long delays between sampling and achievement of results - Time-consuming approach and requires unique expertise with expensive equipment
<i>In situ</i> fluorimeter measuring each sample individually	Enables the analysis of water samples collected individually. Requires a sample bottle to collect water which is then placed into the instrument	- Lightweight - Easy to handle - Acceptable sensitivity depending on the manufacturer - Relatively inexpensive (below 5,000 \$USD)	- It is not suitable for <i>in situ</i>, and continuous measurements - It is not appropriate for the rapid analysis of numerous samples - It is not suitable to identify between present CB species and stage of growth
<i>In situ</i> fluorimeter with continual circulation (flow-through)	A pump moves water to the fluorimeter that remains on board the floating vessel or installed close to the sampling point	- Detect real time variations in the population of CB - Particularly useful to establish an automated tracking system of water quality - Acceptable sensitivity depending on the instrument - Direct measurement of water without the need for filtration and dilution - Differentiate between CB and algae phyla - Direct indication of bloom intensity - Suitable size for field installations	- A correction factor must be applied to the measured values - Subject to measurement biases/interruptions due to air bubbles, large particles and fouling in the pipe, and the pump - Difficulties associated with maintaining the surface of the sensor(s) clean of cells and debris - Not suitable for water profiling and multi-location monitoring - Not suitable to identify between present species and stage of growth - May require constant agitation of samples for accurate results - Heavier than portable probes - Relatively expensive (over 35,000 \$USD up to 90,000 \$USD)

Table 2.2 Monitoring tools for detecting CB (Almuhtaram et al., 2021; Liu et al., 2021; Lou et al., 2017; Te et al., 2015; Zamyadi, 2011; Zamyadi et al., 2016a) (cont'd)

Measurement method	Description	Advantage	Limitation
Submersible <i>in situ</i> fluorimeter probe	Direct measurement of the fluorescence of the chlorophyll and PC in the living CB cells using on-line sensors installed on multiparameter probe. They do not provide quantitative pigment concentration data, but rather supply relative data on the biomass of CB	- Online and real time monitoring of phytoplankton population - Wiper systems available for maintaining the cleanness of sensor surface - Monitoring dynamic CB population over long periods - Depth profiling can be achieved - Acceptable sensitivity depending on the instrument - Particularly suitable for CB monitoring on wide stretches of water where the spatial heterogeneity of CB is challenging - Direct indication of bloom intensity - Suitable to differentiate between CB and algae - Relatively inexpensive (8,000 - 30,000 \$USD)	- Fouling on the multiparameter probe and sensors outer body and variation in maintenance frequency based on antifouling systems - It is not suitable to identify between present CB species and stage of growth - Sources of interference and bias such as: weather conditions and seasonal fluctuations in present/dominant species - Measurements obtained may not truly be representative of the CB presence within the entire monitored water body since probes usually measure one location at a time
Remote sensing	Specific detector of pigment fluorescence is carried by satellites, air plans or helicopters	- Can provide needed spatial and temporal coverage - For mapping CB blooms	- Not suitable for frequent real-time monitoring since it is costly - Detect surface condition only - Clouds limit the satellite view - Low density blooms are not detectable - Bad weather conditions cancel flight plans - Confusion arose regarding the type of bloom prevailing
Adenosine triphosphate (ATP) testing			
Test sticks	- ATP represents total viable planktonic biomass, and it can be used to estimate biomass - ATP is a potential early-warning indicator of increasing planktonic biomass	- Simple and rapid assessment of microbial activity - Low cost - Can be deployed in the field	- Nonspecific measure of biological activity - Moderate correlation to PC - Limited adoption and little guidance available for best practices
Online analyzers		- Real-time automatic measurement of ATP - Can be used to identify diurnal trends and baseline data	- Same limitations as test sticks - Expensive

2.3.2 Cyanotoxin measurement methods

The extent of the blooms cannot define the level or the production rate of cyanotoxin (Catherine et al., 2013). Little is known about what triggers the toxin production in CB cells, albeit the presence of toxin specific genes (Catherine et al., 2013). Light intensity, nitrate concentration, temperature and growth conditions can greatly affect the expression of toxicity of the CB species (Cirés et al., 2016; Ye et al., 2017).

To determine biovolume or cell based threshold based on potential toxicity, it is important to quantify the potential maximum production of toxins per cell, as thresholds are fixed on worse case scenarios of toxin production. Buratti et al. (2017) summarized the microcystins, cylindrospermopsin and saxitoxins cell quota in different CB strains from laboratory tests and field samples. The cyanotoxins cell quota varies greatly. For instance, the microcystins cell quota in small colonies of *Microcystis* sp. could range from 0.1 to 4.0 pg/cell (Buratti et al., 2017). Cyanotoxin may remain in the water source for a long time, and substantial amount of toxins could be released into the water when the CB cells are dead or lysis (McQuaid et al., 2011).

The measurement methods of cyanotoxin are listed in Table 2.3. Biochemical-based methods includes enzyme-linked immunosorbent assay (ELISA) and protein phosphatase inhibition assay (PPIA). The chemical methods are mostly liquid chromatography (high performance liquid chromatography—HPLC) combined with an ultraviolet (UV), fluorescence or mass spectrometric detector (MS). The commonly used technique for the identification and quantification of cyanotoxins is high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) (Fayad et al., 2015).

Table 2.3 Monitoring methods for freshwater cyanotoxins detection (Pacheco et al., 2016; Sanseverino et al., 2017)

Methods	Detectable freshwater cyanotoxin	Advantage	Limitation
Biochemical-based methods			
Enzyme-linked immunosorbent assays (ELISAs)	Microcystins, Cyndrospermopsin, Saxitoxins, Anatoxins, BMAA	- Highly sensitive, rapid and specific - Require low level of expertise - Be able to detect both extracellular and intracellular toxins	- Expensive - Susceptible to interferences
Protein phosphatase inhibitor Assay (PPIA)	Microcystins	- Sensitive and cost effective - Ensure toxin detection within a few hours for a large number of samples - Can be used for the routine screening of microcystin concentration	- Only microcystins can be detected
Chemical methods			
Liquid-based separations (high performance liquid chromatography—HPLC) combined with an ultraviolet (UV), fluorescence or mass spectrometric detector (MS)	Microcystins, Cyndrospermopsin, Saxitoxins, Anatoxins, BMAA	- Highly sensitive - Can identify variants in a mixture - Be able to identify unknown toxins in field samples	- Expensive - Dependent on the availability of analytical standards and well-trained technicians
Molecular methods			
qPCR	Microcystins, Cyndrospermopsin, Saxitoxins, Anatoxins, BMAA	- Fast, simple and cost-effective - Highly sensitive - Different cyanotoxin genes can be investigated simultaneously - Be able to detect the potential toxicity of a bloom	- Efficiency depends on different CB species and the extraction method employed - Gene presence doesn't mean that the production of toxin

2.4 *In situ* fluorescence detection of cyanobacteria

2.4.1 Principle of *in situ* fluorescence detection of cyanobacteria

The principle of *in situ* fluorescence detection can be explained by Perrin Jablonski diagram (Figure 2.1). The diagram illustrates the photon emission process by molecule relaxation from excited states (So et al., 2001). The CB pigments can be excited at the specific wavelength, then quickly (femtoseconds to picoseconds time scale) relaxed to a lower energy level, subsequently emit some secondary photons detected by a fluorescence probe (So et al., 2001). The emission wavelength is always longer than the excitation wavelength due to the Stoke shift (So et al., 2001). The excitation and emission wavelengths are referred to as fluorescence signature of a compound. *In situ* fluorescence probes are extensively employed to monitor and detect organic matter, including diverse phytoplankton groups, in water sources and DWTPs (McQuaid et al., 2011; Zamyadi et al., 2016a).

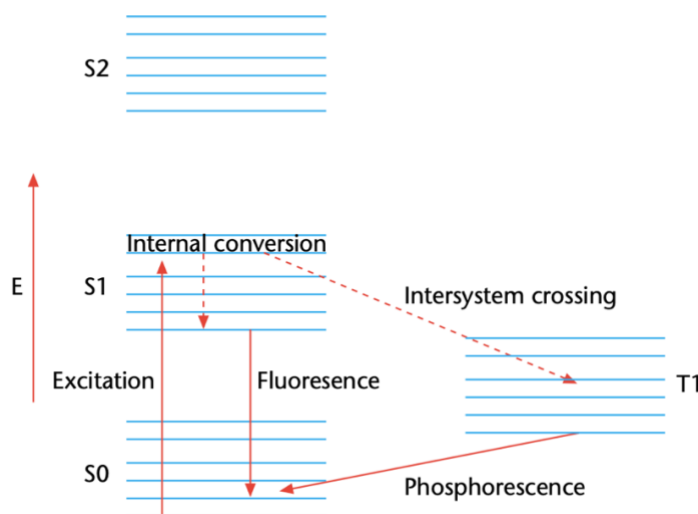


Figure 2.1 Jablonski energy diagram (adapted from (So et al., 2001))

CB possess chlorophyll-a, as well as a unique set of accessory pigments—phycocyanin and phycoerythrin—constituting the organism's main light absorption complex (Bryant, 1982; Chorus et al., 2021b; Gregor et al., 2004; Gregor et al., 2007). CB photosynthetic system contains two photosystems, PSI and PSII. The antenna complex for PSI and PSII is formed as phycobilisomes, located on the thylakoid membrane. Phycobilisomes can be grouped into three classes based on the

different types of polypeptides: 1) phycobiliproteins, 2) linker polypeptides, and 3) PBS-associated proteins (Grigoryeva, 2020). Chlorophyll-a is mostly located in PSI. PC and phycoerythrin are from phycobiliproteins family, most located in PSII, and they have strong fluorescent signatures (Govindjee et al., 2011). Chlorophyll-a can be excited by two peaks at 470 and 660 nm, and has emission at 680–685 nm (Figure 2.2a); PC can be excited by 590–630 nm, and has maximum emission at 650 nm (Figure 2.2b); phycoerythrin can be excited by 550–570 nm, and has emission at 578 nm (Figure 2.2c) (Asai et al., 2001; Beutler et al., 2003; Bryant, 1982; Zamyadi et al., 2016a). Chlorophyll-a as a universal photosynthetic pigment, exists in entire phytoplankton (both eukaryotic phytoplankton and cyanobacteria) (Izydorczyk et al., 2009). So, the chlorophyll-a content in the water sample is not representative of CB concentration.

PC can be found in both fresh and brackish water environments, while phycoerythrin is usually found in marine environments (Bryant, 1982; Carr et al., 1982; Fogg et al., 1973). Although other algae, rhodophyta and cryptophyta, may also contain PC in fresh water (Bryant, 1982), they are hardly found in drinking water reservoirs or lakes (Kong et al., 2014; Magrann et al., 2015). This study is mainly focus on the phycocyanin, as we are studying freshwater cyanobacteria. PC fluorescence detection has been deployed into field probes, enabling continued, *in situ*, real-time monitoring of the CB distribution in water sources and DWTPs (Almuhtaram et al., 2018; Bastien et al., 2011; Bertone et al., 2018; Gregor et al., 2007; McQuaid et al., 2011; Pazouki et al., 2014; Pazouki et al., 2016; Zamyadi et al., 2016a; Zamyadi et al., 2014; Zamyadi et al., 2013b; Zamyadi et al., 2016b; Zamyadi et al., 2012c).

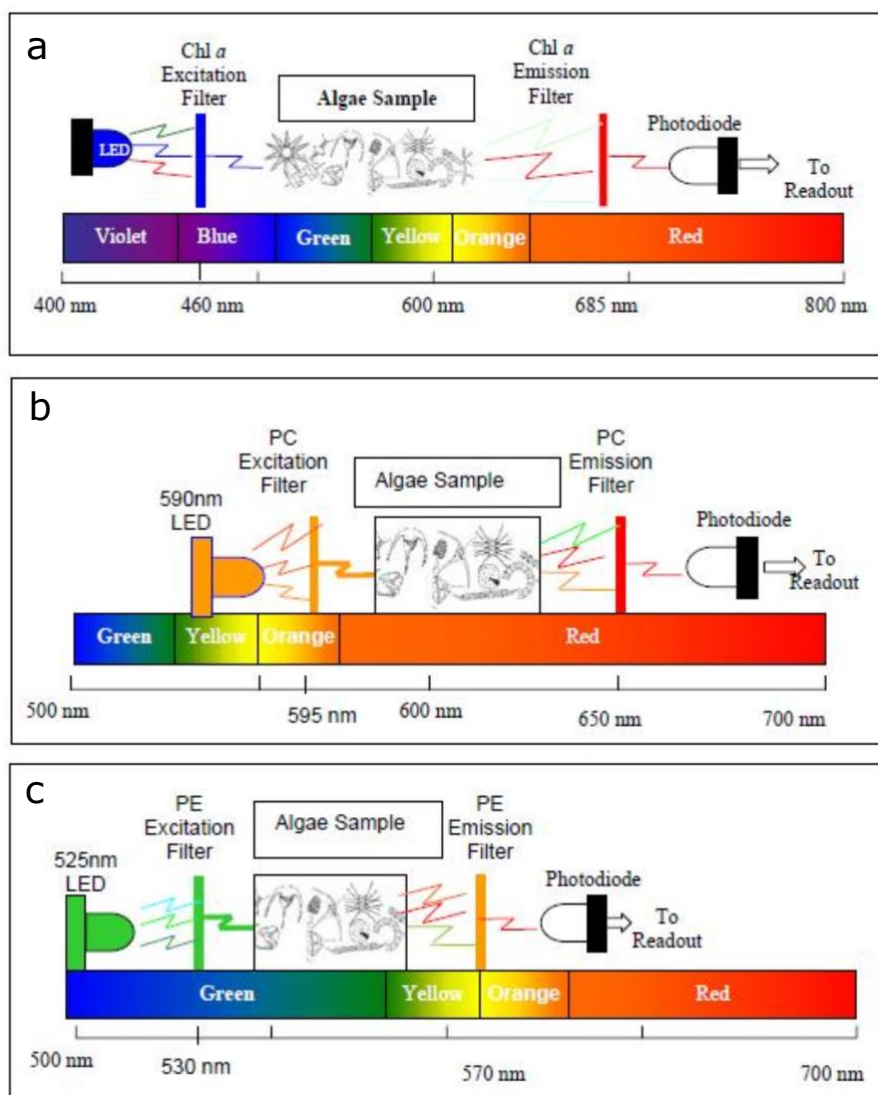


Figure 2.2 Excitation and emission spectra of chlorophyll-a (Chl-a), phycocyanin (PC) and phycoerythrin (PE) (adapted from (Pires, 2010))

Commercially available PC fluorescence probes allow monitoring the spatial-temporal distribution of the CB without sophisticated manipulation. A list of commonly applied *in situ* PC fluorescence probes is summarized in Table 2.4, including their excitation and emission wavelengths and operation assessment. Based upon the emitted PC fluorescence, three formats of data can be usually generated: relative fluorescence unit (RFU), $\mu\text{g PC /L}$, and ppb. Then, CB biomass, biovolume or cell densities can be estimated. We focus on YSI EXO2 probe, as it was mainly used in this work. The YSI EXO2 probe collects the data and stores it onboard, before the data are transferred to a data collection platform (YSI Incorporated, 2019). YSI EXO2 probe can provide both PC RFU and

µg/L as the raw prob reading units. Several studies have shown better correlation with the PC RFU with the CB biovolume and/or cell densities in comparison with µg/L (Bastien et al., 2011; Bowling et al., 2016; Genzoli et al., 2016; Zamyadi et al., 2012b). So, the unit RFU is mostly used in our work.

Table 2.4 Commercial characteristics of commonly applied *in situ* PC fluorescence probes

Manufacturer	Model	Excitation wavelength (nm)	Emission wavelength (nm)	Resolution	Operating range	Detection limit	Reference
Yellow Springs Instrument Company—YSI Inc	6-Series (V6131 phycocyanin)—discontinued	565–605	660±20	0.1 RFU 1 cells/mL	0–100 RFU 0–280,000 cells/mL	0.2 RFU 220 cells/mL	(McQuaid et al., 2011; YSI Incorporated, 2006)
	EXO	590 ± 15	685 ± 20	0.01 RFU 0.01 µg/L	0–100 RFU 0–100 µg/L	0.09 RFU -	(Choo et al., 2018; YSI Incorporated, 2019)
Turner Designs	C3 and C6P	590 ± 30	> 645	-	0–4,500 ppb	2 ppb	(Turner Designs, 2020a)
	CyanoFluor	-	-	-	0–100 µg/L	0.3 µg/L	(Turner Designs, 2017)
	Trilogy	400–600	640 ± 30	-	0–10,000 ppb	2 ppb	(Turner Designs, 2019)
	AquaFluor	≤ 595	630–715	-	0–3,500 ppb 0–20,000 ppb	1 ppb 10 ppb	(Turner Designs, 2018)
	Cyclops	590 ± 30	> 645	-	0–4,500 ppb	2 ppb	(Turner Designs, 2020b)
TriOS	MicroFlu	620	655 ± 5	0.1 µg/L	0–200 µg/L	0.7 µg/L	(Trios Optical Sensor, 2009)
	MicroFlu V2	620	655	-	0–200 ppb 0–500 ppb	0.5 ppb 2 ppb	(Trios Optical Sensor, 2021)
Eureka	Manta II	595	300–1100	-	150–150,000 cell/mL	-	(Hodges, 2016)
Chelsea	TriLux	610	685	-	0–100 µg/L	-	(Hodges, 2016)
Aquaread	Aquaprobe AP-700, 800 and 2000	590	> 655	1 cells/mL	0–300,000 cells/mL	-	(AquaRead, 2021)
bbe Moldaenke*	Algal Online Analyser	590	685 ± 5	0.1 µg chl- <i>a</i> /L	0–200 µg chl- <i>a</i> /L	-	(Zamyadi et al., 2016a)
	Algae Torch	590	680	0.2 µg chl- <i>a</i> /L	0–200 µg chl - <i>a</i> /L	4 µg Chl- <i>a</i> /L	
	FluoroProbe	590	680	0.05 µg chl- <i>a</i> /L	0–200 µg chl - <i>a</i> /L	0.3 µg Chl- <i>a</i> /L	

* All models measure the PC as raw probe readings, except the models from the manufacturer bbe Moldaenke which only measure Chl-*a* as raw probe readings

2.4.2 Sources of interference

Studies have evaluated the PC fluorescence probes' performance with laboratory-cultured unicellular CB species, colonial and mixed species, and in natural waters (Table A.1). In general, PC probe readings are well correlated with the laboratory-cultured unicellular species, and less promising with the colonial species and mixed species. Field samples are more likely to be subject to interferences. The reference method to which the probe readings are compared is also important. In Table A.1, correlations were reported between the PC fluorescence probe readings and the extracted PC concentration, total taxonomic cell counts or total CB biovolume.

According to the previous studies, the major sources of interference fall into the following categories, also listed in Table A.2.

- Natural variation of pigment content and response

The PC content could vary between the same CB species with the same biovolume due to the different growth phases of the cells, and such impact is variable depending on the instruments and references CB species used (Bertone et al., 2019; Chang et al., 2012; Rousso et al., 2022; Ziegmann et al., 2010). For instance, Ziegmann et al. (2010) revealed higher PC content in the decay phase by analyzing the excitation-emission matrix (EEM) spectra of *Microcystis aeruginosa*. While Bertone et al. (2019) noted inconsistent PC content on different growth stages depending on different CB species measured by YSI EXO2 probe, as the authors reported increasing PC content over time (day 7–35) for *Microcystis* sp., but decreasing for *Aphanocapsa* sp. and *Sphaerospermopsis* sp. Chang et al. (2012) observed higher PC during logarithmic phase comparing with the lag and stationary phases for *Microcystis aeruginosa*, *Dolichospermum circinalis* and *Planktothricoides raciborskii* using Turner Designs Model 10 probe. In addition, for different CB species, their cell size and geometry are different, leading to the different pigment content of various species (Sarada et al., 1999). In natural water, CB agglomeration can vary in time and be composed of various species, and the biovolume of these species has a wide range (Zamyadi et al., 2016a). When the CB species aggregate, the fluorescence emission and excitation may not penetrate the whole colony of few hundred millimeter, leading to the underestimation of the PC concentration (Chang et al., 2012; Hodges, 2016). As a results, weaker relations could be found for colonial species (Chang et al., 2012; Hodges et al., 2018), filamentous species (Kring et al., 2014), picoplanktonic sized taxa (Bowling et al., 2016) with total CB biovolume and/or cell

counts in comparison to single-celled CB species. Additionally, due to the less variation of the PC content per unit of cell biovolume than per unit of cell density (Rousso et al., 2022), PC fluorescence readings can be used to better predict the total CB biovolume, than the cell counts, in the mixed culture (Kong et al., 2014; McQuaid et al., 2011).

- Instant and prior prolonged light exposure of cyanobacteria

Light provides the energy for microalgae to grow. Theoretically, low light intensity may stimulate PC production, because cells accumulate more pigments to capture more light as energy. On the other hand, when the light intensity is so high, some species have the potential to degrade PC in order to prevent damage to the cells (Hodges, 2016; Mulders et al., 2014; Zamyadi et al., 2016a). The outcomes are variable depending on the light sources, exposure time, measuring instruments and references CB species used. For instance Rousso et al. (2021) and Misumi et al. (2016) observed a reduction of PC fluorescence for CB cultures exposed to artificial light, attributing the suppression to non-photochemical quenching. Hodges (2016) investigated the PC fluorescence of *Aphanizomenon* sp. under natural light and dark treatments, and the authors reported that the fluorescence measured from the Manta and one of the Turner probes under the light treatment were significantly higher compared to the dark treatment, while the PC content was not modified measured by the other unit of the Turner probe and YSI probe.

Studies also indicate that prior light exposure can affect the PC fluorescence readings. Regarding the prior light exposure to natural light, Zamyadi et al. (2012b) and Brient et al. (2008) reported that exposure up to 7 h has no effect on PC fluorescence production for both CB cells and PC extract. On the contrary, when exposure to artificial light, Brient et al. (2008) and Ma et al. (2015) revealed that PC fluorescence increased with increasing artificial light intensity for both CB cells and PC extract.

- Optical characteristics of the probe

The accuracy of the fluorescence probe measurement can be defined by the sensitivity and specificity of the spectral resolution of the sensor (Zamyadi et al., 2016a). Probe calibration algorithm may lead to the inaccuracy of CB measurement. If the range of the calibration points is too large, a relatively large error may occur at the low cell density; on the other hand, by using the narrow calibration range, the probe detection range will be limited (Zamyadi et al., 2016a).

- Water quality

The other source of interference in the detection of PC concentration of the CB cell is water quality. Chlorophyll-a, water turbidity, water temperature, and dissolved organic matter (DOM) all can influence the accuracy of fluorescence analysis.

The fluorescence peak of chlorophyll-a and PC may overlap at the wavelength of 600–700 nm (Figure 2.3), resulting in some errors in the detection of PC (Choo et al., 2018). A precise spectrometer which can measure a narrow spectrum range may provide more precise data but at a higher cost (Zamyadi et al., 2016a). Previous studies have reported inaccurate PC measurement due to the presence of eukaryotic phytoplankton, ranging from 200% underestimation to 405% overestimation for different species and fluorometers applied (Choo et al., 2018). Correction method was developed to reduce such interference (Choo et al., 2019).

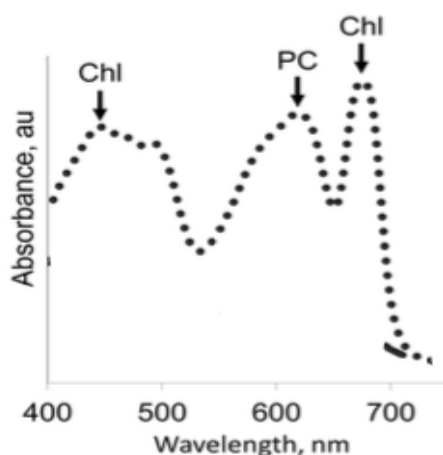


Figure 2.3 The overlap of the absorption spectra of PC and Chl-a (adapted from (Cohen et al., 2014))

Water turbidity is related to the inorganic (clay, silt) and organic matter (plankton, microscopic organisms) in the water source. Turbidity generally could cause the underestimation of the CB fluorescence detection (Brient et al., 2008; Zamyadi et al., 2012b). Turbidity over 50 NTU could make probe measurement ineffective (Bowling et al., 2013; Symes et al., 2016).

Temperature is an important component of fluorescence analysis. A general trend of decreasing PC fluorescence with the increasing temperature was observed in previous studies (Hodges et al., 2018; Zamyadi et al., 2016a).

2.5 Application of *in situ* fluorescence probes for the management of the drinking water

2.5.1 Source monitoring at drinking water intakes or reservoirs

The *in situ* fluorescence probe has been used as a source water monitoring tool for CB detection (Bastien et al., 2011; Brient et al., 2008; McQuaid et al., 2011; Zamyadi et al., 2012b), whereby the probe can be used to report the existing alert levels for municipalities. As described in Table 2.2, conventional monitoring methods for CB abundance are tedious and time consuming, and require highly trained and experienced operator. So online probing with high frequency can be beneficial to manage the water sources which have the potential of CB bloom.

McQuaid et al. (2011) studied the feasibility of using of *in situ* PC probe to monitor the threshold at the Missisquoi Bay. The PC probe reading has a strong correlation with the CB biovolume ($R^2=0.68$, $p < 0.01$, $n=26$). The authors revealed that biovolume of 10 mm³/L corresponds to 10.1 RFU by probe readings and represents the WHO alert level 2. Biovolume of 0.2 mm³/L corresponding to 1.7 RFU by probe readings and represents the WHO alert level 1. However, it should be noticed that this result is quite site-specific and is limited by the CB species in the water sources. Although a good correlation $R^2=0.68$ is obtained, there is still a significant uncertainty in the individual measurements that whether the water source has exceeded the threshold or not.

2.5.2 Drinking water treatment plant monitoring

The *in situ* fluorescence probe can be helpful in plant monitoring in two different ways: 1) online monitoring of CB breakthrough into the DWTPs; and 2) online monitoring of the impact of the treatment within the DWTPs.

- Online monitoring of CB breakthrough into the DWTPs

Cyanobacteria and cyanotoxins might breakthrough into various processes of DWTP. The sludge and in the clarifier all have a great potential for cell accumulation (Zamyadi et al., 2016b). Breakthrough of the cells into the drinking water plant and treated water were being observed, while the source monitoring and treatment processes were applied (Zamyadi et al., 2013b; Zamyadi et al., 2012a). (Zamyadi et al., 2013b) observed a high accumulation of cells in both settlers and filters at relatively low CB concentrations in the source water. Also, (Almuhtaram et al., 2018)

indicated that in four water treatment plants in Great Lakes, low CB cell concentration in the water intake can still result in the significant cell accumulation in clarifier sludge and filler backwash.

- Online monitoring of the impact of treatment changes inside the water treatment plant

The fluorescence probe has been used for online detection of the impact of chemical treatment in few studies. (Zamyadi et al., 2016b) studied the behavior of an *in situ* PC fluorescence probe in a water treatment plant in Melbourne. In order to know the effect of KMnO_4 in CB cell removal, a YSI EXO2 probe was used to monitor the CB concentration at the supernatant sludge thickening tank. The probe measurements were recorded every 15 minutes. KMnO_4 was added into the sludge thicken tank according to the probe reading. The probe monitoring results were compared with the TCC. The result showed that the probe can be used to optimize the addition of the KMnO_4 to reduce the CB content during the water treatment. However, further studies are still required as the probe data varies under different conditions. In fact, the water quality and the CB spatial dispersion are different from the natural water and the water treatment plant.

CHAPTER 3 RESEARCH OBJECTIVES, HYPOTHESES AND METHODOLOGY

3.1 Critical review and problem statement

Research has been carried out to assess the applicability of the *in situ* PC fluorescence probe for monitoring CB biomass in water sources and within DWTPs. Sources of interference associated with the *in situ* fluorescence probe could influence the probe's precision and specificity. Measurement inaccuracy is one of the major issues that has been highlighted in the review. The interferences could be minimized by applying the correction algorithms and by calibrating the probe for the conditions of the targeted water sources. A comprehensive study is required to evaluate the factors that affect the interpretation of the probe reading, and more importantly, to propose a framework to assist plant operators to minimize risk to drinking water in near real time.

Additionally, the reliability of the PC probe readings in identifying threshold alert levels is the primary factor for management purposes, and has not yet been fully studied. How the probe readings are acquired, and how to analyze probe readings, have revealed several uncertainties which include: 1) short-term variations of the probe readings, 2) timing of the grab sample to pair with the probe readings. Time series analysis could improve the interpretation of PC signal trends, and can serve to guide sampling for CB management purposes.

Furthermore, PC probes used at DWTPs were primarily deployed in the reservoir or the inlet, rather than throughout the treatment processes. Few studies investigated the use of probes for treatment validation and control. It is important to use real-time data for the treatment adjustment, and help plant operators to quick response to the increasing CB cells.

3.2 Objectives and hypotheses

3.2.1 General objective

The main objective of this research is to validate to measure CB across a water treatment plant and provide a framework to use resulting signals for the near real-time management of CB risks in drinking water production.

3.2.2 Specific objectives, and hypotheses

Specific objectives and hypothesis and the novelty of the research is summarized in Table 3.1 and Table 3.2 and the chapters corresponding are identified.

3.3 Research strategy and methodology

The YSI EXO2 PC probe (Hoskin Scientific Ltd.) featured with the total algae sensor which measures PC fluorescence content is used in this work. The optics ranges of PC sensor are shown in Table 2.4. The YSI EXO2 probe also features a conductivity/temperature sensor, DO sensor, turbidity sensor and pH sensor. A self-cleaning wiper system is applied in this system. The probe was calibrated according to the manufacturer's manual.

Two aspects of experimental plan have been conducted in this work:

- Cell-culture based laboratory experiments and full-scale DWTPs experiments. CB species *Microcystis aeruginosa* strain CPCC 300 (Figure 3.1a), and *Dolichospermum* sp. strain CPCC 544 (Figure 3.1b) were cultivated in 9 L flasks with BG-11 medium. The culture room is a part of CREDEAU facilities at Polytechnique Montréal. The temperature of the culture room was 20 ± 2 °C. A cool fluorescence light at an intensity of 70 $\mu\text{mol}/\text{S.m}^2$ radiated to the room 12 hours a day. Air diffusers were implemented in the CB cell cultures to provide sufficient oxygen and mixing conditions. Cultures were harvested at the stationary growth phase.
- Full-scale DWTPs experiments were conducted at Bedford, Granby and UWSS DWTPs from July to November 2018. Field grab samples were taken in parallel with probe measurements (Figure 3.2). Samples were collected, prepared, preserved, and transferred to the laboratory for microscopic taxonomic analysis and cyanotoxin analysis.

Field sample microscopic taxonomic analysis were performed at Polytechnique Montréal and Université du Québec à Montréal. Cyanotoxin samples were analyzed at Environmental Chemistry lab at Université de Montréal. Detailed methodology is presented in the following chapters.

The detailed experimental procedure and analytical methods are detailed in each chapter.

Table 3.1 Research hypotheses and the novelties

Hypothesis	Novelty
Hypothesis 1: The uncertainty in the PC probe measurement due to the presence of the NOM and temperature variation can be quantified and is relatively significant	Limited information on the reliability and precision of the PC probe readings is available. This is a critical aspect to be able to apply the data for decision support
Hypothesis 2: The CB community affects the relation between the PC fluorescence probe readings and total CB biovolume	Previous research on the relationships between PC fluorescence probe readings and CB biovolume predominantly studied species-specific PC fluorescence responses from monocultures or the total CB community. Our study covered a broad range of species and morphologies of the CB community to identify the variation of PC content
Hypothesis 3: The raw signal from PC probe must be treated before being used by the DWTP's operators as an alert indicator	Utilities have installed PC probes but face challenges in interpreting the PC readings. The most difficult part is to generate actionable information from PC probe online monitoring. Proper treatment of probe signal should include adequate time series analysis of trends related to the potential consequences of CB breakthrough into drinking water
Hypothesis 4: The successful removal of CB by physical processes in a full-scale DWTPs can be assessed by the PC probe	Insufficient information is available on treatment efficacy evaluated by the PC probe
Hypothesis 5: A more comprehensive CB removal preventative framework based on the PC readings from source to treated water can be implemented in DWTP	Utilities have expressed the need for a fast and efficient method to determine the CB bloom and the timely adjustment to the treatment processes. However, a real-time method for optimization is still unavailable

Table 3.2 Experimental approach to validate the hypothesis and related chapter in thesis

Objective	Experimental Approach	Hypothesis	Chapter
Objective 1: Establish the impact of Suwannee River NOM on the monitoring of CB PC readings and investigate the mechanism of interference	Cultured based: <i>Microcystis aeruginosa</i> and <i>Dolichospermum</i> sp. Analytical approach: Probe, taxonomic cell counts, EEM	1	4
Objective 2: Investigate the impact of temperatures from 6 to 33 °C on PC readings via studying both dissolved and cell-bound PC		1	4
Objective 3: Investigate the relationships between PC fluorescence probe readings and total CB biovolume	Field study: Bedford, Granby and UWSS DWTPs Analytical approach: Probe, taxonomic cell counts, cyanotoxins	2	4
Objective 4: Investigate the use of <i>in situ</i> probe readings to track CB fluctuations in DWTPs on daily and hourly basis		3	5
Objective 5: Evaluate <i>in situ</i> probes for mapping and identifying the critical points for breakthrough and accumulation of cells and their associated toxins using a combination of probe readings and taxonomic cell counts		3	5
Objective 6: Compare the treatment efficacies estimated via grab sampling and integrated mass fluxes which are estimated from PC probe readings		4	6
Objective 7: Propose a PC probe-based framework for CB management in DWTP		5	6

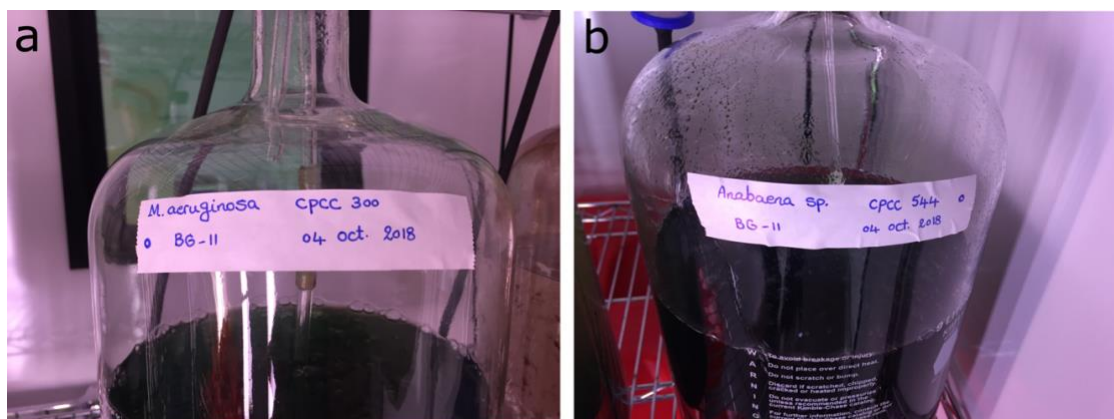


Figure 3.1 Cyanobacterial cultures at CREDEAU, Polytechnique Montréal



Figure 3.2 Grab sampling at UWSS DWTP

CHAPTER 4 ARTICLE 1 - FACTORS AFFECTING THE INTERPRETATION OF ONLINE PHYCOCYANIN FLUORESCENCE TO MANAGE CYANOBACTERIA IN DRINKING WATER SOURCES

This chapter was published as a research paper in the Journal of *Water* on November 18th 2022. Supplementary information is presented in Appendix A.

FACTORS AFFECTING THE INTERPRETATION OF ONLINE PC FLUORESCENCE TO MANAGE CYANOBACTERIA IN DRINKING WATER SOURCES

Liya Ma ^{1,*}, Saber Moradinejad ¹, Juan Francisco Guerra Maldonado ¹, Arash Zamyadi ², Sarah Dorner ¹ and Michèle Prévost ¹

1: Department of Civil, Geological, and Mining Engineering, Polytechnique Montréal, Montréal, QC H3C 3A7, Canada; saber.moradinejad@polymtl.ca (S.M.); juan-francisco.guerra-maldonado@polymtl.ca (J.F.G.M.); sarah.dorner@polymtl.ca (S.D.); michele.prevost@polymtl.ca (M.P.)

2: Faculty of Engineering and Information Technology, University of Melbourne, Melbourne, VIC 3010, Australia; arash.zamyadi@unimelb.edu.au

* Correspondence: liya.ma@polymtl.ca

Abstract

Recently, *in situ* YSI EXO2 phycocyanin fluorescence probes have been widely deployed as a means to determine cyanobacterial abundance in drinking water sources, yet few studies have evaluated the effects of natural organic matter (NOM) and the ambient water temperature on the probe readings. In this study, Suwannee River NOM was added to laboratory cultivated cyanobacterial species to test the performance of the phycocyanin probe. The impact of temperature on phycocyanin fluorescence was evaluated by monitoring the laboratory cultivated cyanobacterial species and extracted phycocyanin pigment. Additionally, *in situ* phycocyanin fluorescence of the field samples from the water intake of a drinking water treatment plant (DWTP) in 2018 were compared with grab sample laboratory taxonomic analyses. We found: (1) the presence of Suwannee River NOM leads to the decrease in cell-bound cyanobacterial phycocyanin readings; (2) increasing ambient water temperature reduces dissolved and cell-bound cyanobacterial phycocyanin readings; (3) field study phycocyanin probe readings significantly correlated with the total cyanobacterial biovolume ($R = 0.73$, $p < 0.1$), and the relationship depends on the biovolume of dominant cyanobacterial species; (4) phycocyanin probe readings have a strong positive correlation with the natural light intensities; and (5) probe users should be fully aware of the sources of interferences when interpreting the results and apply the other physical-chemical parameters data simultaneously generated by the fluorometry to improve the probe's measurements.

Keywords

Cyanobacteria; phycocyanin fluorescence probe; natural organic matter; temperature; biovolume; drinking water treatment plant

Key Contribution

This study is the first to evaluate Suwannee River NOM and water temperature on the YSI EXO2 phycocyanin probe readings.

4.1 Introduction

Cyanobacteria-related illnesses are a rising concern with regards to the quality of drinking water sources. Certain cyanobacterial species can biosynthesize different types of cyanotoxins which pose risks to human and animal health. Cyanobacteria can also produce nuisance taste and odor (T&O) compounds in distributed water, which may reduce water aesthetic quality, and does not meet customer expectation (Harke et al., 2016). It is expected that the frequency, duration, and density of cyanobacterial blooms will increase in the near future, due to global climate change and human activities (Beaulieu et al., 2013; Grattan et al., 2016; J. Alex, 2012; Merel et al., 2013; Paerl et al., 2012; Richardson et al., 2019). To assess the risk posed to drinking water supplies, and to ensure that treatment is adjusted to respond to varying levels of cyanobacteria present, quick detection of cyanobacterial biomass is of critical importance for water utilities.

The technologies used to monitor cyanobacteria can be classified into three main categories: microscopic enumeration and identification; molecular-based methods; and pigment concentration measurements. The counting of taxonomic microscopic cells is time-consuming, expensive, and requires an experienced experimenter (Bertone et al., 2018; Lou et al., 2017; Zamyadi et al., 2016a; Zamyadi et al., 2012c). The results are prone to significant error (up to 40%) because of varying cell morphology, aggregation, presence of the preservation solution, and analyst bias and experience (America Water Works Association (AWWA), 2010; Chorus et al., 2021b; Hawkins et al., 2005; Newcombe, 2009; Sanseverino et al., 2017; Zamyadi et al., 2016a). Considering molecular-based methods, PCR, Real-Time PCR, and high-throughput sequencing can greatly improve the ability to identify the microbial community composition, determine the functional genes, and trace the activity of the cyanobacterial cell genes (Chiu et al., 2017; Jalili et al., 2021; John et al., 2018; Lou et al., 2017; Moradinejad et al., 2020; Romanis et al., 2021; Sanseverino et al., 2017; Serrano-Silva et al., 2018; Srivastava et al., 2013; Te et al., 2015; Tromas et al., 2017; Zamyadi et al., 2019; Zhu et al., 2021). However, molecular-based methods are complex, high-cost, and require skillful analysts (Lou et al., 2017; Srivastava et al., 2013). Moreover, bias could be caused by an incomplete gene bank library, primer selection, amplification, and genomic DNA base composition (Bharti et al., 2021; Moradinejad et al., 2020; Tan et al., 2015). These methods restrict the number of samples that can be analyzed, undermine online assessment, and limit the ability to reflect the cyanobacterial biomass fluctuation. Cyanobacteria can also be monitored by pigment quantification. Phycocyanin is a unique phycobilisome pigment of freshwater

cyanobacteria, and has strong fluorescence signatures. The excitation wavelength of the phycocyanin is between 610 and 630 nm, and the emission wavelength is between 600 and 700 nm (Bryant, 1986; Carr et al., 1982; Prescott et al., 2005). Numerous studies have suggested using fluorescence phycocyanin probes as an online tool for cyanobacterial monitoring due to its operational simplicity, high detection frequency, and low cost (McQuaid et al., 2011; Pazouki et al., 2014; Zamyadi et al., 2014).

It is important to determine sources of uncertainties in order to use phycocyanin probe readings to estimate cyanobacterial levels in water sources. Correlations have been reported between the phycocyanin fluorescence probe readings and the extracted phycocyanin concentration, total taxonomic cell counts, and total cyanobacterial biovolume (Table A.1). In general, phycocyanin probe readings are well correlated with the laboratory-cultured unicellular species, and less promising with the colonial species and mixed species. Field samples are more likely to be subject to interferences. The reference method to which the probe readings are compared is also important. From Table A.1, phycocyanin probe readings are better correlated with biovolume, compared to taxonomic cell counts. Various factors can influence the accuracy of phycocyanin fluorescence analysis (Table A.2). Considerable investigations have been reported into different cells' growth phases, sizes, and geometries, which can result in deviation in the phycocyanin readings (Chang et al., 2012; Hodges et al., 2018; Korak et al., 2015; McQuaid et al., 2011; Rippka et al., 1979; Thomson-Laing et al., 2020; Zamyadi et al., 2012b). Sources of interference relate to several potential environmental factors, which interfere with the probe's capacity to precisely measure phycocyanin pigment content. Such interferences include cell prior light exposure (Brient et al., 2008; Hodges et al., 2018; Misumi et al., 2016; Rouso et al., 2021; Zamyadi et al., 2016a; Zamyadi et al., 2012b), the presence of chlorophyll-a (Bertone et al., 2018; Choo et al., 2018; Choo et al., 2019; Zamyadi et al., 2016a), and water turbidity (Bowling et al., 2013; Brient et al., 2008; Symes et al., 2016; Zamyadi et al., 2012b).

The variation in natural organic matter (NOM) content and water temperature are other critical factors that can influence the accuracy of *in situ* phycocyanin probe monitoring. For this reason, some fluorometer manufacturers specify their operating conditions. For instance, Turner Cyanofluor corrects the interference from dissolved organic carbon (DOC) using a filtrate blank (Turner Designs, 2017). In the actual experimental situation, recent research has shown a significant impact of NOM on phycocyanin probe response to cyanobacterial cells, but the different

water matrix, types of NOM investigated, type and presence of cyanobacterial cells, and type of probe investigated were limited (Bertone et al., 2019; Courtois et al., 2017; Korak et al., 2015). Furthermore, DOC concentration in the study of Bertone et al. (2019) was measured by a YSI EXO2 fDOM sensor, which is not as reliable as direct DOC measurements and does not provide information on the adsorption spectra of the NOM. Temperature was also shown to influence phycocyanin probe readings. Some probe manufacturers incorporate or propose a temperature compensation, and these factors vary between manufacturers and devices. Recent investigations of the impact of temperature on several probe devices showed that corrective factors vary between devices (Hodges et al., 2018; Watras et al., 2017). To the best of our knowledge, this is the first to report on the impact of different ambient water temperatures on the YSI EXO2 phycocyanin probe.

Evaluating the impact of NOM and temperature on the phycocyanin probe measurements is important because climate change and the increased frequency of extreme events in natural water are likely to result in higher temperatures and NOM levels in freshwater (Pachauri et al., 2015; Wauthy et al., 2018). Moreover, in the application of field *in situ* measurement, it is challenging to maintain a constant water temperature, and the probe is generally deployed in a wide range of temperatures.

The main objective of the work was to validate the use of an *in situ* phycocyanin fluorescence probe for cyanobacteria detection in the intake water of a drinking water treatment plant (DWTP). The specific objectives of this study were to investigate: (1) the impact of Suwannee River NOM in ultrapure and lake water on the monitoring of cyanobacterial phycocyanin readings and investigate the mechanism of interference; (2) the impact of temperatures from 6 to 33 °C in ultrapure water on phycocyanin readings on dissolved and cell-bound phycocyanin; (3) relationships between cyanobacterial biovolume and phycocyanin probe measurements; (4) the applicability for the measurement of cyanobacteria within the DWTP by using the fluorescence probe.

4.2 Materials and methods

4.2.1 Cell culture

Microcystis aeruginosa strain CPCC 300, referred to *Microcystis*, and *Dolichospermum* sp. (formerly known as *Anabaena* sp.) strain CPCC 544, referred to *Dolichospermum*, were selected in this study. *Microcystis* and *Dolichospermum* are common unicellular, and filamentous

cyanobacterial species, respectively (Winter et al., 2011). Axenic cultures were cultivated in 9 L flasks with BG-11 medium (Rippka et al., 1979). The temperature of the culture room was 20 ± 2 °C. A cool fluorescence light at an intensity of $70 \mu\text{mol}/\text{S}.\text{m}^2$ radiated to the room 12 h a day. Air diffusers were implemented in the cyanobacterial cell cultures to provide sufficient oxygen and mixing conditions. Cultures were harvested at the stationary growth phase.

4.2.2 Microscopic taxonomic cell counts

Cultures were centrifuged by Sorvall Legend RT at $10,000\times g$ and 4 °C for 15 min to separate the cells from the medium and extracellular organic matter (Qu et al., 2012). The supernatant was discarded, and the pelleted cells were re-suspended in 5 mL of ultrapure water (Millipore Pty Ltd., Burlington, MA, USA) by vortexing for 20 s. An inverted microscopic (Olympus IX71) at $20\times$ was used for taxonomic cell counts in a Sedgwick-Rafter counting chamber. The final counting result was derived from the cell number of the known area to 1 mL of sample. The cell sample was then diluted with ultrapure water or lake water (mentioned below) to low (2000 cells/mL), medium (20,000 cells/mL), and high (100,000 cells/mL) cell concentrations according to the microscopic cell counts results, as described by Zamyadi et al. (2015).

4.2.3 Fluorometer measurements

A YSI EXO2 probe (YSI, Yellow Springs, OH, USA), which featured a phycocyanin/chlorophyll-a sensor, conductivity/temperature sensor, dissolved oxygen (DO) sensor, turbidity sensor, and pH sensor, was used in this study. A self-cleaning wiper was applied to the probe. The probe converts the light emitted from the phycocyanin pigments first into ratio fluorescent unit (RFU), and then to the micrograms per liter phycocyanin pigment concentration ($\mu\text{g}/\text{L}$) via an onboard default conversion factor. Several studies have shown better correlation with the phycocyanin RFU content in comparison with $\mu\text{g}/\text{L}$ (Bastien et al., 2011; Bowling et al., 2016; Genzoli et al., 2016; Zamyadi et al., 2012b). Probe phycocyanin optics has the excitation wavelength of 590 ± 15 nm, and emission wavelength of 685 ± 20 nm (YSI Incorporated, 2019). The probe was calibrated according to the manufacturer's user manual (YSI Incorporated, 2019). Negative phycocyanin RFU readings were observed from the monitoring of ultrapure water. Thus, we calculated the limit of blank (LoB) using Equation 4.1 (Armbruster et al., 2008), and the phycocyanin values that were lower than LoB were adjusted as 0 for all measurements.

$$LoB = mean_{blank} + 1.645(SD_{blank}) \quad \text{Equation 4.1}$$

where $mean_{blank}$ and SD_{blank} are the mean and the standard deviation, respectively, of the measurement of ultrapure water.

All measurements were conducted using the probe calibration cap with a black bottom to eliminate light influence. The probe and its sensors were cleaned with ultrapure water before each sample. The probe data were recorded once the readings were stabilized. The readings were recorded for 10 min at a 0.25 s interval.

4.2.4 NOM addition

To test the interference of NOM on the fluorescence signal of the cyanobacterial cells, assays were performed by using the solution made from Suwannee River NOM (2R101N, International Humic Substances Society, Saint Paul, MN, USA; SRNOM). SRNOM is measured based on the DOC content in this study. The DOC concentrations of the sample were measured using a total organic carbon analyzer (Sievers Analytical Instruments, Boulder, CO, USA), after sample filtration with the pre-rinsed 0.45 μm membrane (Supor 45 μm , PES PALL, Port Washington, NY, USA). In the set of assays, four concentrations of DOC (0, 0.7, 1.7, 11 mg/L) of SRNOM were added in sequence to a range of concentrations of cyanobacterial cells (*Microcystis* and *Dolichospermum*, at 0, 2000, 20,000, and 100,000 cells/mL). Experiments were assessed on two matrixes: ultrapure water and lake water (DOC of 6.31 mg/L). Lake water was collected in May 2019 from Lake Champlain (Quebec).

4.2.5 Water temperature effect

To evaluate the interference of the water temperature with cyanobacterial phycocyanin fluorescence signal, *Microcystis* was spiked into the different temperatures of ultrapure water. Initially, 500 mL ultrapure water samples were placed into containers with temperatures of 6, 12, 33 °C, and room temperature (21 °C). Then, a *Microcystis* cell sample was spiked into each of the different water temperature reactors to reach the cell concentration of 2000, 20,000, and 100,000 cells/mL. In addition, the 1 mg/L C-phycocyanin extract (P2172, Sigma-Aldrich, St. Louis, MO, USA) was dissolved into the above-mentioned ultrapure water with different temperatures.

4.2.6 Fluorescence excitation-emission matrix spectra analysis

The fluorescence spectra of ultrapure water containing *Microcystis* (100,000 cells/mL) with and without SRNOM were measured using fluorescence excitation-emission matrix (EEM) spectra by a fluorescence spectrophotometer (Agilent, Cary Eclipse, Santa Clara, CA, USA). Both excitation spectra and emission spectra were scanned from the wavelength of 200 to 750 nm using a 1 cm quartz cuvette for each sample. The increments of the excitation and the emission spectra were 5 and 2 nm, respectively.

4.2.7 Field sampling

Field sampling was conducted from Bedford DWTP from July to August 2018. Bedford DWTP draws water from Lake Champlain in southern Quebec. Cyanobacterial bloom has occurred in the Bedford DWTP during the summer and fall over the past decade (Bowling et al., 2015; Zamyadi et al., 2014; Zamyadi et al., 2012c). In the DWTP, the data was recorded every 2 h, each round for 30 min at 5 min intervals, by a YSI EXO2 phycocyanin probe.

4.2.8 Data analysis

A non-parametric Kruskal–Wallis test was used to determine if there were significant variations between the set of NOM and temperature essays. Dunn’s post hoc test was used to detect significant differences between each treatment. The level of significance was 0.05.

EEM data processing followed the method reported in Stedmon et al. (2008). In brief, the raw spectra data were subtracted from the blank spectra with ultrapure water. Rayleigh scatter and Raman scatter were removed to eliminate the light scattering. Data manipulations were performed using MATLAB software (MathWorks, USA) and the DOMFlour toolbox.

The correlation scatter plots between the phycocyanin readings and total cyanobacterial biovolume (and cell counts) were performed with the ggscatter R package with the Pearson correlation coefficient.

The correlations between the environmental parameters and phycocyanin readings were conducted using the R function corrplot.

4.3 Results and discussion

4.3.1 Addition of NOM

4.3.1.1 Impact of NOM on phycocyanin probe performance

To investigate the extent of potential interferences by SRNOM on cell-induced fluorescence, the fluctuation of phycocyanin readings as a function of dosage and water matrix are shown in Figure 4.1. The results show that the level of phycocyanin readings decrease in the presence of cells after the addition of SRNOM in both ultrapure water and lake water, and statistical significant results are indicated by the letters a, b, c, d bearing on the boxplots (Figure 4.1). The addition of 11 mg/L of DOC of SRNOM in the high cell concentration (100,000 cells/mL) decreases the median phycocyanin readings by up to 1.7 RFU (1.2 to 1.7 RFU) for both *Microcystis* and *Dolichospermum* suspended in ultrapure water and lake water. The decrease in phycocyanin signal corresponds to a significant underestimation of 6.5–8.8% for high cyanobacterial cell concentrations. Although significant, this underestimation is modest for cell concentrations of 2000 and 20,000 cells/mL. The SRNOM interference is most pertinent when both cell concentration and the SRNOM contents are high.

In comparison with phycocyanin readings in the water matrix without the cells, the addition of 11 mg/L DOC of SRNOM causes an increase of the fluorescence signal of 0.2 RFU in ultrapure water and lake water (Figure 4.1). In lake water, a background fluorescence reading of 0.2 RFU is also noted for DOC of 6.31 mg/L (Figure 4.1b, d). These findings are in agreement with a prior study reporting a 0.8 RFU phycocyanin signal increase in a commercial humic acid solution (DOC of 10 mg/L) (Courtois et al., 2017).

It is notable that the phycocyanin fluorescence signal of *Dolichospermum* suspensions (Figure 4.1c, d) is more dispersed as compared to readings from *Microcystis* (Figure 4.1a, b). Similar observations of greater variability in the fluorescence signals for the *Dolichospermum* were also reported by Kring et al. (2014), as compared to unicellular *Microcystis aeruginosa*. These authors attributed their results to the lack of species-specific calibration (fingerprint) of the probes and the filamentous nature of *Dolichospermum*. Additionally, the cell-induced phycocyanin fluorescence in the lake matrix is even more dispersed than the tests that were conducted in ultrapure water for both *Microcystis* and *Dolichospermum*. In lake water, the addition of SRNOM in the presence of NOM increases fluorescence signal for *Dolichospermum* at low DOC (1.7 mg/L), suggesting an

interaction between the cells and the NOM. Optical measurements in natural water can be influenced by the presence of the suspended particles in the natural water, which can lead to the scattering and absorption of the fluorescence (De Oliveira et al., 2018; Zamyadi et al., 2016a).

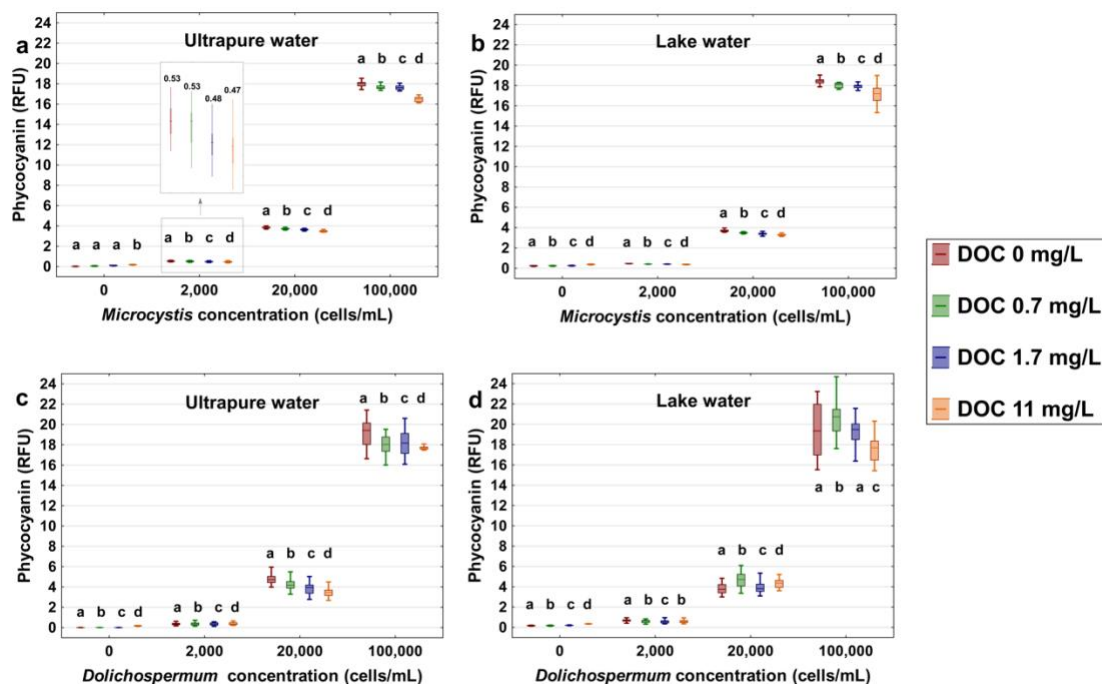


Figure 4.1 Phycocyanin readings (RFU) by using the YSI EXO2 probe of 0 (no cells added), 2000, 20,000, and 100,000 cells/mL of cyanobacterial species with spike 0, 0.7, 1.7, 11 mg/L dissolved organic carbon (DOC) of Suwannee River NOM (SRNOM): (a,b) *Microcystis aeruginosa* strain CPCC 300 suspended in 500 mL ultrapure water and Lake Champlain water; (c,d) *Dolichospermum* sp. strain CPCC 544 suspended in 500 mL ultrapure water and Lake Champlain water. The probe measured each of the sample at 250 ms interval for 10 min. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$)

4.3.1.2 Fluorescence EEM studies

Fluorescence EEM spectroscopy is used to investigate the fluctuation of the *Microcystis* fluorescence by the addition of SRNOM. The excitation-emission results of ultrapure water in the presence of *Microcystis* cells (with and without SRNOM) are presented in Figure 4.2. Figure 4.2 demonstrates that the peak location is not changed by adding 11 mg/L DOC of SRNOM in the 100,000 cells/mL *Microcystis* samples. Both EEM spectra show the phycocyanin fluorescence measured at excitation wavelength of 625 nm, and emission wavelength of 650 nm, exactly matched the phycocyanin optical window. Strikingly, the addition of SRNOM decreases the fluorescence intensity by up to 33%, reflecting the decrease of fluorescence signal in Figure 4.1.

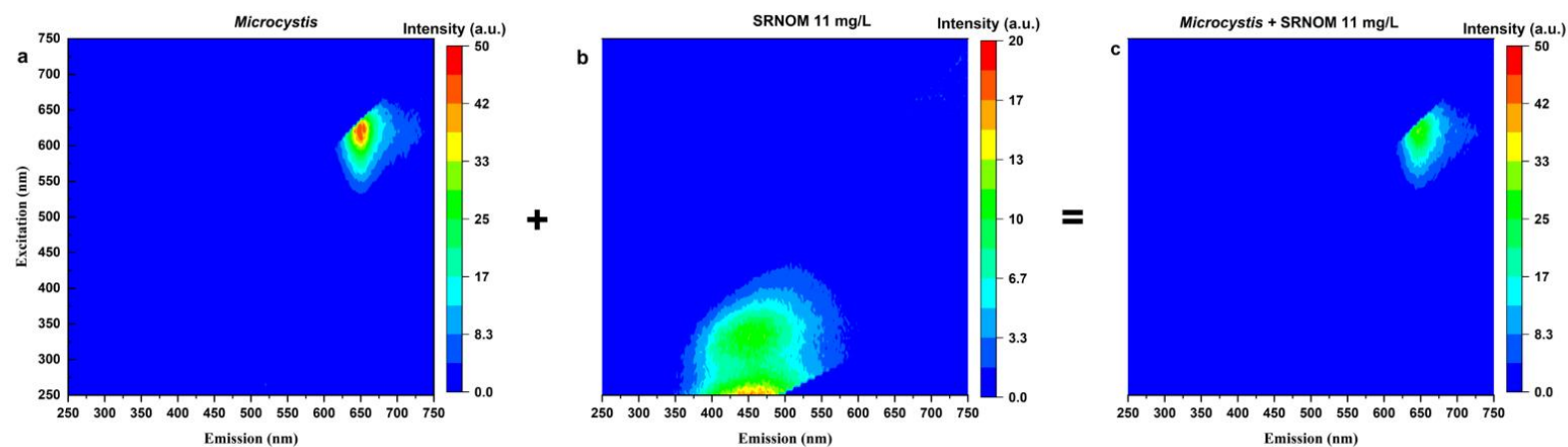


Figure 4.2 Excitation-emission matrix for (a) 100,000 cells/L *Microcystis aeruginosa* CPCC 300 suspension in ultrapure water, (b) 11 mg/L DOC of SRNOM, (c) 11 mg/L DOC of SRNOM added to 100,000 cells/L *Microcystis aeruginosa* CPCC 300 suspension in ultrapure water. Note the *Microcystis aeruginosa* intensity is twice as high as that of SRNOM

SRNOM exhibits fluorescence maximum at an excitation wavelength of 250 nm and emission wavelengths ranging from 430 to 480 nm (Figure 4.2b), which aligns with the wavelength reported in Yoo et al. (2016). Cyanobacterial phycocyanin fluorescence signatures occur at high wavelength range (excitation and emission wavelength of 625 and 650 nm, respectively; Figure 4.2a). Comparison between the wavelengths of SRNOM and cyanobacterial phycocyanin indicates that their fluorescence is unlikely to overlap, precluding the interference from these compounds, provided quenching effects are accounted for. Similar investigations into whether the presence of NOM affects phycocyanin fluorescence readings have been undertaken by Korak et al. (2015) by analyzing the EEMs. These authors found that DOM from river water (DOC of 2.65 mg/L) has no fluorescence interference with the extracted 1 mg/L phycocyanin pigment; however, with the *Microcystis aeruginosa* intracellular organic matter (IOM), the phycocyanin fluorescence intensity decreased by more than an order of magnitude. In addition to decreased intensity of *Microcystis aeruginosa* IOM, new fluorescence behavior was noted from the tests of other two species (*Oscillatoria* sp. and *Lyngbya* sp.) (Korak et al., 2015). The authors attribute this to the quenching of cell IOM phycobiliprotein fluorescence (Korak et al., 2015). Furthermore, Korak et al. (2015) demonstrated that the decrease in phycocyanin fluorescence monitored in a DWTP could be used as a good indicator for the IOM release. However, our study found that the variation in NOM level could also cause a decline in fluorescence intensity of cyanobacterial cells (cell-bound phycocyanin). Thus, the previously found phycocyanin reduction may not be due to the release of IOM for a system that contains NOM.

The magnitude of the interference caused by NOM also depends on both the number of cells and the aromaticity of NOM. Bertone et al. (2019) found that concentrated DOM from lake water (SUVA₂₅₄ of 4.21 L/(mg.m)) results in underestimation of up to 14% of the phycocyanin signal measured by a YSI EXO2 probe (with 8,000,000 cells/mL *Raphidiopsis raciborskii*). The authors also reported that, the more aromatic and humic the DOM (higher SUVA₂₅₄), the lower the measured phycocyanin signal. However, the concentrations of DOM were not specified in this study (Bertone et al., 2019). In our study, both cell concentrations (100,000 cells/mL) and SUVA₂₅₄ value (SRNOM is 3.6 L/(mg.m)) were lower than in Bertone et al. (2019). As a result, lower suppression of phycocyanin was expected to be observed. Our observations complement the findings from these studies and consolidate the fact that the source of NOM may determine the extent of the impact on the phycocyanin fluorescence signal.

To quantify the importance of NOM interference, important points to consider include: the concentration of NOM added, aromaticity of NOM (SUVA₂₅₄), the ratio of added NOM to the number of cells, and prior exposure of the cells to NOM for the testing of the interference. Firstly, it is difficult to identify the concentration of NOM in some studies, as different DOC measurement methods are used. Bertone et al. (2019) used an fDOM sensor to measure DOC concentration but did not report on the concentration. fDOM sensors may not be as reliable as direct DOC measurements, as fDOM sensors need to be validated for the interference from ambient temperature, turbidity, and suspended particles (Bertone et al., 2018; De Oliveira et al., 2018; Downing et al., 2012). Secondly, as shown in Figure 4.1, the species present (*Microcystis aeruginosa* and *Dolichospermum* sp.) and the background of DOC in the test water define the extent and the trends for fluorescence quenching. Thirdly, the interference caused by the addition of NOM is not impacted by the ratio of the added NOM to the number of cells, which varies from 0.11 to 5.5 µg DOC/cell.

The use of a phycocyanin probe to detect cyanobacteria in the presence of NOM will likely result in an underestimation of cyanobacteria. High concentrations of NOM (DOC of 11 mg/L) may lead to significant underestimation of bloom densities that require prompt response from managers. However, Figure 4.1 shows that significant false negative readings are associated with considerable NOM levels. An interference in the low probe signals of less than 1 RFU would not result in any change in terms of cyanobacteria surveillance and management solutions. Moreover, in a real-world application, it is unlikely that a sudden increase in NOM extent will occur in the source water. According to our field sample results, DOC concentration ranges from 5.3 to 7.9 mg/L, provided that the NOM-induced interference appears negligible. The calibration to a standard DOC solution may reduce the interference by the presence of NOM in the background water.

4.3.2 Varying water matrix temperature

The phycocyanin readings of *Microcystis* were measured at different ultrapure water temperatures. Figure 4.3a shows that the level of phycocyanin readings decreases at higher water temperatures. Kruskal–Wallis tests show a statistically significant effect in all the sets of assays. Dunn’s post hoc tests reveal significant differences between each assay. For the high *Microcystis* density (100,000 cell/mL), the phycocyanin readings decrease by 8.4 RFU when water temperature increases from 6 to 33 °C. The decrease in phycocyanin signal corresponds to a significant underestimation of

36.8% for the 100,000 cells/mL cyanobacterial cell concentration. The underestimation is 50.0% for 2000 cells/mL and 20.5% for 20,000 cells/mL of *Microcystis*.

It is important to know whether this phenomenon is due to the physiological state of the cells or the radiative/non-radiative decay of the excited fluorophore. To understand the phenomenon, 1 mg/L phycocyanin was dissolved in ultrapure water and phycocyanin signals were measured at different temperatures (same condition as with *Microcystis* cells added). Figure 4.4a shows that the phycocyanin signal decreases with increasing water temperature, which confirms the trends shown in Figure 4.3a. Temperature may impact the excited fluorophore energy decay pathways. In other words, the dependence of the phycocyanin probe signal on the temperature variation is due to the radiative decay of the excited fluorophore rather than the physiological state of the cells (So et al., 2001). In the latter case, fluorescence readings of the phycocyanin extract are expected to be the same as the variation in the ambient water temperature. So et al. (2001) explained that, when phycocyanin is excited at a specific wavelength, it releases energy either as radiative decay (fluorescence emission) or as nonradiative decay (heat); the temperature variation can shift the energy releasing pathway, so that as temperature increases, the nonradiative decay increases, which in turn reduces the fluorescence intensity.

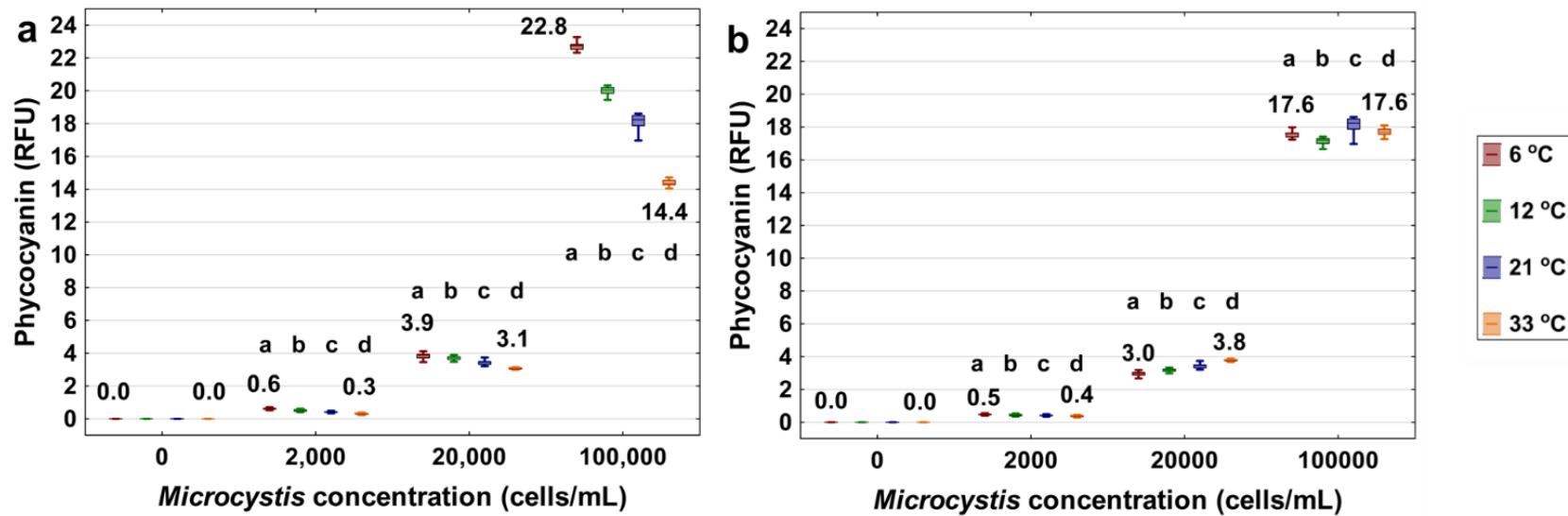


Figure 4.3 Phycocyanin readings (RFU) with the YSI EXO2 probe of the 0, 2000, 20,000 and 100,000 cells/mL of *Microcystis aeruginosa* strain CPCC 300 suspended in 6, 12, 21, 33 °C of ultrapure water. The probe measured each of the samples at a 250 ms interval for 10 min (a). In (b) the temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation 4.3. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$)

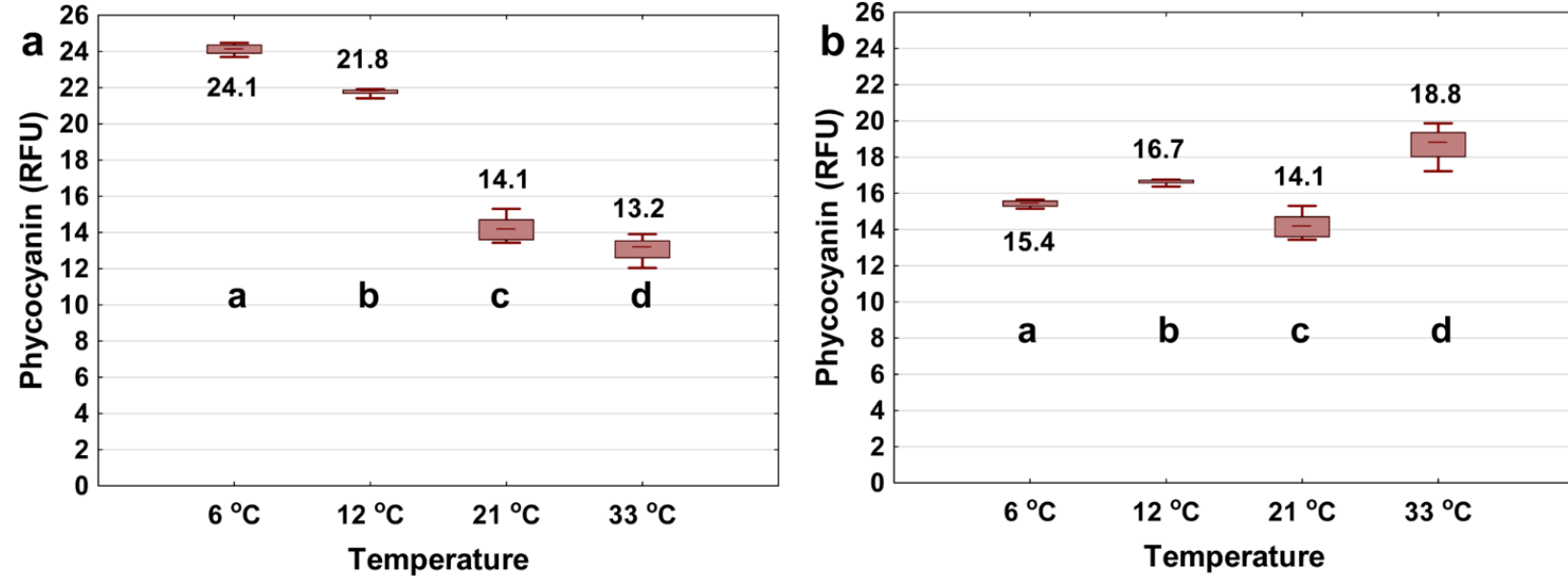


Figure 4.4 Phycocyanin readings (RFU) using the YSI EXO2 probe of 1 mg/L extract phycocyanin pigment in 6, 12, 21, 33 °C of ultrapure water. All samples were tested over the period of 10 min. The probe measured each of the sample at a 250 ms interval for 10 min (a). In (b) the temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation 4.3. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$)

Few studies have quantified temperature interference on phycocyanin fluorescence readings. Watras et al. (2017) proposed a detailed demonstration in which the impact of temperature on a Turner Cyclops-7 probe can be compensated by adjusting raw phycocyanin fluorescence data to a reference temperature. The authors report a linear correlation (Equation 4.2) with temperature for extracted phycocyanin pigment and phycocyanin from lake water, and an exponential correlation (Equation 4.3) for laboratory-cultured cyanobacteria *Synechococcus leopoliensis*. We inferred that the algorithms derived from Watras et al. (2017) could be also applied to *Microcystis aeruginosa* and YSI EXO2 probe. Thus, we applied Equation 4.2 and Equation 4.3 to assess its ability to compensate fluorescence readings with the YSI EXO2 probe, using the 21 °C test as the reference temperature.

$$F_r = F_m / (1 + \rho(T_m - T_r)) \quad \text{Equation 4.2}$$

$$F_r = F_m / (e^{\alpha(T_m - T_r)}) \quad \text{Equation 4.3}$$

where F_r stands for reference fluorescence (RFU), F_m stands for measured fluorescence (RFU), T_r stands for reference temperature (°C), T_m stands for measured temperature (°C), ρ (between -0.018 and -0.037), and α (between -0.017 and -0.030) represents the temperature coefficient (Watras et al., 2017).

As shown in Figure 4.3, after fitting with the exponential algorithm (Equation 4.3), the measured phycocyanin difference dropped from 50.0% to 20.0% for the 2000 cells/mL, slightly increased from 20.5% to 26.7% for the 20,000 cells/mL, and dropped from 36.8% to 0% for the 100,000 cells/mL of *Microcystis*, for temperatures ranging from 6 to 33 °C. For the dissolved extract phycocyanin in ultrapure water, the difference dropped from 45.2% to 22.1% (Figure 4.4). In addition, we applied the linear algorithm (Equation 4.2) to the *Microcystis* (Figure A.1) and extracted phycocyanin (Figure A.2) fluorescence readings. Results show that the exponential model was most efficient in reducing temperature interference for both *Microcystis* cells and extracted phycocyanin.

Watras et al. (2017) observed that the adjusted readings of cyanobacterial cells at other temperatures are higher than the readings at the reference temperature. The difference in fluorescence increased with the difference between the reference temperature and the tested temperature (approximately 4 to 33 °C). The deviations were more prominent at high cell densities.

In our study, the corrected readings show different trends depending on cell densities: an under/over estimation at 20,000 cells/mL, and a systematic but small underestimation at 100,000 cells/mL (Figure 4.3b). Hodges et al. (2018) investigated two units of the same Turner Cyclops-7 phycocyanin probe as used by Watras et al. (2017). On both units, he did not report significant differences in readings for a narrower temperature range (13.8, 17, and 23.5 °C) using laboratory-cultured *Microcystis aeruginosa* (181,000 to 828,000 cells/mL). By comparison, phycocyanin readings at 4 °C were higher than those at other adjusted temperatures for the other unit tested. The authors also assessed Eureka Manta II and TriLux probes, but no significant differences were found among all the temperatures tested (4 to 23.5 °C). Prior work and this study show that phycocyanin probe readings can be significantly affected by temperature in the range of their typical application (6–33 °C). Clearly, some models appear to have incorporated correction algorithms (Eureka Manta II and TriLux), while others, such as the model tested in this study, do not. When deploying and interpreting the probe readings, users should be fully aware of the probe features.

According to our controlled validation testing, the use of uncorrected temperature readings can lead to an overestimation (up to 26.0%) and underestimation (20.4%), compared to the readings at the reference temperature (21 °C) when the temperature ranges from 6 to 33 °C. The reliability of phycocyanin probe measurements is subject to the temperature variation through time. During the cyanobacteria monitoring, if the probe is deployed, and the temperature varies during the deployment period, the phycocyanin values should be interpreted carefully considering the temperature variation. The significance of the difference depends on the application; in particular, refer to the alert levels used for cyanobacteria management plans. According to McQuaid et al. (2011), probe reading 1.7 RFU represents WHO alert level 1 (published in 1999), and probe reading 10.1 RFU represents WHO alert level 2. The use of the probe tested for temperatures between 6 and 33 °C could lead to false detection of cyanobacteria at around 6000 cells/mL if 1.7 RFU is applied as a guideline. If a higher threshold is used, for instance, 10.1 RFU, the cyanobacteria at 34,000 cells/mL need to be carefully checked. However, during our field cyanobacteria sampling, the intake water temperature ranged from 22.9 to 28.2 °C, which is the typical temperature during bloom season. Such a narrower temperature range indicates that the estimation error appears to be reduced, compared with the results in our study.

Finally, it is important to consider that laboratory cultured cyanobacterial cells, such as *Microcystis*, are usually unicellular, whereas they may aggregate and form colonies in natural blooms.

Therefore, when applying the results from the laboratory fluorescence test to the *in situ* monitoring, these discrepancies and complexities need to be considered. Readings in the laboratory-cultured cyanobacterial cells may not represent real bloom conditions, and the monitoring frameworks should be validated using real bloom assemblages. Even for the temperature-corrected readings, other factors such as the different genera present, single species versus mixed blooms, and the presence of NOM may impact the temperature deviation of the phycocyanin readings. Modification of existing correction algorithms to incorporate other external conditions, such as cyanobacterial cell species and status, probe character, reference temperature chosen, and water quality, require further investigation.

4.3.3 Validation of probes with field samples

4.3.3.1 Relationships between cyanobacterial biovolume and phycocyanin probe measurements

Phycocyanin fluorescence probe readings were validated with taxonomic cell counts results on Bedford intake water from 24 July to 26 August 2018. The values of the phycocyanin in RFU from the YSI EXO2 probes are plotted against the main cyanobacterial species biovolume and total cyanobacterial biovolume in Figure 4.5. A significant relationship was observed between phycocyanin and total cyanobacterial biovolume ($R = 0.73$, $p = 0.098$). Correlations have been extensively reported in the literature between the phycocyanin fluorescence probe readings and the extracted phycocyanin concentration, total cyanobacterial biovolume, and total cyanobacterial cell counts (Table A.1). Phycocyanin probe readings are generally well adjusted with the laboratory-cultured unicellular species (Bastien et al., 2011; Bertone et al., 2019; Choo et al., 2018; Hodges et al., 2018; Kong et al., 2014; Thomson-Laing et al., 2020; Zamyadi et al., 2012b). However, the correlation is less promising with the colonial species (Hodges et al., 2018), mixed cultured species (Kong et al., 2014), and field-collected species (Almuhtaram et al., 2018; Bastien et al., 2011; Bowling et al., 2013; Bowling et al., 2016; Carr et al., 1982; Cotterill et al., 2019; Hodges et al., 2018; Korak et al., 2015; McQuaid et al., 2011; Rousso et al., 2021; Song et al., 2013; Thomson-Laing et al., 2020; Zamyadi et al., 2014; Zamyadi et al., 2016b; Zamyadi et al., 2012a). A variety of factors can affect probe measurement for environmental samples. Differences in phycocyanin content in cyanobacteria have been reported in the literature. For example, the phycocyanin quota may vary due to different growth phases (Bertone et al., 2019; Chang et al., 2012; Rousso et al.,

2022; Ziegmann et al., 2010). Ziegmann et al. (2010) revealed higher phycocyanin content in the decay phase by analyzing the EEM spectra of *Microcystis aeruginosa*. Such findings are consistent with those of Bertone et al. (2019), who used the same probe model as in our study (YSI EXO2), and reported increasing phycocyanin content over time (day 7–35) for *Microcystis* sp. However, Bertone et al. (2019) noted inconsistent phycocyanin content in different growth stages depending on different cyanobacterial species, as the authors also found decreasing phycocyanin content over time (day 7–35) for *Aphanocapsa* sp. and *Sphaerospermopsis* sp. These results differ from those reported by Chang et al. (2012) for *Microcystis aeruginosa*, *Dolichospermum circinalis*, and *Planktothricoides raciborskii* using a Turner Designs Model 10 probe. Interference with probe readings can be also due to the spatial dispersion of the cyanobacteria. Cyanobacterial agglomeration can vary in time and be composed of various species. The biovolumes of such species span a wide range, which leads to the inaccuracy of the estimation (Kasinak et al., 2015; McQuaid et al., 2011). When cyanobacterial species aggregate (such as *Microcystis*), the fluorescence emission and excitation may not penetrate the whole colony of a few hundred millimeters, leading to the underestimation of the phycocyanin concentration (Chang et al., 2012; Hodges, 2016). These are all attributes that differ greatly between environmental samples and may explain the weaker correlation compared to the more controlled laboratory-cultured samples.

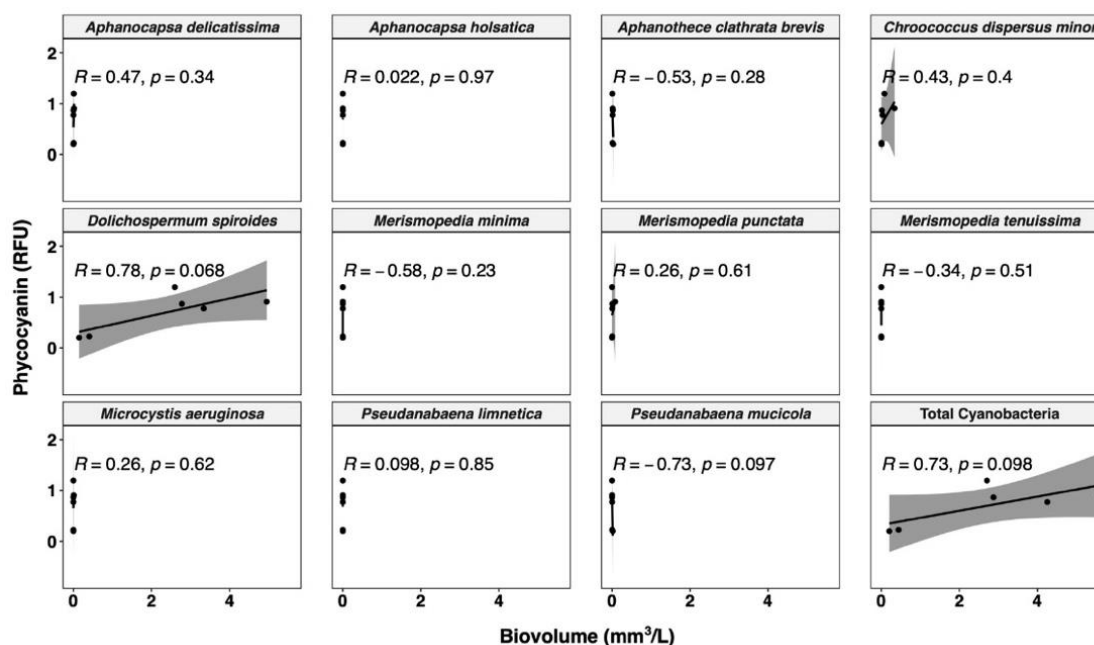


Figure 4.5 Relationship between phycocyanin probe readings and cell biovolume for different cyanobacterial species. Shaded area around line represents 95% confidence interval

The cyanobacterial community composition has a considerable influence on the phycocyanin measurements. The R value of the best-fit line between biovolume and phycocyanin for each species ranges from -0.73 to 0.78 (Figure 4.5). Only with high biovolume species are high R values observed, as expected, since a low count or biovolume is not expected to correlate with a global fluorescence reading. The strongest relationship occurs in the species *Dolichospermum spiroides*, which accounts for 93.5% of the total cyanobacterial biovolume. Such a wide range may be due to the difference in cell size and phycocyanin cell quota between species (Kong et al., 2014; Rouso et al., 2022), which can be validated by comparing the phycocyanin signal to cell counts of each species.

In terms of cell counts, the samples exhibit more diverse cyanobacterial communities (Figure 4.6). The most abundant species is *Aphanothece clathrate brevis* (33.1%), followed by *Aphanocapsa delicatissima* (31.6%), and *Dolichospermum spiroides* (19.0%). The different patterns between the cell counts and cell biovolume may be due to the different cell size (Kong et al., 2014). For instance, in our study, *Dolichospermum spiroides* has the highest biovolume of $179.6 \mu\text{m}^3/\text{cell}$. This contrasted sharply with the most abundant *Aphanothece clathrate brevis* and *Aphanocapsa delicatissima*, with very low cell biovolumes of 0.5 and $0.3 \mu\text{m}^3/\text{cell}$, respectively. Cell

phycocyanin quota has been demonstrated to be proportionally related to cell volume, so species with larger cell sizes have more phycocyanin per cell (Brient et al., 2008). Furthermore, much weaker correlations are observed between phycocyanin RFU and total cyanobacterial cell counts ($R = 0.41$, Figure 4.6) than for total cyanobacterial biovolume ($R = 0.73$, Figure 4.5), in line with previous studies (Kasinak et al., 2015; Kong et al., 2014; Macário et al., 2015; McQuaid et al., 2011; Zamyadi et al., 2012b). Rousso et al. (2022) assessed four cyanobacterial species using the same probe as in our study, and reported less variability in phycocyanin fluorescence per unit biomass (or per unit biovolume) among species than per cell. Therefore, conversion of phycocyanin RFU to biovolume may result in a reduced bias than conversion to cell counts. Moreover, the WHO alert level has recently updated its cyanobacterial monitoring framework. Compared with the WHO guidance level published in 1999 (Chorus et al., 1999), the recently published WHO alert level incorporates cyanobacterial cell biovolume, instead of taxonomic cell counts, as an indicator, due to the substantial variation in the cell size (Chorus et al., 2021b). Our findings, together with those of earlier research, suggest that phycocyanin per cell varies greatly between species, primarily due to pigment concentration varying with cell size. Biovolume is a better indicator of phycocyanin fluorescence than cell counts. Nevertheless, phycocyanin per biovolume can still differ greatly between species, as observed by Thomson-Laing et al. (2020), which showed more than 100-fold phycocyanin fluorescence per biovolume differences between the studied 12 species. Overall, for improved speciation and biomass correlation, *in situ* phycocyanin probe readings require more validation with robust methods.

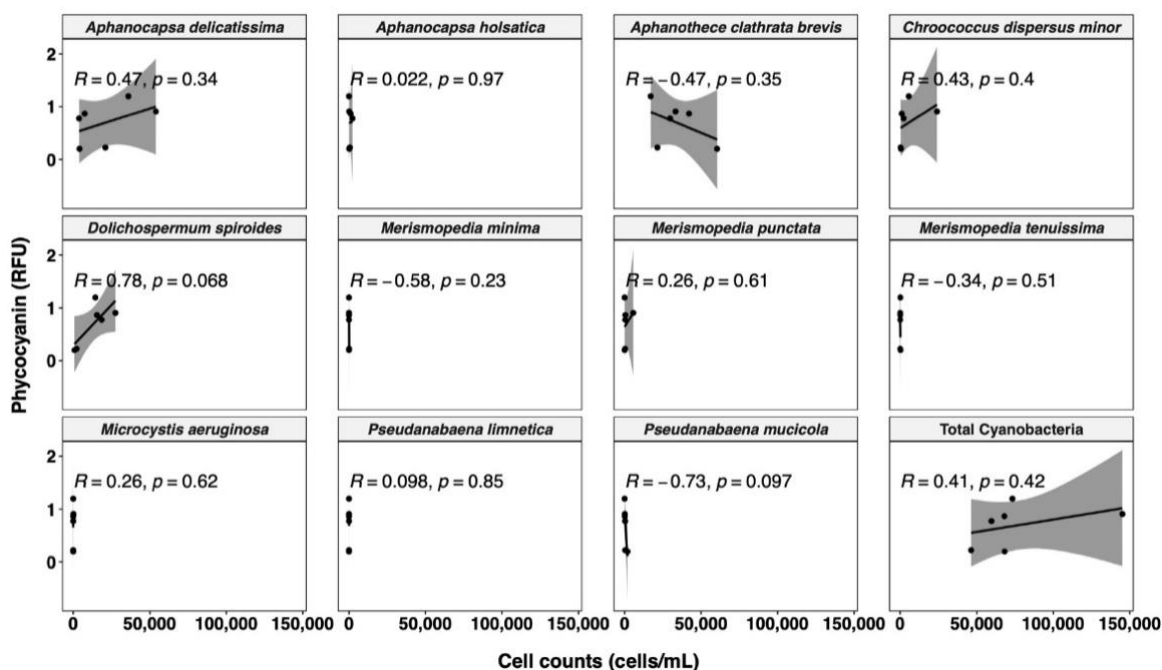


Figure 4.6 Relationship between phycocyanin probe readings and cell counts for different cyanobacterial species. Shaded area around the line represents the 95% confidence interval

In addition, the timing of the phycocyanin fluorescence probe measurements and the grab sample collection is an important factor to consider. Samples for taxonomic cell counts were collected at a fixed time using a short flushing of about 2 min followed by the collection of 1 L. At the Bedford DWTP, the probe recorded data for 30 min every 2 h with 5 min intervals, as multiple flows were measured. We performed the comparison of the grab samples with the probe data by averaging the set of six data points taken at the time closest to the collection of the grab sample. Both were taken from the same raw water sampling line. This means that the samples were not truly paired in terms of volume collected and exact time of collection. As cyanobacterial biomass may vary rapidly (Genzoli et al., 2016; Miller et al., 2013), these differences in sample volume and timing may be significant.

4.3.3.2 Comparison of field phycocyanin measurements and other water quality parameters

The potential impact of several other water quality attributes measured by the YSI EXO2 probe on the phycocyanin readings was also considered. The concentration ranges and mean values of the intake water quality for Bedford for the period from 24 July to 26 August 2018 are summarized in

Table 4.1. Phycocyanin, chlorophyll-a, and other physicochemical parameters of the intake water correlations are illustrated in Figure 4.7. Since DOC results were obtained on certain days' grab samples, such results were not compared with the hourly phycocyanin probe readings. Phycocyanin was found to be positively correlated with chlorophyll-a ($R = 0.67$, $p \leq 0.01$), turbidity ($R = 0.60$, $p \leq 0.01$), temperature ($R = 0.35$, $p \leq 0.01$), pH ($R = 0.25$, $p \leq 0.01$), and DO ($R = 0.10$, $p \leq 0.01$); negatively correlated with total dissolved solids (TDS) ($R = 0.11$, $p \leq 0.01$); and no significant correlation was found with conductivity ($R = 0.00$, $p = 0.89$). In addition, we obtained daily total photosynthetically available radiation (PAR) (W/m^2) from NASA POWER open source, and we found a positive correlation between phycocyanin readings and PAR ($R = 0.51$, $p \leq 0.01$).

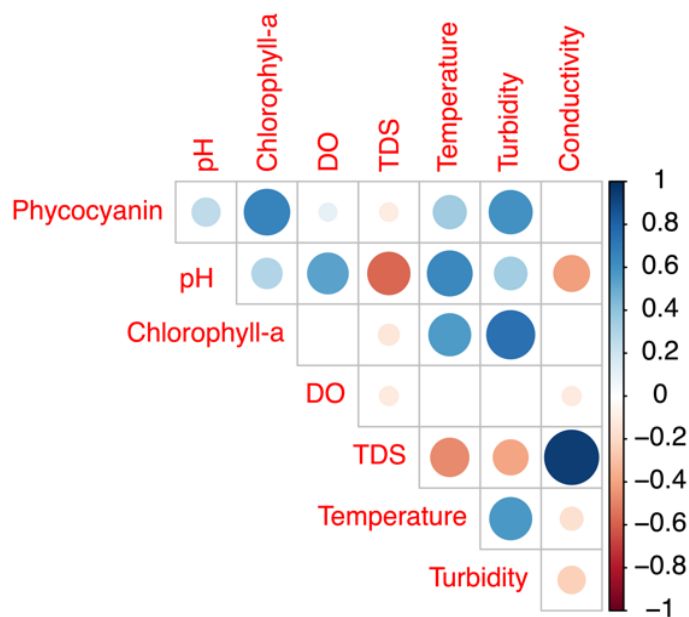


Figure 4.7 Correlation plot of physicochemical variables for the raw water of Bedford drinking water treatment plant (DWTP) during July and August of 2018

Table 4.1 Physico-chemical variables for the raw water of Bedford DWTP during July and August of 2018

Parameters	Units	Instruments	Data Value	Mean	Min	Max
Phycocyanin	RFU	YSI EXO2 probe	2-h average	1.1	0	38.2
Chlorophyll-a	RFU			1.3	0.2	4.5
DO	mg/L			5.8	0.5	14.5
pH				7.3	6.5	9.4
TDS	mg/L			91	84	106
Temperature	°C			25.7	22.9	28.2
Turbidity	NTU			7.3	1.6	63.6
Conductivity	µS/cm			141.8	132	165.5
DOC	mg/L	Total organic carbon analyzer	Data collect on certain days	6.9	5.3	7.9
PAR	W/m ²	NASA POWER open source	Daily	96.7	46.3	137.3

Cyanobacteria contain both phycocyanin and chlorophyll-a pigment (Chorus et al., 2021b). Eukaryotic phytoplankton, which contribute chlorophyll-a but not phycocyanin pigment, would have also been present at the time of our experiments. In addition, phytoplankton cells may lead to increased turbidity measurements. Thus, phycocyanin readings are expected to be positively correlated with chlorophyll-a and turbidity. Previous studies have reported inaccurate phycocyanin measurement due to the presence of eukaryotic phytoplankton, ranging from 200% underestimation to 405% overestimation for different species and fluorometers applied (Chang et al., 2012; Choo et al., 2018; McQuaid et al., 2011; Zamyadi et al., 2016a). In our study, the impact of eukaryotic phytoplankton is unlikely to be large, as the highest chlorophyll-a level is 4.5 RFU, and the mean value is 1.3 RFU, indicating only a small total eukaryotic phytoplankton biomass was present during our monitoring period. The reminder of that portion of the chlorophyll-a would have been from cyanobacteria.

Investigations also suggest that light intensity can affect phycocyanin fluorescence measurements (Donkor et al., 1996). The growth of cyanobacteria depends on the amount of light available and used. Theoretically, low light intensity may stimulate phycocyanin production, because cells accumulate more pigments to capture more light as energy; conversely, when the light intensity is high, some species have the potential to degrade phycocyanin in order to prevent damage to the cells (Hodges, 2016; Mulders et al., 2014; Zamyadi et al., 2016a). The outcomes are variable

depending on the light source, exposure time, measuring instrument, and reference cyanobacterial species used. In our study, we found positive relationships between *in situ* phycocyanin readings and natural light intensity (Figure 4.8). Such an outcome is also observed in other studies. Ma et al. (2015) revealed that higher artificial light intensity ($90 \mu\text{mol m}^{-2}\text{s}^{-1}$) resulted in an increase in phycocyanin content in *Nostoc sphaeroides*. Brient et al. (2008) used a TriOS probe, whereby dissolved phycocyanin solution and *Planktothrix agardhii* culture were exposed to various intensities of artificial light ($0\text{--}150 \mu\text{mol m}^{-2}\text{s}^{-1}$), and the authors showed that fluorescence increased with increasing light intensity. Hodges (2016) investigated the phycocyanin fluorescence of *Aphanizomenon* sp. under natural light and dark treatments, and the authors reported mixed trends. The fluorescence measured from the Manta and one of two Turner probes with light treatment were significantly higher compared to the dark treatments; whereas the phycocyanin readings were not modified using the other Turner probe and the YSI probe (Hodges, 2016). Rouso et al. (2021) and Misumi et al. (2016) observed a reduction in phycocyanin fluorescence for cyanobacteria cultures exposed to artificial light, attributing the suppression to non-photochemical quenching. To the best of our knowledge, this is the first study to report on the variation in phycocyanin fluorescence readings in environmental water under natural light intensities. Previous research on light interference have predominantly studied species-specific phycocyanin fluorescence responses from monocultures, which may not cover a broad range to clearly identify the variation in phycocyanin content reported throughout the range of species and morphologies. In our study, the daily total PAR ranged from 46.3 to 137.3 W/m^2 , which are equivalent to around 98 to $287 \mu\text{mol m}^{-2}\text{s}^{-1}$, assuming 45% of total solar irradiation is photosynthetically active (Thimijan et al., 1983). When the light intensity is high, the cyanobacteria photosynthetic rate is rapid, and, consequently, the carbon dioxide will decrease and DO will increase (Aparicio Medrano et al., 2016; Dervaux et al., 2015), leading to the increase in cyanobacterial biomass (Qi et al., 2018a). Such a contribution may explain the positive correlation between phycocyanin, and pH and DO.

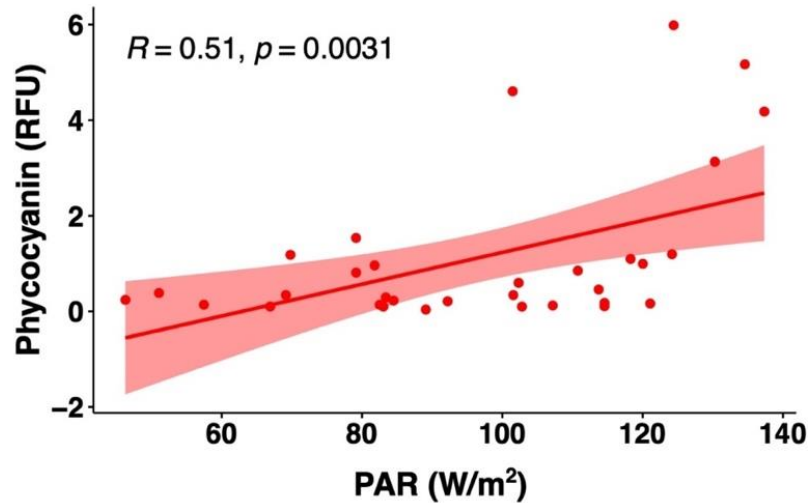


Figure 4.8 Correlations between photosynthetically available radiation (PAR) and phycocyanin probe readings for the raw water of Bedford DWTP during July and August of 2018. Shaded area around the line represents the 95% confidence interval

Phycocyanin fluorescence probes are equipped to concurrently measure other quality parameters, including water temperature, DO, pH, turbidity, and specific conductivity. Such synchronous measurements may be used to improve phycocyanin estimation accuracy. Few studies have assessed the bias in phycocyanin readings by synchronously measured water quality data. Bowling et al. (2013) deployed a YSI 6600 V2 probe in a river in Australia during November 2008 to May 2009, and the authors found similar positive trends between phycocyanin and chlorophyll-a ($R^2 = 0.077, p < 0.05$), turbidity ($R^2 = 0.07, p < 0.05$), and pH ($R^2 = 0.04, p < 0.05$). Although Bowling et al. (2013) found a weak positive correlation between phycocyanin readings and conductivity ($R^2 = 0.05, p < 0.05$), this study did not. Besides the above-mentioned study, there are limited studies on the relationship between water quality attributes and phycocyanin probe readings. Most studies have focused on the effect of environmental factors on the formation of cyanobacteria. For example, Rome et al. (2021) and Guo et al. (2016) found that bloom occurrence is associated with higher water temperature (above 25 °C), elevated pH (7 to 9), and super-saturated DO. Zhao et al. (2014) indicated that nitrate concentration and air temperature are positively correlated with bloom occurrence, whereas wind speed, relative humidity, and conductivity are negatively correlated with bloom occurrence. Ndong et al. (2014) applied a YSI 6600 V2-4 probe in the same water body as in our study (Missisquoi Bay), and the authors showed the maximum probability of 68% that the transport of cells to the plant intake were associated with the water temperature and wind that drive

lake hydrodynamics and water column mixing. Although these researchers have shown the factors related to the physical presence of cells at a given location, the current study also demonstrates that the factors influencing the phycocyanin probe readings must be considered simultaneously. Interestingly, it is reported that water temperatures act as a major controlling factor in cyanobacterial growth (Gao et al., 2018; Huisman et al., 2018; Newcombe et al., 2010; Rome et al., 2021; Zhao et al., 2014). Our field study also showed a positive correlation ($R = 0.35$, $p \leq 0.01$) between water temperature and phycocyanin readings. However, our laboratory tests with a fixed number of cyanobacterial cells revealed that phycocyanin readings decrease with elevated temperature. Thus, the improvement in the accuracy of phycocyanin readings using water temperature may partially stem from the inherent interactions between the temperature and cyanobacterial growth.

In order to develop a comprehensive model to estimate the cyanobacteria, future work might employ other available commercial probes and different cyanobacterial species to better understand the impacts of varied attributes. When designing or applying management criteria based on phycocyanin, it is critical to acquire preliminary data on the general community composition, dominant cyanobacterial species, and other possible environmental conditions that may impact readings. It may be feasible to conduct a series of experiments and incorporate the results into algorithms to reduce the errors. Fluorometers cannot effectively provide reliable estimation of cyanobacteria without proper compensation of the effect discussed above, especially in environments where physical-chemical attributes vary, and with more complicated species.

4.4 Conclusions

- SRNOM can quench and interfere with the reliability of the cell-bound phycocyanin readings. High SRNOM content in the water matrix can lead to an underestimation of cyanobacterial quantification measured by a phycocyanin probe, especially for high cyanobacterial cell concentrations.
- The concentration of NOM added, the aromaticity of NOM, the species present, and the background DOC in the test water define the extent and the trends for fluorescence quenching.

- In the case of a source with high naturally occurring NOM, DWTP managers need to assess the interference caused by NOM, and the phycocyanin RFU-based thresholds can be adjusted accordingly.
- Phycocyanin probe readings can be significantly affected by temperature in the range of their typical application (6–33 °C). Applying the correction algorithms can yield high success rates to decrease the interference. The relative importance of such interference varies between commercially available probes.
- The correlation between probe readings and taxonomic cell counts is unreliable, and only biovolume-based estimations are a relevant option for management purposes.
- A simultaneous measurement of the other physical-chemical parameters may help increase the accuracy of phycocyanin readings.

**CHAPTER 5 ARTICLE 2 - MONITORING OF CYANOBACTERIA
BREAKTHROUGH AND ACCUMULATION BY *IN SITU*
PHYCOCYANIN PROBE SYSTEM WITHIN FULL-SCALE
TREATMENT PLANTS**

This chapter was submitted as a research paper in the Journal of *Environmental Monitoring and Assessment* on April 13th 2023, and published on August 17th 2023. Supplementary information is presented in Appendix B.

**MONITORING OF CYANOBACTERIA BREAKTHROUGH AND ACCUMULATION BY
IN SITU PHYCOCYANIN PROBE SYSTEM WITHIN FULL-SCALE TREATMENT
PLANTS**

Liya Ma ^{1,*}, Juan Francisco Guerra Maldonado ¹, Arash Zamyadi ², Sarah Dorner ¹, and Michèle Prévost ¹

1: Department of Civil, Geological, and Mining Engineering, Polytechnique Montreal, Montreal, Quebec, H3C 3A7, Canada

2: Department of Civil Engineering, Monash University, Clayton Campus, Melbourne, Australia

* Correspondence: liya.ma@polymtl.ca

Abstract

Worldwide, there has been an increase in the presence of potentially toxic cyanobacterial blooms in drinking water sources and within drinking water treatment plants (DWTPs). The objective of this study is to validate the use of *in situ* probes for the detection and management of cyanobacterial breakthrough in high and low-risk DWTPs. *In situ* phycocyanin YSI EXO2 probes were devised for remote control and data logging to monitor the cyanobacteria in raw, clarified, filtered, and treated water in three full-scale DWTPs. An additional probe was installed inside the sludge holding tank to measure the water quality of the surface of the sludge storage tank in a high-risk DWTP. Simultaneous grab samplings were carried out for taxonomic cell counts and toxin analysis. A total of 23, 9 and 4 field visits were conducted at the three DWTPs. Phycocyanin readings showed a 93-fold fluctuation within 24 hours in the raw water of the high cyanobacterial risk plant, with higher phycocyanin levels during the afternoon period. These data provide new information on the limitations of weekly or daily grab sampling. Also, different moving averages for the phycocyanin probe readings can be used to improve the interpretation of phycocyanin signal trends. The *in situ* probe successfully detected high cyanobacterial biovolumes entering the clarification process in the high-risk plant. Grab sampling results revealed high cyanobacterial biovolumes in the sludge for both high and low-risk plants.

Keywords

Cyanobacteria; Phycocyanin; Fluorescence probe; Water treatment plant

Key Contribution

The originality of this study lies in (1) the application of a single phycocyanin probe to monitor the cyanobacteria at four sites throughout the DWTP; and (2) the systematic evaluation of the cyanobacterial cells and their associated toxins at different sites in both high and low-risk DWTPs.

5.1 Introduction

In recent years, an increase in the occurrence of potentially toxic cyanobacterial blooms has been reported in water supply systems worldwide (Akyol et al., 2021; Kim et al., 2021; Zamyadi et al., 2019). Climate change and human activities may intensify the frequency and duration of harmful cyanobacterial blooms (Griffith et al., 2020; Nwankwegu et al., 2019). Concerns related to the impacts of cyanobacteria in water supply systems are related to human health risks (e.g., skin diseases, gastroenteritis, Alzheimer's disease, liver damage) and esthetic water quality (e.g., taste and odor) (Chernoff et al., 2017; Chorus et al., 2021b; Kim et al., 2021). Due to such effects of toxic cyanobacterial blooms, several monitoring frameworks were developed over the last two decades to aid the management of cyanobacterial blooms in drinking water supplies (Chorus et al., 2021b; Ellis, 2009; Ministry of Health, 2020; NHMRC, 2022).

The action plans based on the different alert levels consist of weekly grab sampling once the waterbody or raw water has been tested for positive toxins or potentially toxigenic cyanobacteria (Chorus et al., 2021b; Ministry of Health, 2020). The grab sampling frequency is not standardized. To the authors' knowledge, the highest sampling frequency suggested by management strategies is twice per week (Newcombe et al., 2010). Such recommended monitoring frequencies may not be appropriate to estimate the cyanobacterial concentration in the source water and inside the drinking water treatment plants (DWTPs), since the bloom conditions may change rapidly, and the sampling schedules did not always correspond to the peak bloom period (Genzoli et al., 2016; Zamyadi et al., 2014). Furthermore, the processing time needed by laboratory taxonomic cell counts and toxin analyses could contribute to a longer response time. These limitations are critical for the cyanobacteria management for water utilities, given the need for prompt intervention and management to prevent the presence and accumulation of cyanobacteria inside the DWTPs. Thus, intensive *in situ* fluorescence measurement tools for the rapid detection of cyanobacteria-specific pigment, i.e. phycocyanin, have been increasingly used for cyanobacterial quantification. Besides the high detection frequency, the advantages of the *in situ* phycocyanin probes are inexpensive and operational simplicity (Bertone et al., 2018; Zamyadi et al., 2016b).

Extensive studies have demonstrated the application of the *in situ* phycocyanin fluorescence probe to prevent the entry of cyanobacterial cells within the DWTPs by source water or raw water monitoring (Bowling et al., 2016; Cotterill et al., 2019; Gregor et al., 2007; Izydorczyk et al., 2009; McQuaid et al., 2011; Thomson-Laing et al., 2020). However, there are limited applications of using the probe within the full-scale DWTPs. The occurrence of blooms may affect the cyanobacterial removal efficiency, leading to cyanobacteria and cyanotoxins breakthrough into various processes of DWTPs. Reported instances of cyanobacteria accumulation were observed in filter media, surface of clarifier, surface of filter, sludge thickener and supernatant of sludge thickener (Jalili et al., 2021; Pestana et al., 2016; Zamyadi et al., 2013a; Zamyadi et al., 2019). Almuhtaram et al. (2018) measured the phycocyanin fluorescence of the raw water, surface of clarifier and filter, and treated water at four Great Lakes region DWTPs. But the measurements were only recorded at each sampling visit. Such discrete probe monitoring frequencies compromise the online assessment, and were insufficient to identify the intermittent cyanobacterial breakthrough within the DWTPs. Zamyadi et al. (2013b) and Zamyadi et al. (2014) used online probe data throughout the treatment process, but only 1 data were recorded every 4 hours. Such frequency may not capture the actual cyanobacterial dynamic, due to the hypothesized highly temporal fluctuation of the cyanobacteria biomass in the water source. In addition, the phycocyanin level for the surface of the sludge holding tank was not measured by the *in situ* probe from these studies (Almuhtaram et al., 2018; Zamyadi et al., 2014; Zamyadi et al., 2013b). This study further explored the applicability of phycocyanin probes when applied across the treatment train in both high and low cyanobacterial risk DWTPs and provides a quantitative evaluation of cyanobacterial cell accumulation and breakthrough, allowing the operators to use the probe to evaluate the need for treatment adjustments.

The main objective of this work is to apply time series analysis on continuous phycocyanin probe data to investigate cyanobacteria dynamics inside the high and low-risk full-scale DWTPs, including bloom timing and magnitude at various locations within the DWTPs. The specific objectives are to: (1) investigate the use of *in situ* probe readings to track cyanobacterial fluctuations

in DWTPs on daily and hourly basis. (2) evaluate *in situ* probes for mapping and identifying the critical points for breakthrough and accumulation of cells and their associated toxins using a combination of probe readings and taxonomic cell counts.

5.2 Materials and methods

5.2.1 Water source and site description

Sampling campaigns were undertaken at three full-scale DWTPs in Canada (Figure B.1) during the summer and fall of 2018. The three DWTPs can provide the opportunity to study both low and high-risk incoming cyanobacterial cells into their intake water. The treatment processes in the three plants are listed in Table B.1.

DWTP A is located on Missisquoi Bay of Lake Champlain. Due to the high concentration of phosphorus and nitrogen in the bay, *Microcystis* cyanobacterial blooms occur during the summer and fall in recent decades (Jalili et al., 2021; McQuaid et al., 2011). The plant employs conventional treatment trains as presented in Figure 5.1. The processes are: powdered activated carbon (PAC) injection followed by coagulation, flocculation, and sedimentation, then chlorination. The sludge supernatant is discharged into the lake, while the sludge is transferred to the wastewater treatment plant. The source water is directed into the raw water tank prior to treatment.

DWTP B draws water from Lemieux reservoir which is fed by the North Yamaska River. The Yamaska River is classified as eutrophic (Remmal et al., 2017). Lime is added to the reservoir prior to treatment to control the pH. Solar mixers are installed in the reservoir to prevent the creation and proliferation of cyanobacteria. An overview of the treatment processes of DWTP B and the location of sampling points is illustrated in Figure B.2.

DWTP C is in Southwest Ontario. It supplies potable water for Leamington, Lakeshore, Kingsville and Essex. The plant draws water from Lake Erie, and the water is pre-chlorinated before entering the plant. During the summer bloom season, the cyanobacteria start forming in the shallow warm

waters of Lake Erie close to Ohio and then are transported throughout Western Lake Erie. Cyanobacterial blooms have been observed annually in recent years in Lake Erie (Jankowiak et al., 2019). An overview of the treatment processes of DWTP C and the location of sampling points is illustrated in Figure B.3.

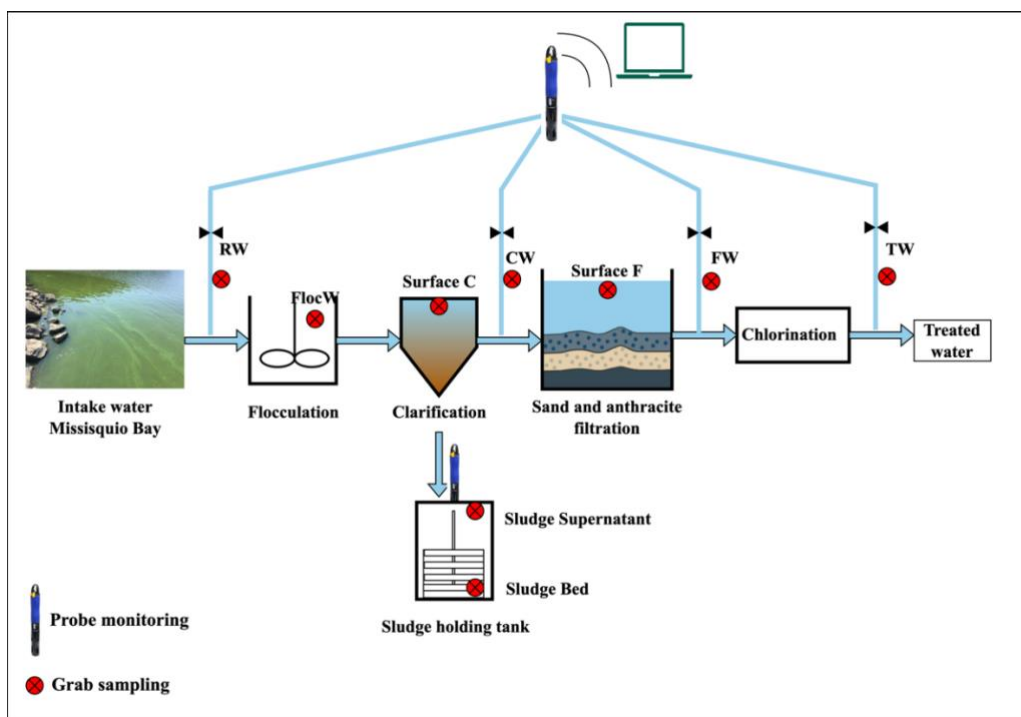


Figure 5.1 Schematic of the treatment processes of DWTP A, grab sampling and probe monitoring locations. The water intake is from Missisquoi Bay. Sampling points are indicated by: raw water (RW), flocculated water (FlocW), clarified water (CW), filtered water (FW), treated water (TW), surface of clarifier (Surface C), surface of filter (Surface F), supernatant of sludge storage tank (Sludge Supernatant) and sludge bed of sludge holding tank (Sludge Bed)

5.2.2 *In situ* phycocyanin monitoring

YSI EXO2 water quality multi-probe (YSI, Yellow Springs, OH, USA) fitted with *in situ* phycocyanin fluorescence sensor was used in this study. Phycocyanin optics has the excitation and emission wavelength of 590 ± 15 nm and 685 ± 20 nm, respectively (YSI Incorporated, 2019). In each of the three studied DWTPs, a YSI EXO2 probe with its remote control and data logging was installed to measure the phycocyanin RFU of raw water (RW), clarified water (CW), filtered water (FW), and treated water (TW). Each probe was equipped with a self-cleaning wiper. In-

plant samples were almost not exposed to light, and the flow chamber of the probe has a black bottom to prevent light influence. Figure B.4 demonstrates the probe monitoring system. The switches are used for controlling the electronic solenoids, so that RW, CW, FW and TW can continuously go through the probe flow cell, separately. Four types of water went through the probe flow cell sequentially (30 min cycle) and probe readings were recorded at 5-minute intervals. Therefore, for each water type, six measurements were recorded every 2 hours. The flow rates through the probe flow cell were adjusted to ensure the absence of air bubbles in the probe cell and to fully flush the probe cell when switching between water types. Probe data were continuously recorded, transmitted via a modem, and downloaded remotely. Negative phycocyanin readings which represent readings out of the probe's detection limit were replaced with zero.

For DWTP A, an additional YSI EXO2 phycocyanin probe was installed inside the sludge holding tank to measure the water quality of the supernatant of sludge storage tank (Sludge Supernatant), as shown in Figure 5.1.

5.2.3 Sampling procedure and analysis of water quality parameters

From July to October of 2018, a total of 23, 9, and 4 field visits were conducted at A, B, and C DWTPs, respectively. These visits were conducted to calibrate the probe and collect grab samples. Grab samples were taken in parallel with probe measurements from the RW, CW, FW and TW from all three DWTPs. They were collected from sampling taps available inside the DWTPs and were fully flushed before collection.

In addition, targeted grab samples were also collected at several sites throughout the DWTPs to evaluate the distribution of cyanobacterial levels and the treatment performance. The additional grab sample sites were different within each DWTP and are listed as follows.

For DWTP A: flocculated water (FlocW), apparent accumulation at the surface of clarified water (Surface C), apparent accumulation at the surface of filtered water (Surface F), supernatant of sludge storage tank (Sludge Supernatant) and the underlying sludge bed of sludge holding tank

(Sludge Bed). For DWTP B: underlying sludge bed of sludge holding tank (Sludge Bed). For DWTP C: apparent accumulation at the surface of clarified water (Surface C), apparent accumulation at the surface of filtered water (Surface F), and underlying sludge bed of sludge holding tank (Sludge Bed).

Taxonomic cell counts (in singlicate) and toxin analysis (in duplicate) were undertaken on all samples. The taxonomic cell count samples were preserved with Lugol's iodine in the dark at room temperature (21 °C). The analyses of taxonomic counts, species identification and biovolume calculation were achieved at Université du Québec à Montréal's (UQAM) Biological Sciences Department. The method is according to Lund (1959); Planas et al. (2000). The reproducibility of taxonomic cell counts has been discussed by Zamyadi et al. (2012a) and was conducted with the same protocol and technician as in our study.

With regards to the microcystin (MC) analyses, an on-line solid-phase extraction ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (On-line SPE-UHPLC-MS/MS, Thermo TSQ Quantiva) was used to determine the total MCs concentrations. More details on the cyanotoxin analysis methods are explained in Munoz et al. (2017) and Roy-Lachapelle et al. (2019).

5.2.4 Data analysis

Statistical analysis was done using R (version 4.2.1). Moving average statistics were calculated using the `rollmean` function in the `zoo` package (version 1.8.10). ANOVA was performed with the factor of the hour of the day, and Tukey's HSD test was used as the post hoc multiple comparison, with the significance level $\alpha = 0.1$.

5.3 Results

5.3.1 Time series analysis of the intake water of a high-risk DWTP

Individual phycocyanin fluorescence readings varied greatly within a 24-h period during the 32 days of monitoring in July and August 2018 (Figure 5.2). Quick and substantial fluctuations can

occur as shown on August 11, when phycocyanin readings increased 93-fold, from 0.6 RFU at 12:30 to 55.8 RFU at 16:30 based on raw probe measurements (exact time), exceeding the mean RFU reading of 1.1 RFU for the whole period. Specifically, phycocyanin values over 1.8 RFU equivalent (calculated from the site-specific equivalency derived from McQuaid et al. (2011) to the Alert Level 1 of WHO, 2021), were measured in 13.5% of the readings on 11/32 days of monitoring. Readings exceeding 5.0 RFU (calculated from the site-specific equivalency derived from McQuaid et al. (2011) to the Alert Level 2 of WHO, 2021), were less common and of shorter duration. Values exceeding 5.0 RFU were measured in 5.0% of the readings on 7/32 days of monitoring. Notably, the longer-lasting high values all occurred during the afternoon period.

As probes produce high frequency readings, the interpretation of measurements could be facilitated by using daily moving average, weekly moving average, 2, 6, 12 and 18-h moving averages as shown in Figure 5.2. During the monitoring period, the phycocyanin readings varied within the range of 0–55.8 RFU using raw measurements. The highest readings decreased by using moving averages. For instance, the highest phycocyanin reading was 38.1 RFU using 2-h moving average, and the highest phycocyanin reading was 5.6 RFU using daily moving average.

To investigate whether high phycocyanin readings are observed during some periods of the day, phycocyanin readings were sorted per hour over a 7-day period in July and a 25-day period in August (Figure 5.3). Higher mean phycocyanin values were observed during the afternoon period (after 16 h), for both July and August. ANOVA tests show statistically significant differences in phycocyanin readings during the different hours of the day during July, and Tukey's HSD post-hoc tests reveal significant differences in the comparisons of 18 h and the other measured time (Figure B.5).

Additionally, the correlations between total cyanobacterial biovolumes and the probe readings were investigated (Figure B.6). As multiple flows were measured by the probe, the phycocyanin readings for the raw water were recorded 30 min every 2 h with 5 min intervals, so different time-series statistics of the phycocyanin readings were considered. The readings most closely

associated with the 2-h moving average ($R = 0.73$, $p = 0.098$) to account for the differences in the timing of grab sampling and probe readings.

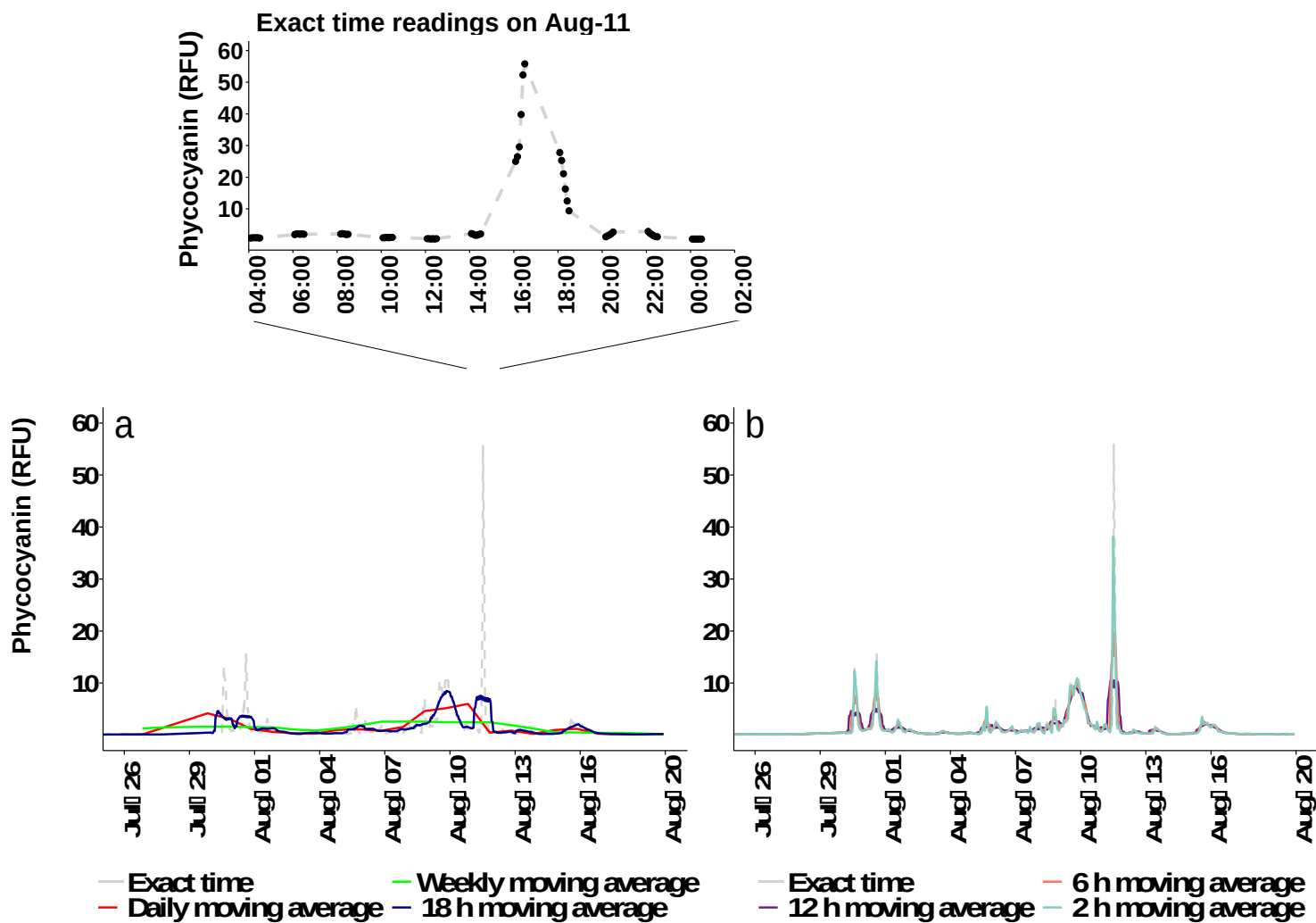


Figure 5.2 Temporal monitoring of different time-series analysis of *in situ* YSI EXO2 phycocyanin probe readings (RFU) at the raw water intake of the DWTP A during July and August of 2018. (a) exact time, daily, weekly, and 18-h moving averages; (b) exact time, 2, 6, 12-h moving averages

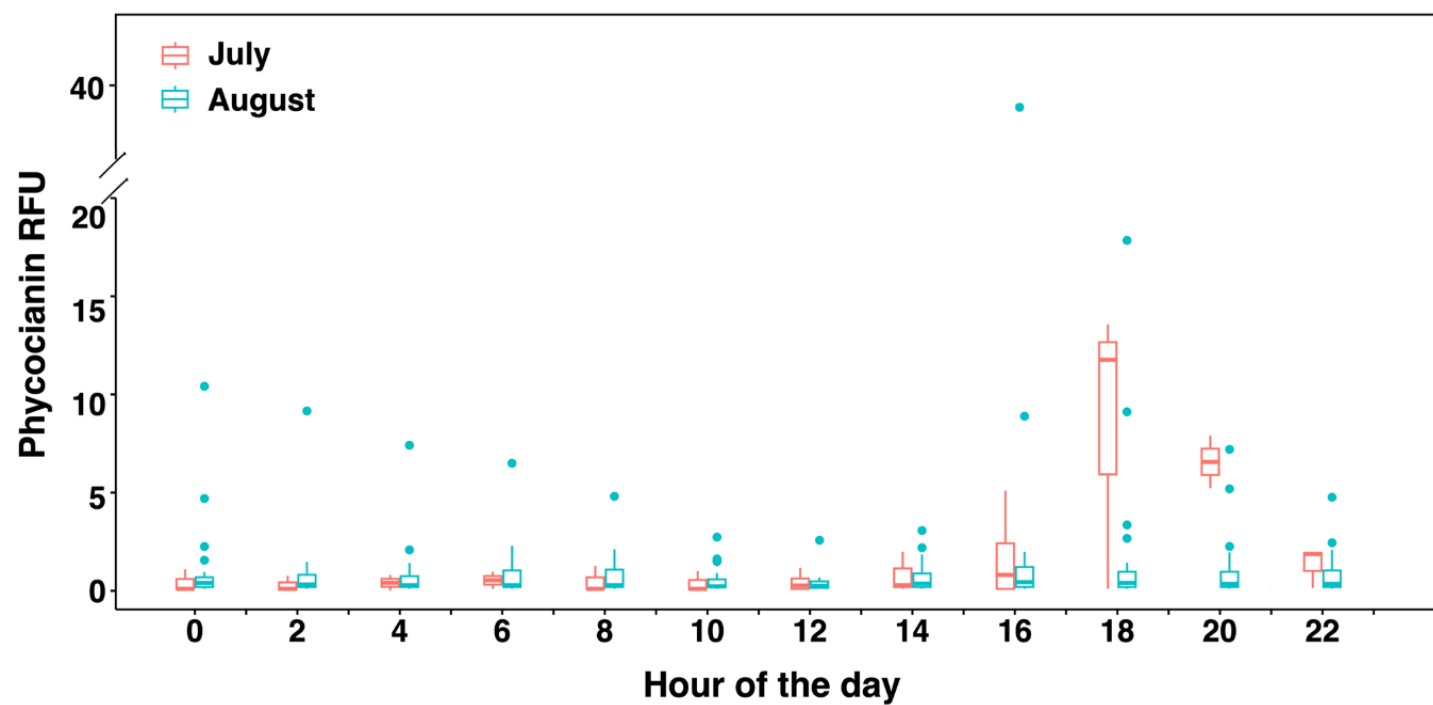


Figure 5.3 Phycocyanin (RFU) by hour of the day at DWTP A raw water intake during July (n=7 per hour) and August (n=25 per hour) of 2018. The bottom and top of each box represent the 25–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value

5.3.2 Applying the probe readings to estimate the risk of cyanobacteria within a high-risk DWTP

5.3.2.1 Cyanobacterial cells and cyanotoxins in the RW, CW, FW, and TW in a high-risk DWTP

The 2-h moving average of phycocyanin in the RW, CW, FW and TW at DWTP A during July and August 2018 is shown in Figure 5.4a. The plant experienced high incoming cyanobacterial densities during late July and early August. On July 30 and 31 and August 5, 8, 9, 10 and 11, the probe observed remarkable increases in phycocyanin in the RW, and their phycocyanin values surpassed the alert level 2 of 5.0 RFU. Moreover, a distinct increase in phycocyanin up to 10.1 RFU on July 30 was recorded in CW (Figure 5.4a).

While probe readings were proceeding online, grab samples were taken for taxonomic cell counts and toxin analysis (Figure 5.5). The total cyanobacterial biovolume in RW on July 31 was 2.7 mm³/L, August 1 was 2.9 mm³/L, August 2 was 5.6 mm³/L and August 7 was 4.3 mm³/L (Figure 5.5b), close to or surpassed WHO alert level 2 for drinking water sources of 4.0 mm³/L (Chorus et al., 2021b). In addition, on 5 days during September and October, total cyanobacterial biovolumes in RW were higher than alert level 2. However, valid probe data was lacking in parallel with the grab sampling results during September and October.

Cell counts of flocculated water taken from the flash mix were generally lower than those measured in the corresponding RW, possibly because of the impact of aggregation on the ability to enumerate or because of the impact of the coagulants used as shown recently by Le et al. (2021). During the monitoring period, the probe readings for FW and TW were lower than 0.1 RFU (Figure 5.4a). In accordance, very low cyanobacterial biovolumes (lower than 0.01 mm³/L) were detected in FW and TW (Figure 5.5b).

With regards to the total MCs in the water samples, between July 25 and August 25, total MCs in the RW remained modest, ranging from 88.4 to 286.4 ng/L. During September and October, total MCs in the RW were higher, and reached up to 2456.1 ng/L on October 16. Total MCs in CW and FW were all below 900 ng/L, and in TW were below 300 ng/L during the whole sampling period (Figure 5.5c).

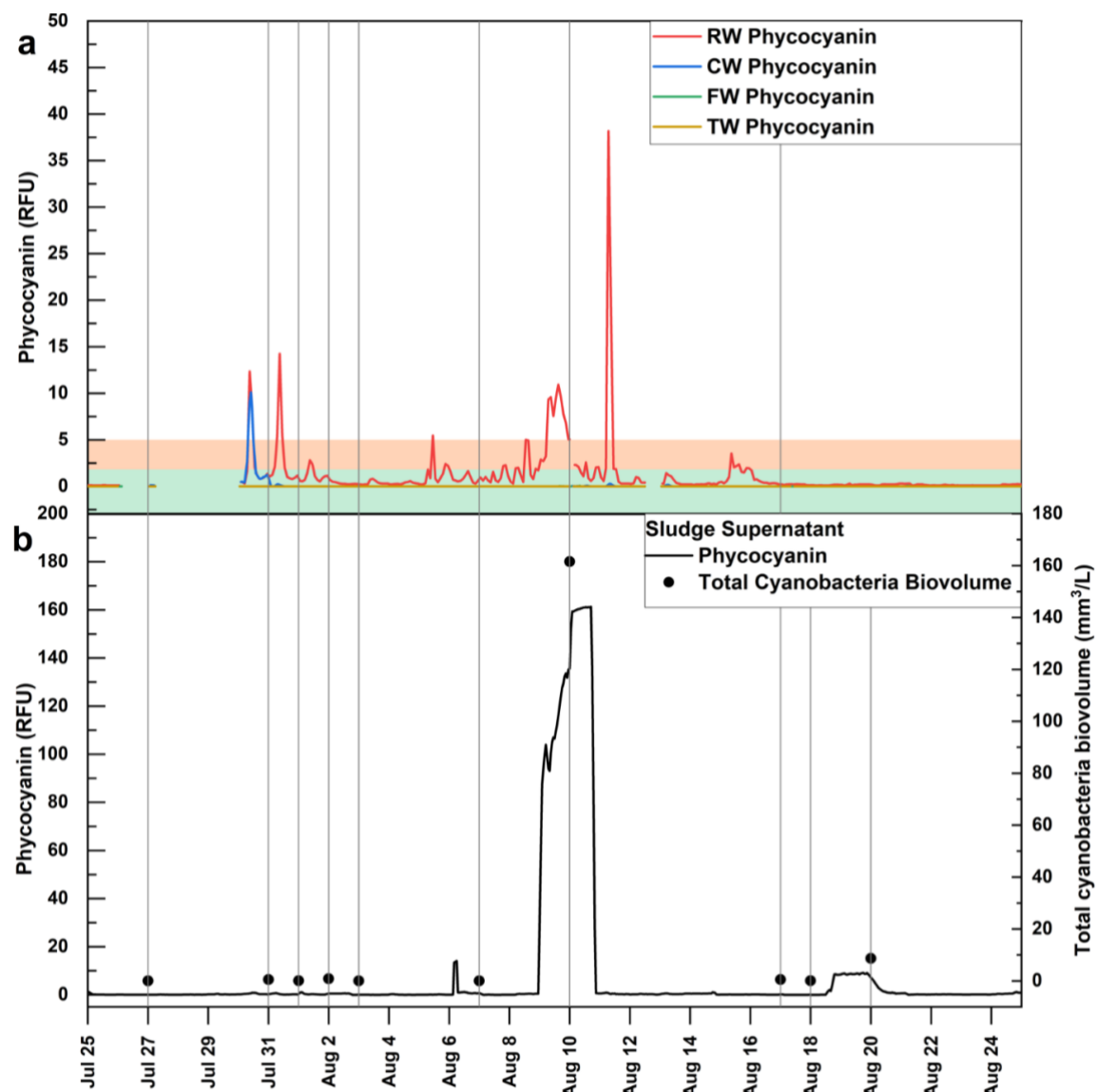


Figure 5.4 Temporal monitoring of 2-moving average *in situ* phycocyanin fluorescence (RFU) of the (a) raw water (RW), clarified water (CW), filtered water (FW), and treated water (TW), (b) supernatant of sludge storage tank (Sludge Supernatant) and its total cyanobacterial biovolumes (mm³/L) inside the DWTP A during the bloom season of 2018. Gray vertical lines indicate when grab samplings were collected. Background colors indicate the equivalent phycocyanin RFU alert levels (1.8 and 5.0 RFU) to WHO 2021 alert levels

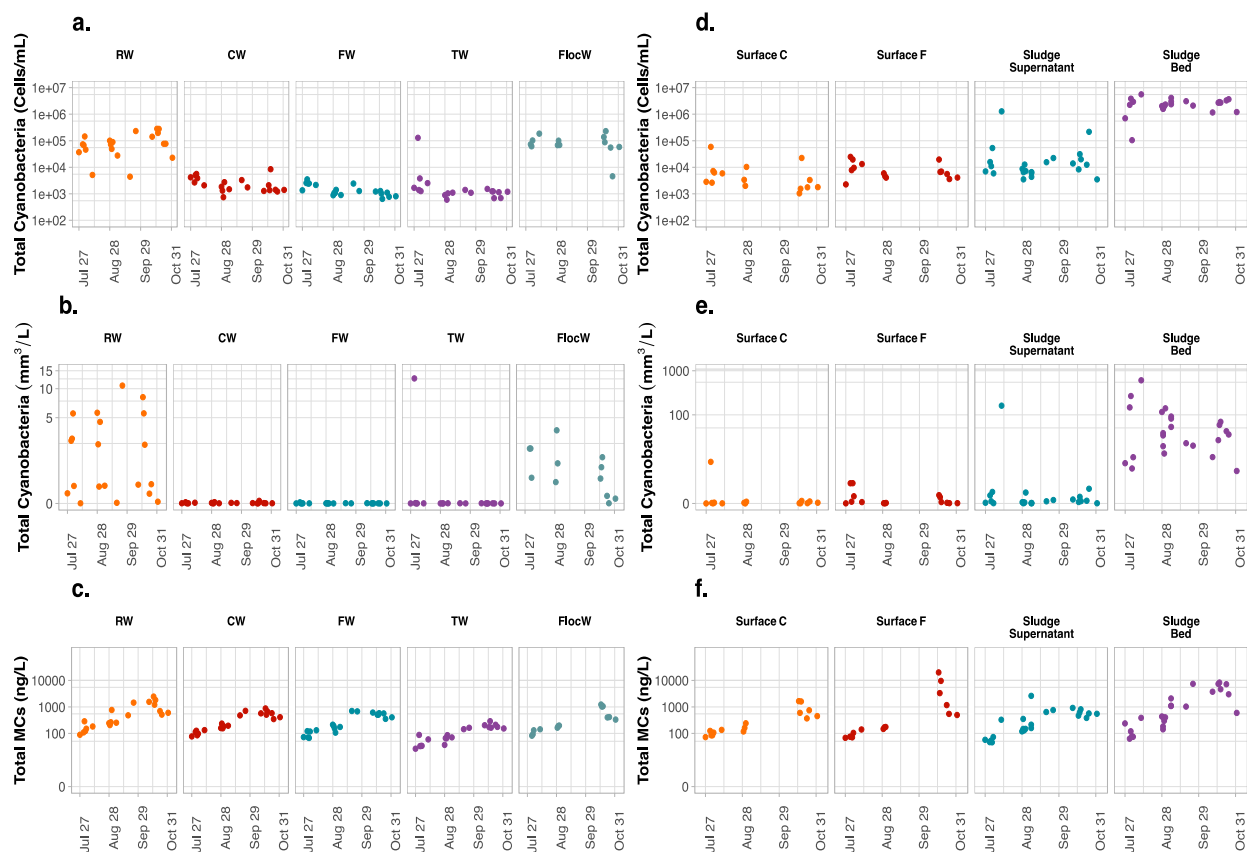


Figure 5.5 Total cyanobacterial cell counts, cell biovolumes and total MCs in water raw water (RW), clarified water (CW), filtered water (FW), treated water (TW), flocculated water (FlocW), apparent accumulation at the surface of clarified water (Surface C), apparent accumulation at the surface of filtered water (Surface F), supernatant of sludge storage tank (Sludge Supernatant), and sludge bed of sludge holding tank (Sludge Bed) of the DWTP A

5.3.2.2 Cyanobacterial cells and cyanotoxin in the surface of separation processes and in the sludge in a high-risk DWTP

The surface of separation processes and sludge in the holding tank were also sampled. Following the high incoming density of cyanobacteria arriving in the DWTP A on July 30 and the subsequent rise of cyanobacteria in CW, accumulation at the surface of these processes was noted with high cell biovolume with 7.7 mm³/L in Surface C and 1.9 mm³/L in Surface F on July 31 (Figure 5.5e). During the other sampling dates, total cyanobacteria were all lower than 0.1 and 0.5 mm³/L in Surface C and Surface F, respectively.

An additional probe was used to monitor the cyanobacterial concentration in Sludge Supernatant inside the high-risk DWTP A. Phycocyanin probe readings started to increase at the Sludge

Supernatant on August 9 and reached the highest level on August 11 at 2 h with 161.3 RFU, then decreased to 0.7 RFU on August 11 at 6 h (Figure 5.4b). Like the data for the other sites, all available data regarding grab sampling in the Sludge Supernatant and Sludge Bed were compiled. Trends of cyanobacterial biovolumes result in the Sludge Supernatant conformed well with that of probe readings (Figure 5.4b). When the phycocyanin level in the Sludge Supernatant reached high values on August 10, the total cyanobacterial biovolume was also found to be at the highest value with 161.5 mm³/L on August 10. Furthermore, the highest number of cyanobacterial biovolume was observed in the Sludge Bed with 608.7 mm³/L on August 10. Notably, they were 62,304 and 234,811 times greater than the RW. During the other days, total cyanobacterial biovolumes in the Sludge Supernatant were generally lower than in RW, whereas Sludge Bed samples always had higher values (1.8–1609 times) than that in RW.

The significant increase in phycocyanin readings in the Sludge Supernatant on August 10 also corresponds to the increase in cyanotoxin concentration. On August 10, total MCs in Sludge Supernatant and in the Sludge Bed reached 329.2 ng/L and 387.1 ng/L, a two-fold increase than measured in RW (183.1 ng/L). For other dates between July and August, total MCs in Sludge Supernatant and Sludge Bed were all lower than that in RW, with below 72 ng/L in Sludge Supernatant and below 120 ng/L in Sludge Bed (Figure 5.5f). During September and October, on the 5 days that cell biovolumes in RW were higher than 4 mm³/L, the MCs in the Sludge Bed were also higher than that in RW, with up to 8288.2 ng/L in the Sludge Bed.

5.3.3 Applying the probe readings to estimate the risk of cyanobacteria within two low-risk DWTPs

5.3.3.1 Cyanobacterial cells and cyanotoxins in the RW, CW, FW, and TW in low-risk DWTPs

In situ phycocyanin probes were also used to monitor the presence of cyanobacteria at DWTPs B and C. The phycocyanin values for either of the plants were lower than 0.4 RFU during the entire monitoring period (Figure 5.6). Indeed, the NOAA real-time satellite imagery did not detect any bloom at the Canadian site of Western Lake Erie (intake water for DWTP C) (NOAA, 2018). Likewise, grab sampling total cyanobacterial biovolumes with lower than 0.1 mm³/L for RW, CW, FW and TW of the DWTP B and C confirmed the low phycocyanin readings (Figure 5.7b and Figure 5.8b). Such low values precluded any meaningful correlation relation between phycocyanin

and biovolumes. DWTPs B and C provided the opportunity to monitor the low incoming level of cyanobacterial cells to the DWTPs.

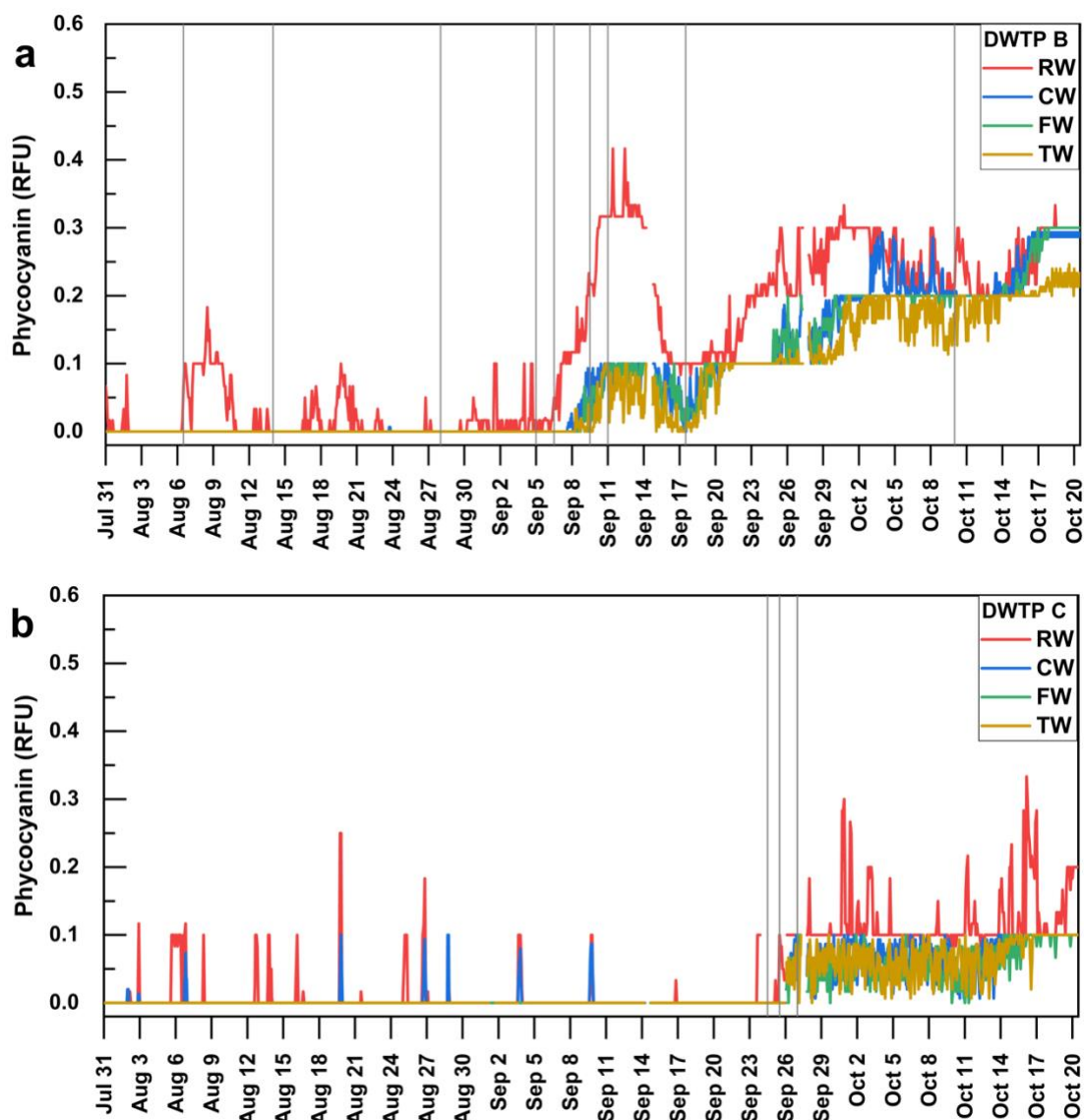


Figure 5.6 Temporal monitoring of 2-h moving average *in situ* phycocyanin fluorescence (RFU) of the raw water (RW), clarified water (CW), filtered water (FW), and treated water (TW) inside the (a) DWTP B, and (b) DWTP C during the bloom season of 2018. Gray vertical lines indicate when grab samplings were collected

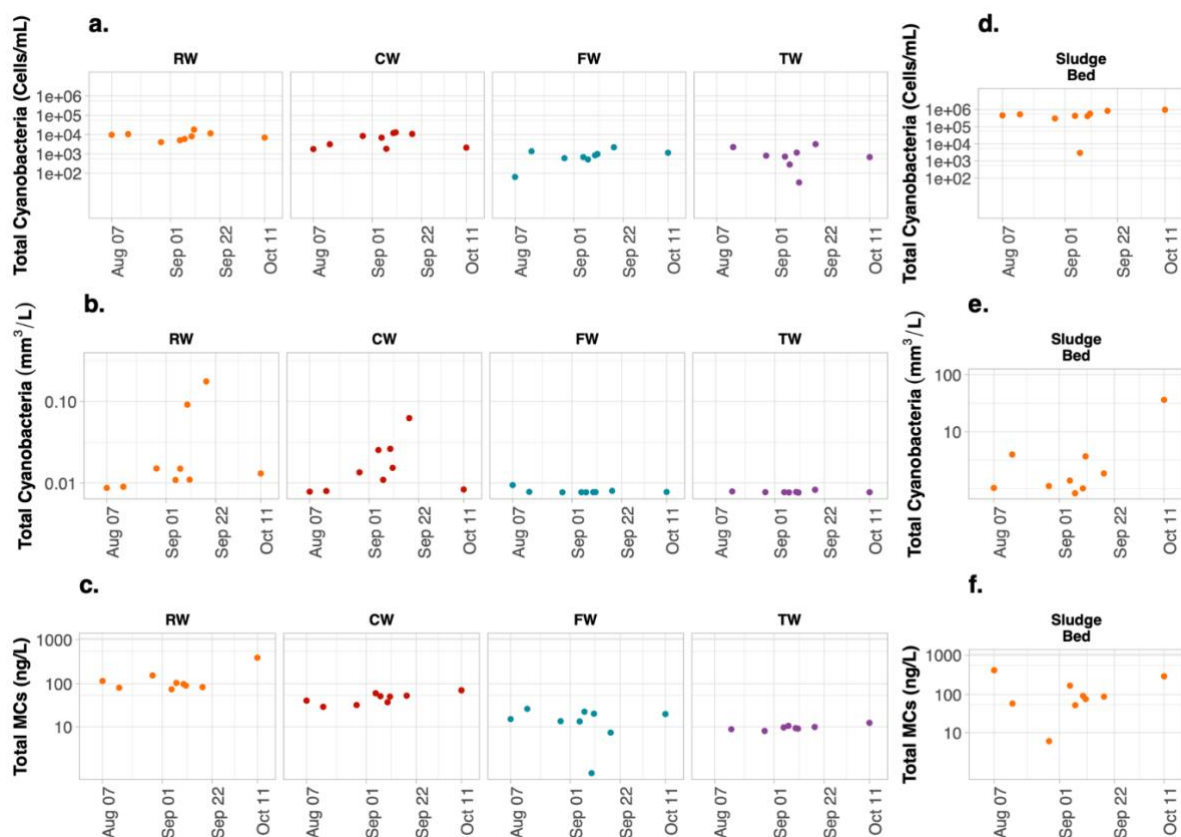


Figure 5.7 Total cyanobacterial cell counts, cell biovolumes and total MCs at raw water (RW), clarified water (CW), filtered water (FW) treated water (TW), and sludge bed of sludge holding tank (Sludge Bed) of the DWTP B

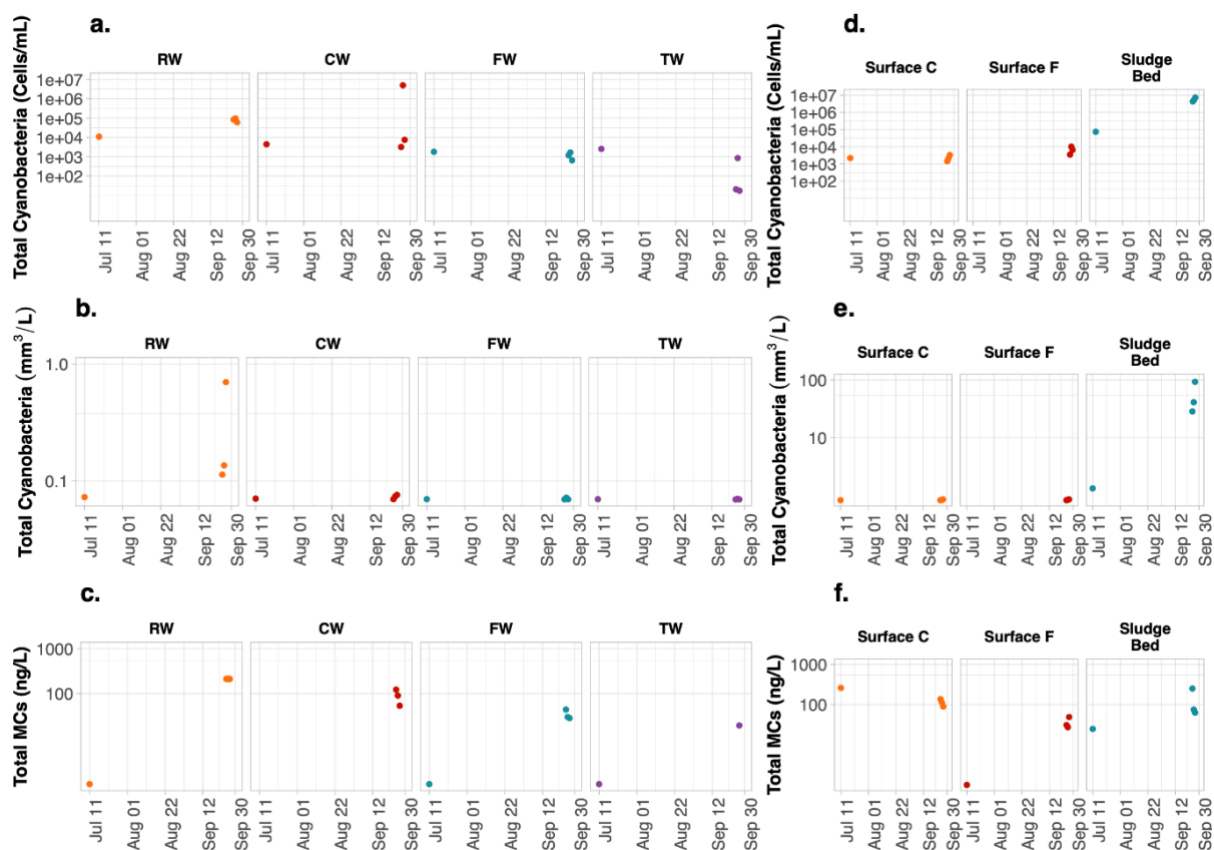


Figure 5.8 Total cyanobacterial cell counts, cell biovolumes and total MCs at raw water (RW), clarified water (CW), filtered water (FW) treated water (TW), surface of clarified water (Surface C), surface of filtered water (Surface F), and sludge bed of sludge holding tank (Sludge Bed) of the DWTP C

5.3.3.2 Cyanobacterial cells and cyanotoxin at the surface of the separation processes and in the sludge in low-risk DWTPs

With regards to Surface C and Surface F, we only have results for low-risk DWTP C, the cyanobacterial biovolumes for Surface C and Surface F were all lower than that in RW, and their values were lower than 0.1 mm³/L (Figure 5.8b, e).

Sludge in the holding tanks is the most problematic site for cell accumulation for these two low-risk plants. At DWTPs B and C, all the Sludge Bed contained total cyanobacterial biovolumes greater than that of the RW, while the other measured locations had lower values than that of RW (Figure 5.7 and Figure 5.8). Given the low cell flux into these two DWTPs, the observations of cyanobacteria in the Sludge Bed were surprising. The average total cell biovolume of sludge samples was 5.1 mm³/L at DWTP B (Figure 5.7e). Even more significant accumulations were found in the Sludge Bed of DWTP C, with an average of 41.1 mm³/L (Figure 5.8e). The largest discrepancy happened at DWTP C on September 27 when the probe showed 0.1 RFU and the total biovolume was 0.8 mm³/L in RW, whereas an elevated 92.8 mm³/L total biovolume was measured in the Sludge Bed. Higher total cyanobacterial biovolumes and counts were observed in the Sludge Bed samples from these two low-risk plants. The concentration factors reached 1828 times for DWTP B and 219 times for plant C.

Although cell biovolumes significantly increased in the Sludge Bed of the two low-risk DWTPs, total MCs were not high. On most of the days, the total MCs in the Sludge Bed were lower than that in RW (Figure 5.7c and f and Figure 5.8c and f). Our study did not find exceedance of drinking water standards of 1 µg/L MCs corresponding to alert level 1 (Chorus et al., 2021b).

5.4 Discussion

5.4.1 Daily and hourly variations affect the ability to assess risk

Large daily and hourly fluctuations in phycocyanin probe readings and cell counts were observed in the RW of the high-risk DWTP. Daily fluctuation of up to 55 phycocyanin RFU was noted (Figure 5.2). Higher phycocyanin readings occurred during the afternoon compared to other times of the day (Figure 5.3), and statistically significant differences were found between 18 h and the other times of the day during July (Figure B.5). Zamyadi et al. (2014) found probe-based cyanobacterial cell concentrations also varied widely within a day, with the highest RFU during

the afternoon. Hydrodynamic modeling of cyanobacterial blooms at Missisquoi Bay noted the cyclic nature of risk to the drinking water intake (Ndong et al., 2017). The observations are coherent with prior reports that cyanobacterial concentration can vary yearly (Bertani et al., 2017), seasonally (Gagné et al., 2018), daily (Genzoli et al., 2016) and hourly (Qin et al., 2018). Light availability, temperature, nutrient, wind, and shallow lake migration may explain these patterns (Aparicio Medrano et al., 2016; Ndong et al., 2014; Qi et al., 2018b; Qin et al., 2018; Rousso et al., 2021). Significant efforts have been deployed to model cyanobacterial dynamics in freshwater lakes (reviewed by (Rousso et al., 2020)), and those approaches may be applied to predict future scenarios and help better the management of cyanobacteria in the DWTPs. Without the input of a dynamic measurement of incoming cyanobacteria, one event of peak cyanobacterial loading was not identified leading to the breakthrough of cyanobacterial cells and toxins as the operators failed to timely implement treatment responses.

These wide phycocyanin variations question the ability of grab samples or single probe measurements to be used against action thresholds to determine the need for corrective actions or advisories. Clearly, increases in cyanobacteria that occur in the interval between grab sampling may receive, suggesting the risk of delay or missing high cyanobacterial biomass. Routine grab sampling only provides a snapshot of the concentrations at any given time and is not indicative of cumulative or peak loadings of cyanobacteria entering the DWTP. Cumulative loadings are indicative of the amount of cyanobacteria and toxins that will be concentrated in the DWTP processes such as the clarifier and sludge, while peak loadings are indicative of periods of high risk for breakthrough. For DWTP management purpose, online monitoring or composite sampling would be better indicators of loadings.

Most guidelines advise utilities to conduct weekly sampling for cyanobacterial cell counts or biovolumes and, in some cases, toxin concentrations. For example, Australian guidance proposes a maximum sampling frequency of twice per week (Newcombe et al., 2010). For implementation reasons, utilities are likely to conduct sampling on fixed days and times, which can entirely miss short-lived and longer lasting cyanobacterial events. Sampling once or twice a week on a fixed schedule may provide misleading results. In our study, grab samplings were conducted in the morning (8 to 10 h), while cyanobacteria increased in the afternoon period at the high-risk DWTP A intake water, which indicates that peak concentrations occurring in the afternoon were unlikely to be ever measured. For example, on August 10, a maximum 2-h moving average of 10.9 RFU

should warrant plant operator based on exceedance of the alert level of 5.0 RFU (Figure 5.2a), while grab sampling result of 0.02 mm³/L was lower than the vigilance level (Figure 5.5b).

Furthermore, the difference in time series trends shows the importance of the method for evaluating the risk of cyanobacteria entering the plants. The appropriate statistical treatment should be chosen in accordance with the requirement (Figure 5.2). One single prominent phycocyanin peak based on raw measurements is not representative of the cyanobacterial loadings entering the plant. Moving averages for 2, 6, 12, and 18-h could detect all increases in phycocyanin for various operational purposes. In this study, 2-h moving average provides actionable information to the plant manager to be vigilant, indicating the need for immediate corrective action, such as adjusting coagulation and PAC dosages. Elevated phycocyanin values using the 6-h moving averages indicate that the bloom is persistent and may affect all separation processes over time. Daily and weekly moving averages smooth out the short-term fluctuations which may be useful for longer term trending. In some cases, progressive increases of daily moving averages of RFUs are indicative of upcoming large peaks of phycocyanin concentration. For example, the daily moving average started to increase on July 27 and August 8 and occurred prior to the large peaks on July 30 and August 11 based on raw measurements (exact time). Similar results were found for weekly moving averages which provided increased signals prior to the raw measurements. However, the weekly moving average consistently underestimated the phycocyanin readings and cannot detect the peaks.

5.4.2 Relationships between phycocyanin RFU and total cyanobacterial biovolumes

Conversion of phycocyanin fluorescence readings to total cyanobacterial cell counts and/or biovolumes is required when using phycocyanin estimations to relate readings to cyanobacterial risks in a water treatment plant. Numerous studies have provided a strong correlation between phycocyanin readings and cyanobacterial cell counts/biovolumes for field-collected samples (Cotterill et al., 2019; Kong et al., 2014; Thomson-Laing et al., 2020; Zamyadi et al., 2014). However, only a few studies have assessed the statistical treatment of phycocyanin readings to improve the interpretation of grab sample cyanobacterial biovolumes. Genzoli et al. (2016) established different statistics to characterize phycocyanin patterns in the Klamath River in the western United States to predict the risk of toxins in source water. The authors compared

taxonomic cell counts to YSI 6600 phycocyanin probe readings considering hourly means, daily means, daily 10% trim means, minimums daily, maximums daily, 25th and 75th quantiles of daily, daily medians, and moving averages (4, 8, and 14 days). Although the authors noted similar correlation coefficients (ranging from 0.69 to 0.77) between cell counts and all statistics phycocyanin readings, low grab sampling frequency (weekly or biweekly) could hardly represent the phycocyanin conditions. These findings apply to the prediction of risk at the source. In our study, only the 2-h moving average was best correlated with the total cyanobacterial biovolumes (Figure B.6). We consider the relative use of different statistics of the probe readings to predict the level of challenge to the separation processes in a DWTP. A shorter 2-h moving average was indicative of the incoming loadings. But for clarified and filtered water, a correlation cannot be established between the biovolume and the RFU probe readings, because most readings were below the detection limit.

Although not the focus of this study, fluorometric interferences (e.g. temperature, presence of eukaryotic algae, and DOM), light exposure, and turbidity are the factors that can influence the phycocyanin readings (Choo et al., 2019; Ma et al., 2022; Rouso et al., 2021; Symes et al., 2016). In our study, the extent of such interference may have influenced the results was low. With regards to DOM and temperature, as the sampling was conducted between July and October, a sudden change of DOM level was unlikely to happen, and the maximum temperature difference in the raw water of the studied DWTPs was 5 °C (data not shown). The impact of eukaryotic algae in our study is unlikely to be large, as the highest chlorophyll-a was in the raw water of the high-risk DWTP A with 4.5 RFU (data not shown), and the average chlorophyll-a at the other sites of three studied DWTPs was all approaching 0. Almost no light exposure was applied to the in-plant samples because of the sampling point locations within the treatment plant. Turbidity over 50 NTU can make probe measurement ineffective (Bowling et al., 2013); but in our study, for the raw water of DWTP A, there was only one point (5 mins on August 16) with turbidity over 50 NTU. It was also reported that the nutrients could have an effect on phycocyanin production of a microalgae (Wicaksono et al., 2019). However, in our study, the nitrate and phosphate concentrations of all the samples were ranging from 0.04 to 0.36 mg/L and 0 to 0.05 mg/L, respectively (data not shown), much smaller than that of 12.5 to 100 mg/L and 0.25 to 2 mg/L in the study of Wicaksono et al. (2019). So the interference of nutrients could also be neglectable.

Besides the interference from the environment, different species present and colony formation can also contribute to the uncertainty of phycocyanin readings (Chang et al., 2012; Hodges et al., 2018; Rouso et al., 2022). Cyanobacterial density evaluated as biovolumes in the three studied DWTPs displayed considerable variability within each site over the course of sampling (data not shown). Ma et al. (2022) provided data concerning the various species present in the RW in DWTP A. Such variations of cyanobacterial biovolumes and species were also noted in other studies during DWTP monitoring campaigns (Jalili et al., 2021; Zamyadi et al., 2013a). Nevertheless, this interspecific difference in phycocyanin readings could be reduced by converting cell counts to biovolumes (Ma et al., 2022; Macário et al., 2015).

5.4.3 Mapping the location of cyanobacterial cells in the high and low-risk DWTPs

In high-risk DWTP A, high numbers of total cyanobacterial biovolumes on the Surface C reached $7.7 \text{ mm}^3/\text{L}$ as observed on July 31 (Figure 5.5e). The Surface C accumulation may lead to a risk of cyanobacterial cells passing into the CW as clarified water is collected on top of these clarifiers (Zamyadi et al., 2013b). Indeed, on that day, we observed relatively high phycocyanin levels in the CW with up to 1.2 RFU. Furthermore, this greater breakthrough resulted in higher total cyanobacterial biovolume on the Surface F with $1.8 \text{ mm}^3/\text{L}$. However, probe readings and grab sampling taxonomic results show that cyanobacteria did not pass through into FW during all events monitored. The low cyanobacterial level in FW may due to adequate capture by the filters or by the dilution of the limited accumulation of cyanobacteria in the volume of water over the filters (Zamyadi et al., 2013b).

Regarding the two low-risk DWTPs, cell accumulation was not noted on the surface of clarifiers (Surface C) or filters (Surface F). Almuhtaram et al. (2018) and Zamyadi et al. (2013b) also studied other low-risk DWTPs with cyanobacterial concentration in the RW lower than 1000 cells/mL. Almuhtaram et al. (2018) also reported no accumulation at the surface of clarifiers and filters in four DWTPs, while Zamyadi et al. (2013b) reported cyanobacterial scums with a concentration of up to 1.5×10^6 cells/mL in one of two plants. Pre-oxidation was identified as an approach to damage to cyanobacterial cells, affecting the cells' integrity and buoyancy, thus decrease the cyanobacterial accumulation at the surface of clarifiers and filter (Moradinejad et al., 2019). Indeed, pre-chlorination was implemented for the intake water of the DWTPs in our study and

Almuhtaram et al. (2018), while the DWTP in Zamyadi et al. (2013b) was not equipped with pre-oxidation.

As expected, the concentrated particles in the sludge in the high and low-risk DWTPs led to higher cyanobacterial biovolumes in the Sludge Bed than in the RW. Despite the higher concentration of cyanobacteria in the sludge, typically contained lower levels of total MCs than in the RW, except for the worst-case scenarios for the high-risk DWTP A. These observations are in agreement with Jalili et al. (2021) who reported low MCs in Sludge Bed (below 281 ng/L) in the same DWTP A, although the Sludge Bed experienced 3–31 times higher cell numbers as compared to RW. Similar patterns of MCs were also observed in the low-risk DWTPs where MCs cell quota in the Sludge Bed of 0.05 pg/cell was around 70 times lower than in the RW of 3.3–4.2 pg/cell (Almuhtaram et al., 2018), indicative of elevated cell numbers yet low MCs in the Sludge Bed. Lower levels of MCs in the sludge can result from the degradation, absorption on PAC as shown by Jalili et al. (2021). Although the high cyanobacterial densities in the sludge did not always represent a considerable risk of toxins, several investigations have shown that cyanobacterial cells may survive and even proliferate at least 10 days in the sludge storage tank, and release cyanotoxins (Jalili et al., 2022; Pestana et al., 2016). The presence of nutrients could influence the dynamics (Dreyfus et al., 2016; Pestana et al., 2016). We also observed high total cyanobacterial biovolumes and total MCs in the Sludge Supernatant when the cell concentrations in the Sludge Bed were high. Therefore, the stored sludge should be considered for cyanotoxin release source when: (1) recycling the sludge supernatant to the head of the DWTP, and (2) discharging the sludge bed to the wastewater treatment plant.

5.5 Conclusions

- Phycocyanin readings in the raw water fluctuated up to 93-fold (from 0.6 to 55.8 RFU) within 24 hours. The wide daily and hourly variations in phycocyanin readings show the dynamic of cyanobacterial risk during a bloom period.
- Monitoring for risks at the raw water or across the plant cannot be achieved using daily grab sampling, as it could only give a snapshot of the cyanobacterial concentrations at a given time. And most importantly, grab sampling cannot provide an estimate of the dynamic flux of the cyanobacteria entering a DWTP.

- A 2-hour moving average of the phycocyanin readings was proposed to improve the interpretation of phycocyanin signal trends and guide treatment response, avoiding responding to peaks of very short duration.
- An *in situ* phycocyanin probe was applied to successfully detect high cyanobacterial biovolumes entering a high-risk DWTP and the subsequent removal of cyanobacteria by clarification, filtration and chlorination.
- Significant cell accumulations will occur in the sludge in both high and low-risk DWTP. Such accumulations were not always associated with elevated MCs.

**CHAPTER 6 ARTICLE 3 - A FRAMEWORK TO USE *IN VIVO*
PHYCOCYANIN READINGS FOR CYANOBACTERIAL RISK
ASSESSMENT WITHIN DRINKING WATER TREATMENT PLANTS**

This chapter was submitted as a research paper in the Journal of *Environmental Management*, on May 18th 2023 Supplementary information is presented in Appendix C.

**A FRAMEWORK TO USE *IN VIVO* PHYCOCYANIN READINGS FOR
CYANOBACTERIAL RISK ASSESSMENT WITHIN DRINKING WATER TREATMENT
PLANTS**

Liya Ma ^{*}, Sarah Dorner, and Michèle Prévost

Department of Civil, Geological, and Mining Engineering, Polytechnique Montréal, Montréal, QC
H3C 3A7, Canada

^{*} Correspondence: liya.ma@polymtl.ca

Abstract

In situ phycocyanin (PC) probes have been deployed as a cost-effective and efficient way to monitor cyanobacterial (CB) abundance in drinking water sources and to identify periods of potential risk at drinking water treatment plants (DWTPs). Monitoring CB removal efficacies in near real-time by sequentially using a single probe for multiple streams across the treatment plant provides a more useful assessment of CB risk breakthrough in treated water. Removal efficacies were measured in three DWTPs using integrated mass fluxes estimated from PC readings and grab sample total CB biovolume estimations in raw, clarified, filtered, and treated water. Selective CB species removal during the treatment processes was also evaluated. In addition, relationships between physio-chemical parameters (turbidity, pH, dissolved oxygen, conductivity and chlorophyll-a) and PC across the treatment processes were investigated. Finally, a framework to use *in situ* multi-stream PC monitoring is proposed to manage risks of CB cells breakthrough in treated water. Estimates of 2-hour moving mean PC metrics are proposed to provide short term alert in raw water and establish periods of treatment vulnerability or dysfunction, while daily mean PC values can be used to estimate total and process specific log removals to justify treatment adjustments. Benefits and limitations of the tested *in situ* probes for the application of vigilance and alert levels are discussed and key knowledge gaps for future research and guidance are identified.

Keywords

Multi-stream phycocyanin probes; Treatment removal of cyanobacteria; Drinking water treatment plant; Risk reduction.

Key Contribution

This study proposed a phycocyanin probe-based framework to assist plant operators to minimize cyanobacterial risk to drinking water in near real time.

6.1 Introduction

In recent years, there has been an increase in the occurrence of potentially toxic cyanobacterial (CB) blooms in water supply systems around the world (Akyol et al., 2021; Kim et al., 2021; Zamyadi et al., 2019), due to climate change and human activities (Griffith et al., 2020; Nwankwegu et al., 2019). Cyanotoxins can cause a range of health problems, including skin diseases, gastroenteritis, Alzheimer's disease, and liver damage (Chorus et al., 2021b). Microcystins are the most commonly detected cyanotoxins across the world (Graham et al., 2010; Meriluoto et al., 2017). Up to 70% of the freshwater CB blooms are toxic (Pham et al., 2015; Vareli et al., 2012).

CB proliferation in water sources raises serious consequences due to its impact on the management of water treatment (Catherine et al., 2013; Steffen et al., 2017). Incoming water quality to DWTPs can change rapidly, creating an unexpected shock load (Ma et al., 2023). These events impact normal plant operations and are known to cause treatment process problems. For instance, CB breakthrough at different steps of the treatment process can deteriorate treated water quality and affect sludge treatment (Almuhtaram et al., 2018; Jalili et al., 2021; Zamyadi et al., 2014; Zamyadi et al., 2019). Routine monitoring based on grab sampling only provides a snapshot of the CB density at a given time, often leading to an underestimation of CB entering or present inside the DWTPs (Ma et al., 2023). *In situ* probes equipped with sensors able to detect and quantify total CB using phycocyanin (PC) fluorescence, a CB-specific pigment, can better detect shock loading events than the discrete grab sampling (Ma et al., 2023; McQuaid et al., 2011). Key advantages of *in situ* probes are their high detection frequency, low cost and operational simplicity (Bertone et al., 2018).

Management strategies must be implemented to ensure the water quality in the presence of CB and their associated toxins in the source water and within the DWTPs. Countries and regions have developed guidelines to prevent and control CB and cyanotoxins in drinking water. Management of CB at the water intake is typically done by: (1) assessing cell counts and/or cell biovolumes present in raw water in reference to pre-set thresholds; and (2) measuring the level of toxins present in a grab sample in treated water (Chorus et al., 2021b; Health Canada, 2021; Ministry of Health, 2020; NHMRC, 2022; Ohio EPA, 2022). These pre-set thresholds are set using hypotheses such as the type of CB present and their maximum potential for cyanotoxin production.

Proactive management should include the monitoring of CB treatment efficacy across the different treatment barriers present in a DWTP. Grab sampling cell counts have been used to evaluate the efficacy of clarification, filtration and sludge separation (Jalili et al., 2021; Zamyadi et al., 2012a). On the other hand, probe based readings have been used to monitor CB biomass sporadically or in semi-continuous mode to observe trends in clarified, filtered, chlorinated and sludge supernatant (Almuhtaram et al., 2018; Zamyadi et al., 2014; Zamyadi et al., 2013b; Zamyadi et al., 2016b). Finally, limited information is available on species-dependent removal in treatment plants. (Zamyadi et al., 2013a) and (Zamyadi et al., 2012a) reported that *Aphanizomenon* sp. and *Aphanothece* sp. were more difficult to be removed by clarification than *Microcystis* sp. and *Dolichospermum* sp. in one DWTPs. Our study further explored the trends in species-specific removal across the treatment processes in three DWTPs with varying operating conditions and source water.

The main objective of this work is to investigate the interpretation of *in situ* PC probe readings for CB risk assessment within the DWTPs. The novelty of this work is to use the probe readings to assess risk based on the mass of CB captured and stored across the various treatment processes of three DWTPs. The specific objectives are: (1) to assess the performance of full-scale DWTPs for the removal of CB by grab sampling total CB cell biovolume, and compare it with the integrated mass fluxes estimated from PC probe readings; (2) to evaluate the impact of CB cell speciation on the treatment efficacy of different barriers; (3) to propose an *in situ* PC probe-based monitoring framework to assist plant operators to minimize the CB risk into drinking water; (4) to assess the relationships between physio-chemical parameters between sites inside the DWTPs as an indication of the change in CB risks to water quality. Finally, we identify the key knowledge gaps for future research and guidance for the application of *in situ* probe for DWTP process monitoring.

6.2 Materials and Methods

6.2.1 Water source and site description

The three DWTPs, named as DWTP A, DWTP B, and DWTP C, have been described previously (Ma et al., 2023). DWTP A draws water from Missisquoi Bay of Lake Champlain. DWTP B draws water from Lemieux reservoir which is fed by the Yamaska River. DWTP C draws water from Lake Erie. The three DWTPs are representative of typical drinking water sources and treatment processes in Canada. Briefly, DWTP A can be regarded as a high CB risk DWTP; while DWTP B

and C can be regarded as low CB risk DWTPs. All of them employ powdered activated carbon (PAC) injection, followed by conventional treatment processes consisting successively of coagulation, flocculation, sedimentation, and post-chlorination steps, as presented in Figure C.1. DWTP C also employs pre-chlorination at the very beginning of the treatment.

6.2.2 *In situ* phycocyanin monitoring

Monitoring was performed between July and October in 2018. *In situ* YSI EXO2 probe (YSI, Yellow Springs, OH, USA) was used in this study. The probe featured a PC/chlorophyll-a (Chl-a) sensor, turbidity sensor, conductivity/temperature sensor, pH sensor and total dissolved oxygen (DO) sensor. The probe was also fitted with a self-cleaning wiper. In each of the three DWTPs, a YSI EXO2 probe was devised for remote control and data logging to monitor the CB in raw, clarified, filtered, and treated water (Figure C.1). Four types of water went through the probe flow chamber sequentially, in 30 mins cycle. Probe readings were recorded at 5-minute intervals. Therefore, six measurements were recorded for each type of water every 2 hours. Probe readings were continuously recorded, transmitted via a modem, and downloaded remotely. Negative PC values which represent readings out of the detection limit were substituted with 0.001. The limit of blank (LoB) and limit of detection (LoD) were calculated according to the methods of (Armbruster et al., 2008). The LoB and LoD are 0.13 and 0.16 RFU, respectively.

6.2.3 Grab sampling procedure and taxonomic analysis

Grab samples for taxonomic analysis were taken in parallel to probe measurements from the raw, clarified, filtered, and treated water from all three studied DWTPs. The samples were taken from sampling taps located within the DWTPs thoroughly flushed prior to collection. A total of 23, 9, and 4 sampling events were conducted at DWTP A, B and C, respectively.

The taxonomic cell counts samples were preserved in the dark at room temperature (21°C) using Lugol's iodine. Taxonomic cell counts, species identification, and biovolumes calculations were carried out at the Department of Biological Sciences at the Université du Québec à Montréal (UQAM) using the methodology outlined in (Planas et al., 2000) and (Lund, 1959).

6.2.4 Data analysis

Contour graphs, correlation analyses and principal component analysis (PCA) were performed using R (version 4.2.1). Log removal was calculated by Equation 6.1.

$$\text{Log removal} = \text{Log}_{10}\left(\frac{\text{influent concentration}}{\text{effluent concentration}}\right) \quad \text{Equation 6.1}$$

6.3 Results and discussion

6.3.1 Treatment efficiencies evaluated from grab sampling

6.3.1.1 Total cyanobacterial cell removal by treatment processes

CB density evaluated as cell biovolumes from paired samples across the treatment processes can be used to compute log removal efficacies for clarification, filtration and post-chlorination (Figure 6.1). Higher mean clarification log efficacies of 2.2 ± 1.0 were found at the high-risk DWTP A, while lower clarification log efficacies were observed with 0.4 ± 0.3 and 1.2 ± 0.6 at the low-risk DWTPs B and C (Figure 6.1). Filtration efficacies were 0.6 ± 0.5 , 1.5 ± 0.6 , and 0.6 ± 0.6 at the DWTP A, B and C, respectively. Likewise, post-chlorination log efficacies were 0.1 ± 0.3 , 0.2 ± 0.6 , and 0.6 ± 0.5 at the DWTP A, B and C, respectively. When considering overall cumulative log removals, 2.7 ± 1.4 , 1.9 ± 0.6 , and 2.4 ± 1.3 were achieved in the DWTP A, B and C, respectively. Such results are in agreement with previously reported treatment efficacies between 92% and 99% (Jalili et al., 2021; Sun et al., 2012; Zamyadi et al., 2013a). Overall, the CB cells were efficiently removed by the three barriers, so that the cell biovolumes were all lower than the WHO alert level 1 of 0.3 mm³/L in the treated water. These high levels of removal efficacies confirm that conventional treatment can be extremely efficient to remove CB cells in high and low-risk DWTPs as recommended by WHO (Chorus et al., 2021b).

The three DWTPs surveyed offer three situations as shown by Figure 6.1 for CB and Figure 6.3 for turbidity. DWTP A is characterized by median turbidity and varying CB biovolumes levels, DWTP B has systematically low turbidity and biovolumes, while DWTP C has high turbidity and low CB biovolumes.

When combining all observations, treatment efficacies for CB biovolumes were dependent on the density of incoming cells into each treatment process. The incoming (raw water) cell biovolumes

were found to be positively correlated with clarification efficacy, with $R = 0.76$ ($p \leq 0.001$) (Figure 6.2a). The incoming cell biovolumes to the filtration process (clarified water) were also found to be positively correlated with filtration efficacy, with $R = 0.61$ ($p \leq 0.001$) (Figure 6.2b). The key steps for CB removal by clarification are most probably the efficient destabilization of the CB cells by coagulant and the presence of sufficient particles to facilitate flocculation, at least in the absence of a sludge blanket clarifier (DWTPs A and B). High CB biomass or elevated turbidity resulted in clarification efficacies consistently high at levels greater than 2.0 log. However, when the incoming biovolumes were below 0.5 mm³/L, CB removal efficacies ranged from 0.2 log to 2.4 log, depending on the incoming turbidity. Indeed, at the highest turbidity of 35.9 NTU with low CB biovolume of 0.1 mm³/L, high CB biovolume removal of 2.0 log was observed. Similarly, high biovolume of 10.7 mm³/L with low turbidity of 1.7 NTU also resulted in excellent biovolume removal efficacy of 3.2 log. Likewise, better removals via filtration occurred when biomass levels were greater than 0.01 mm³/L, showing a much lower threshold for a process with a lower cut-off for particle removal. It is therefore probable that the determining factor for clarification efficacy is a sufficient dose of coagulant to ensure particle destabilization and the presence of a sufficient number of particles to facilitate aggregation during flocculation. The log removal performance of physical treatment processes is commonly assumed to be a first-order process with respect to the influent concentration of a particulate contaminant (Haas et al., 1998). These findings confirm that for high-risk DWTP, a properly optimized clarifier, as recommended by the WHO (Chorus et al., 2021b), is adequate for the elimination of CB cells.

At DWTP B, the negative removal of cells in clarified water on Sep 5 shown on Figure 6.1 was due to the increase of *Aphanothece clathrata brevis* cells in the clarified water than in the raw water, and it may be due to the release of cells from the sludge (Zamyadi et al., 2012a). As a result, on this day, the clarification failed to function adequately, and the filtration took over. Species selective removal is further described in section 6.3.1.2.

Turbidity removal efficacies were also computed and are presented in Figure 6.3. The incoming high turbidity did not correspond to high incoming CB density. For instance, turbidity in the raw water of DWTPs A and C is similar (Figure 6.3), but with varying CB biomass (Figure 6.1). Turbidity measurements cannot differentiate the presence of CB in a natural water matrix from other organic and inorganic sources of turbidity. Hence, turbidity is not suitable for triggering remedial actions to control CB. Although (Ahn et al., 2007) reported positive correlations between

turbidity and probe readings, there was no significant sources of inorganic turbidity presence in the water source (Ahn et al., 2007). Also, Zamyadi et al. (2014) found that high turbidity can be associated with increasing CB density in raw water, but stressed that turbidity threshold levels cannot be used to manage CB. In addition, no relationship was found between the removal of turbidity and the removal of cells (Figure C.2). For instance, in DWTP A, low turbidity removal (lower than 0.3 log) could be found on July 5, 27, 31, Aug 1 and 2, but the biomass removal was high (higher than 1.1 log). These findings suggest that the CB biomass was overall better removed than the turbidity.

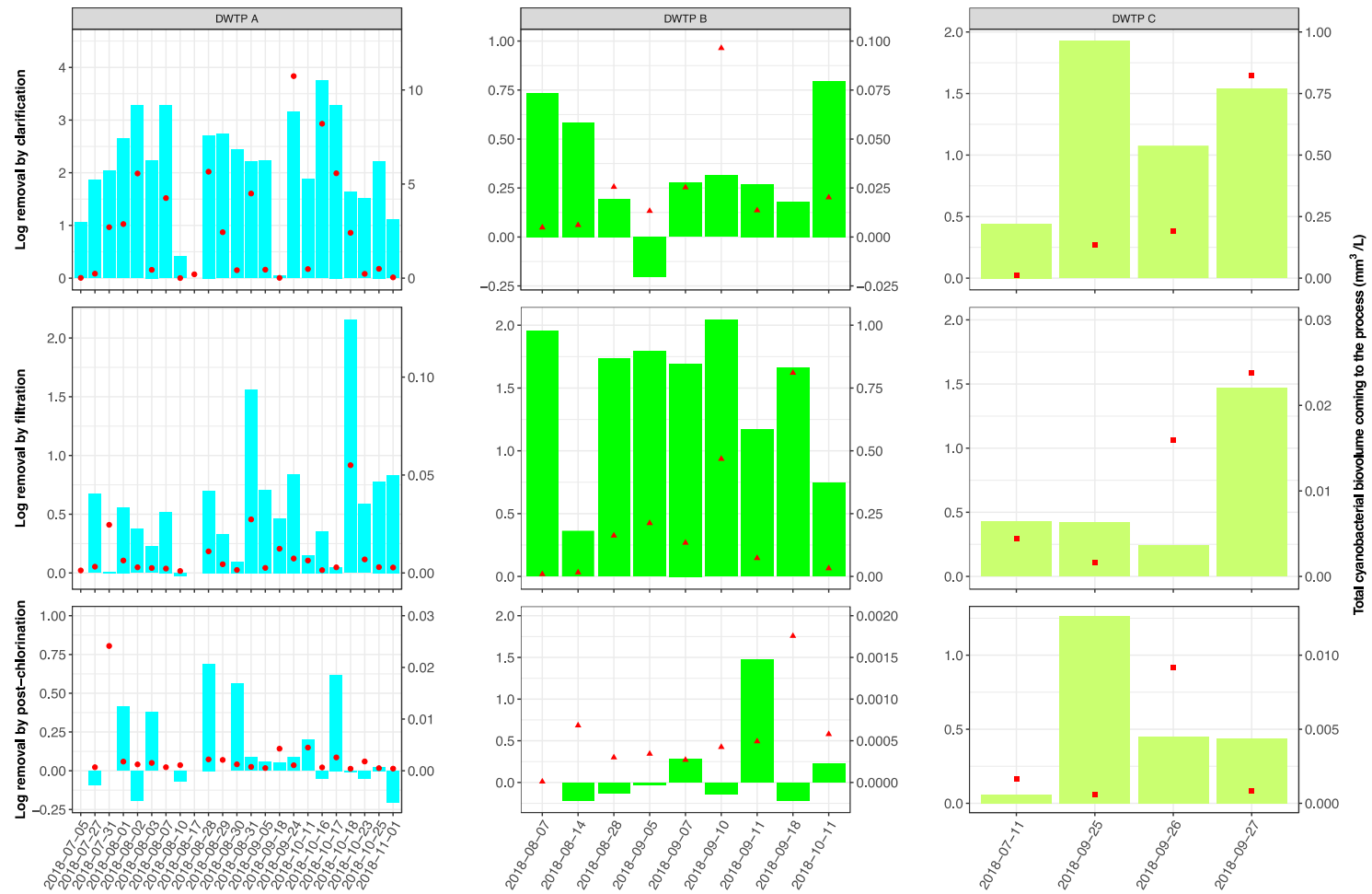


Figure 6.1 Log removal of total cyanobacterial biovolumes (mm³/L) by clarification, filtration, and post-chlorination in studied three DWTPs. Red dots, triangles, and squares indicate the total cyanobacterial cell biovolumes (mm³/L) coming into the corresponding treatment processes. n = 23, 9, and 4 for DWTP A, B and C

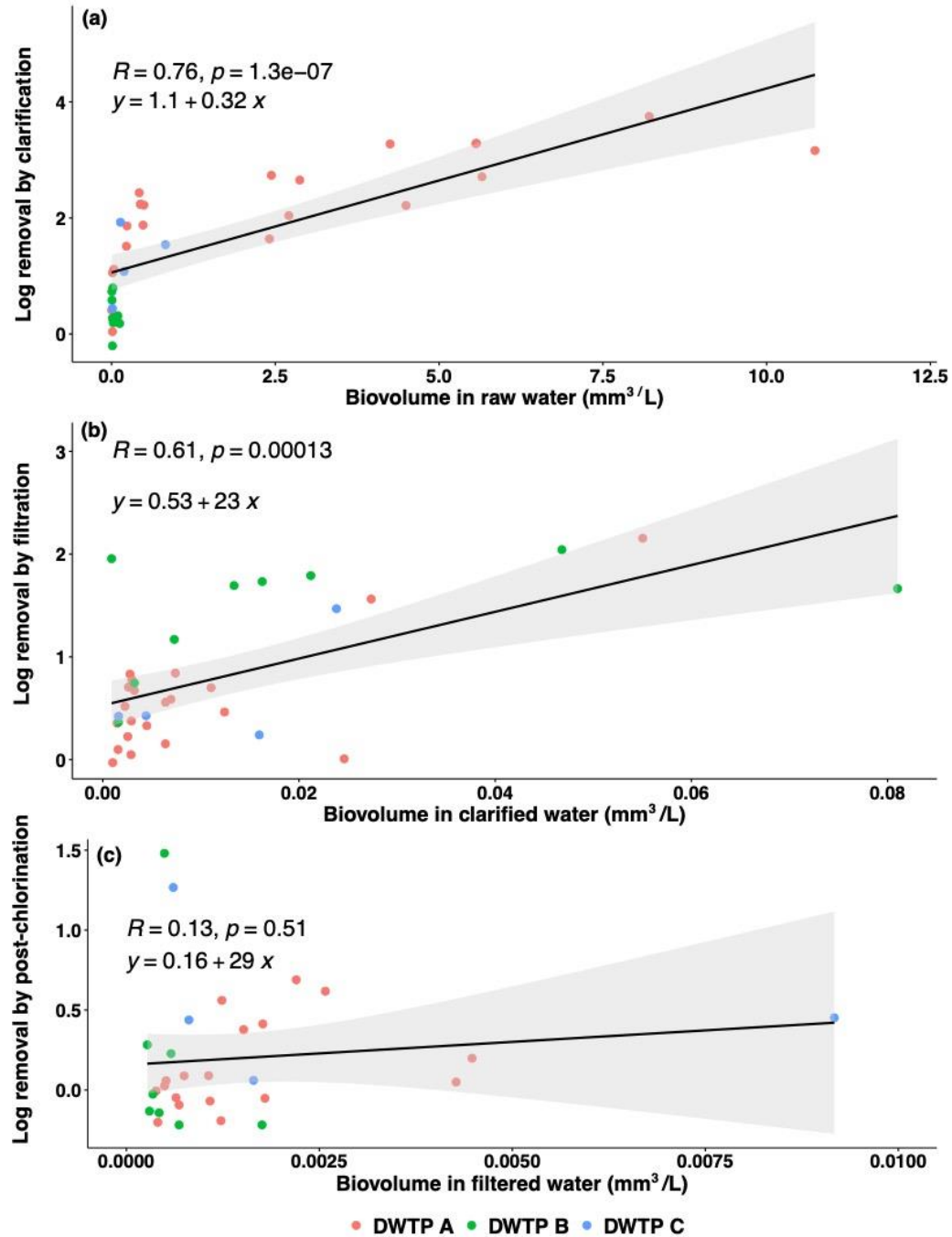


Figure 6.2 Log removal of total cyanobacterial biovolumes (mm^3/L) by (a) clarification, (b) filtration, and (c) post-chlorination versus the incoming cell biovolumes (mm^3/L) coming into the corresponding treatment processes. Paired data are from the samples of all three DWTPs

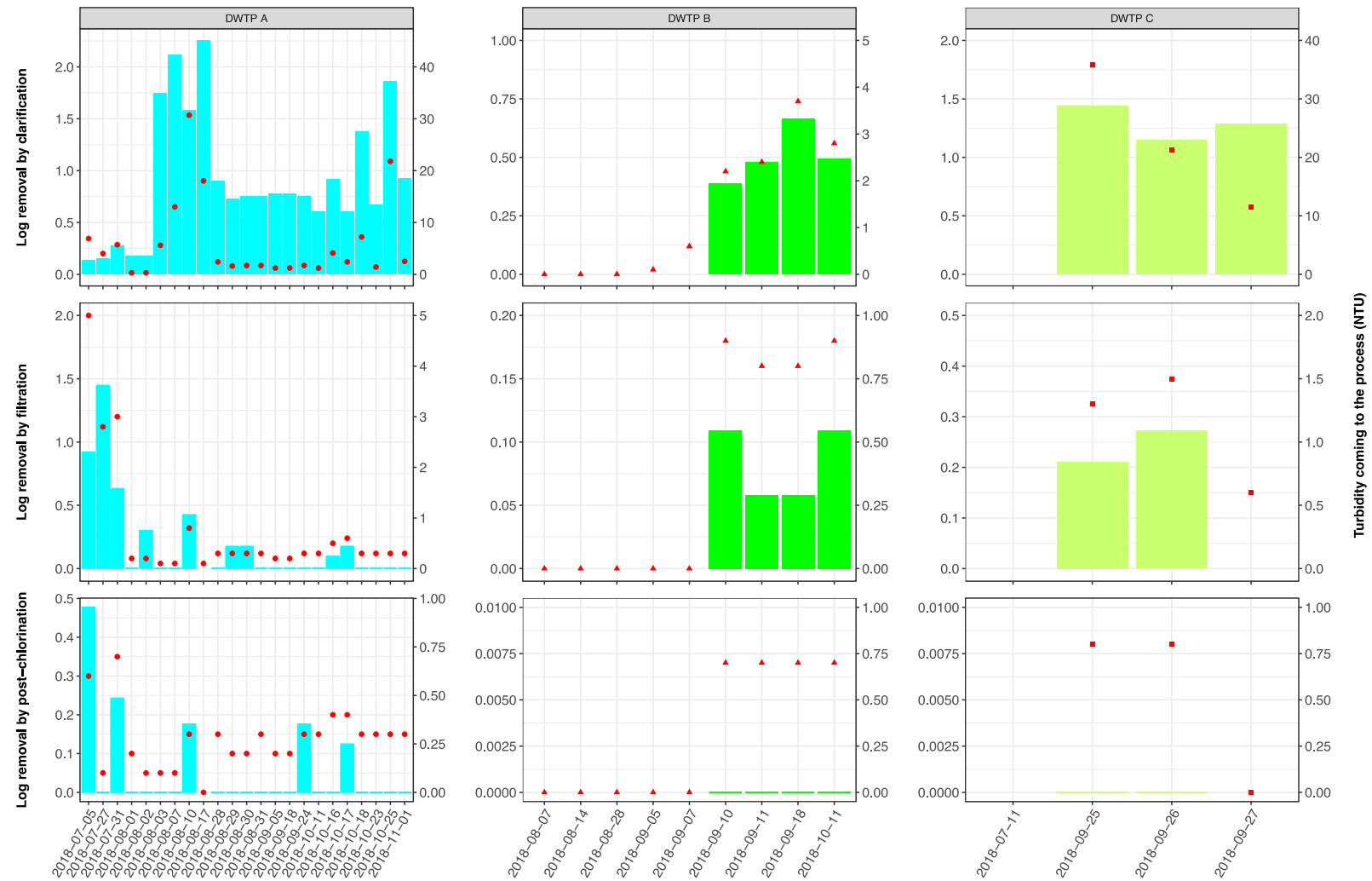


Figure 6.3 Log removal of turbidity (NTU) by clarification, filtration, and post-chlorination in studied three DWTPs. Red dots, triangles, and squares indicate the turbidity (NTU) coming into the corresponding treatment processes. n = 23, 9, and 4 for DWTP A, B and C

6.3.1.2 Trends in cyanobacterial species-specific removal across the treatment processes

The speciation of CB as the water progresses through the treatment processes inside the high-risk DWTP A, provides information on the selective removal and accumulation of the CB cells. During the entire studied period, the CB community entering the DWTP A consisted of multiple CB species. To facilitate interpretation, the most 8 dominant species according to cell counts (Figure 6.4) and cell biovolume (Figure 6.5) are shown. The dominant species differ whether you consider cell counts and biovolume, due to the large difference between the cell sizes of the species present (up to 600-fold) (Kong et al., 2014; Ma et al., 2022). Likewise, Figure C.3 to C.6 show the composition of species in different treatment processes in the low-risk DWTP B and C. The detailed removal efficacies for each species in three DWTPs were provided in Table C.1, C.2 and C.3.

With regards to cell counts (Figure 6.4, Figure C.3 and Figure C.5), *Aphanothece clathrata brevis* was the most dominant species in the raw water of all three studied DWTPs, and it was mirrored in clarified, filtered and treated water. *Microcystis aeruginosa* was among the top 3 dominant species in the raw water of all three studied DWTPs. While for biovolume (Figure 6.5, Figure C.4 and Figure C.6), *Dolichospermum spiroides*, and *Microcystis aeruginosa* were among the most dominant species in all three studied DWTPs.

With regards to treatment efficacies for each species, for DWTP A, *Aphanocapsa delicatissima*, *Microcystis aeruginosa*, *Dolichospermum spiroides*, *Chroococcus dispersus minor*, *Aphanocapsa holsatica* and *Chroococcus minimus*, were removed over 2.6 log (with maximum ± 0.7 log) by clarification. These levels are coherent with reported log removal of total toxin producers ranging between 1.5 log and > 5.5 log (Szlag et al., 2015). However, both clarification and filtration were partially effective to remove *Pseudanabaena mucicola* and *Aphanothece clathrata brevis*. *Pseudanabaena mucicola* was the least removed by clarification with 1.0 ± 1.1 log, followed by *Aphanothece clathrata brevis* with 1.2 ± 0.5 log. In most cases, the small biovolumes and counts in some cases increased in filtered water possibly because of the accumulation in filters as reported by (Zamyadi et al., 2012a) or breakage of filaments. For the low-risk DWTPs B and C, *Aphanothece clathrata brevis* was also among the least removed species with only 0.2 ± 0.4 log in DWTP B.

The mean total CB biovolumes in clarified water were systematically low as shown by the maximum biovolume of 0.1 mm³/L observed on September 18 in DWTP B, well below the WHO alert biovolume level of 0.3 mm³/L. Treated chlorinated water was even lower. However, relatively small average cell concentrations ranging from 831.2 to 1312.0 cells/mL of *Aphanothece clathrata brevis* were found in treated water, corresponding to very small biovolumes of less than 0.002 mm³/L in the three studied DWTPs (Figure 6.5, Figure C.4 and Figure C.6). Other studies in DWTP A also found *Aphanothece* was more difficult to be removed by clarification as compared to other CB species (*Microcystis* and *Dolichospermum*) (Jalili et al., 2021; Zamyadi et al., 2013a; Zamyadi et al., 2012a), possibly due to the differences in cell morphology, biovolume (0.5 µm³/cell), charge, resistance to sheer stress, and motility (Henderson et al., 2008a). Although these observations imply that the consistent removal of *Aphanothece clathrata brevis* is challenging, the actual biovolumes are negligible as compared to the WHO alert level 1 and additionally, this species is not known to produce toxins.

Overall, longitudinal monitoring with taxonomic cell counts showed significant temporal variability of clarification and filtration efficacy across several months. They highlight the need for combined optimized clarification and filtration to achieve consistent high level removals of cell concentrations and biovolumes. Chlorination was not systematically effective in lowering cells and biovolume. Optimized filtration to achieve low water turbidity and the potential use of filter aid should be recommended to ensure minimal passage of CB cells into treated water, especially if clarification is operating at sub-optimal performance.

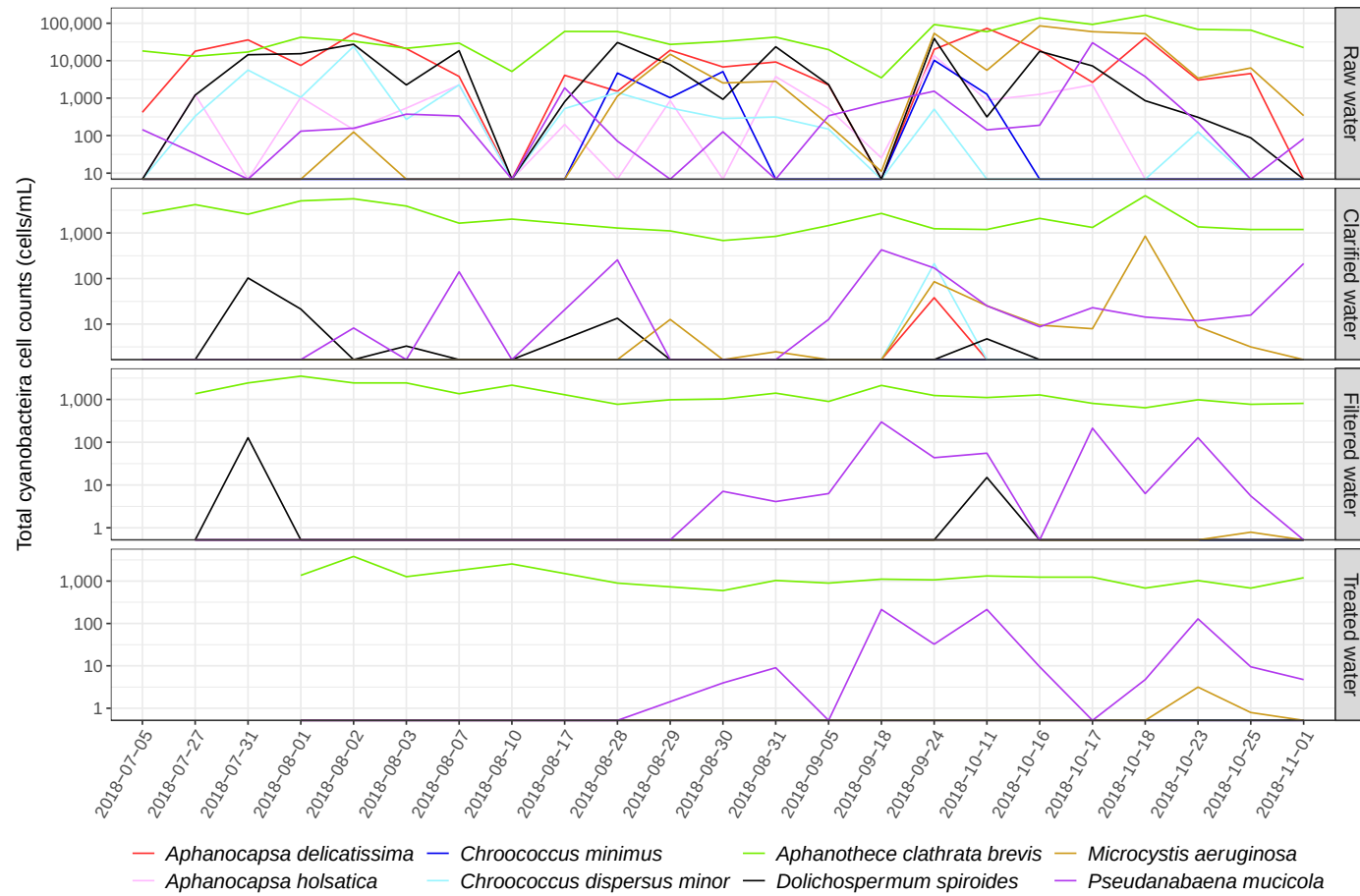


Figure 6.4 Cyanobacteria speciation evaluated as cell counts (cells/mL) in the raw, clarified, filtered, and treated water in DWTP A

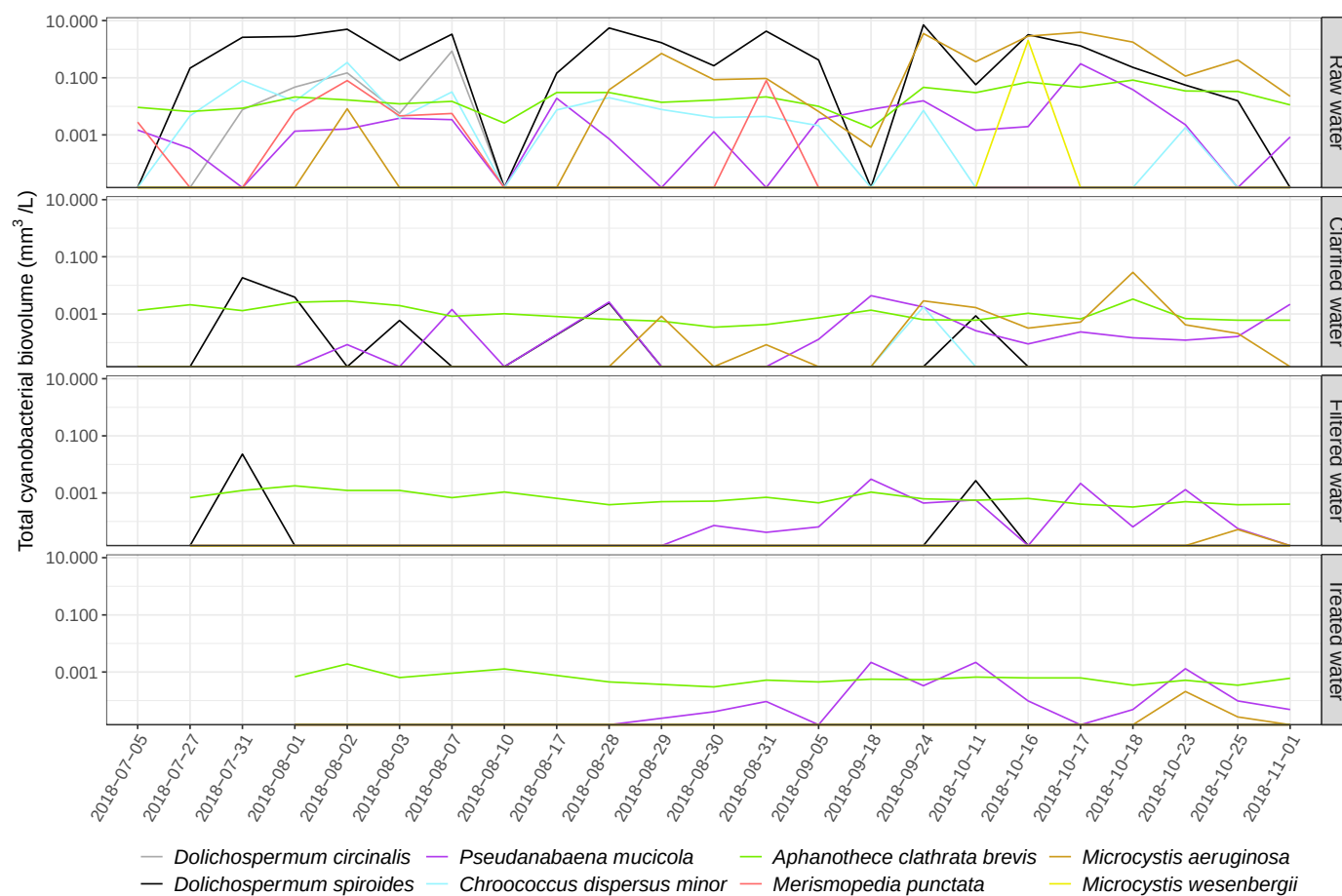


Figure 6.5 Cyanobacteria speciation evaluated as cell biovolumes (mm³/L) in the raw, clarified, filtered, and treated water in DWTP A

6.3.2 Treatment efficacies evaluated by *in situ* phycocyanin probes and biovolumes

Process efficacies were evaluated using *in situ* PC measurements of the various effluent flows in the DWTPs by two metrics: (1) 2-hour moving average PC readings to illustrate the short term periods distribution (Figure 6.6a); (2) daily PC mean (Figure 6.6b), as incoming PC readings could fluctuate significantly across a day (Ma et al., 2023). Trends in removal efficacies differ between DWTPs and scattering depends on the type of metric used. In general, higher estimated log removals were observed for all processes in DWTP A as compared to DWTP B and C. These figures also show the relative contribution of the various processes to the removal of CB. For DWTP A, clarification is the main process removing CB (mean 2.2–2.4 log), whereas less efficient clarification contributes to lower total overall removals in DWTPs B (mean 0.5–0.6 log) and DWTP C (mean 0.1–0.2 log). Less scattering is observed in Figure 6.6b (daily mean removals) as compared to Figure 6.6a (2-hour moving average removals). This is attributed to the fact that shorter periods of lower performance happening in a single day are averaged out in daily mean removals. Both metrics provide valuable information to operators.

Finally, significant correlations were found between log removals derived from grab sampling biovolumes and time corresponding PC probe readings for the clarification process, but not for filtration (Figure 6.7). Daily biovolumes removals were better described by daily mean PC metrics (Figure 6.7b) than 2-hour metrics (Figure 6.7a).

Monitoring removal efficacies based on near real-time *in situ* PC detection can be easily implemented at relatively low costs using multi-stream reactor and a multi-parameter probe. Treatment regimes (e.g., coagulant dosages) for surface water are typically adjusted to meet turbidity removal goals that drive primary compliance for disinfection and are unlikely to be adjusted to meet a specific goal for CB removal. Unfortunately, turbidity and CB removal were not correlated (Figure C.2). Using the probe-based assessment of daily CB removal would provide an easy metric for operators to monitor the treatment performance.

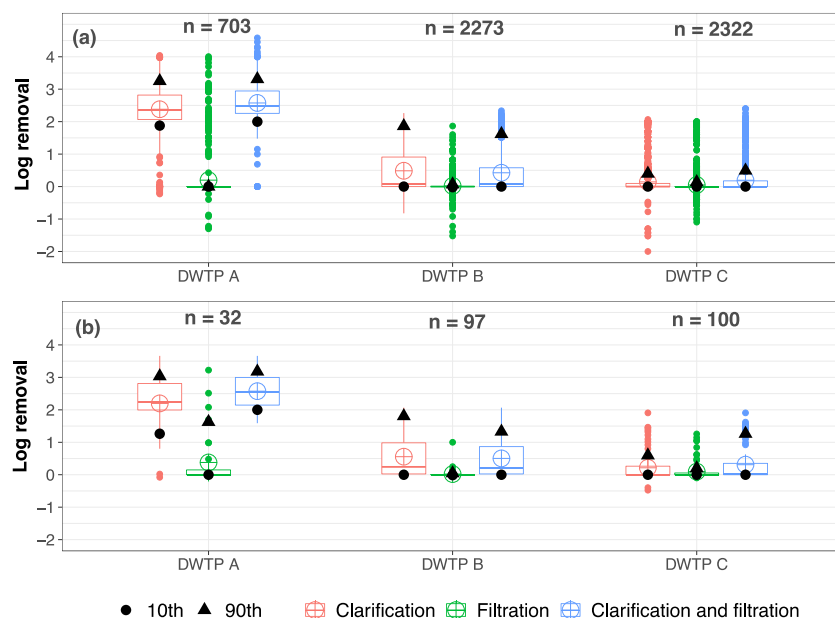


Figure 6.6 Phycocyanin RFU log removal efficacies estimated from (a) 2-hour moving average, (b) daily mean. Boxes represent the 25–75th percentile. The line within each box corresponds to the median value, and the circle corresponds to the mean value. The number of observations is indicated by n

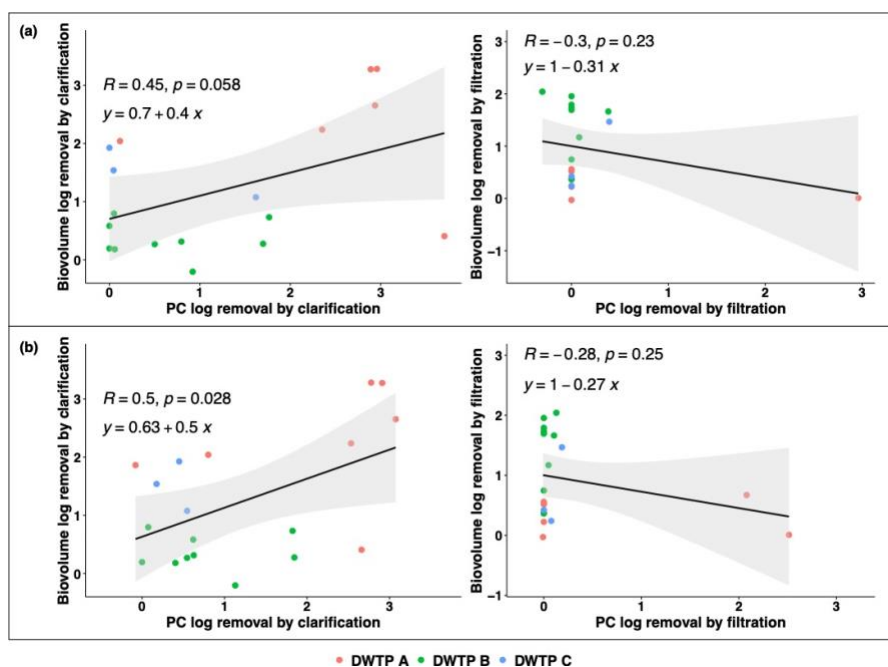


Figure 6.7 The clarification and filtration log removal efficacies evaluated by cell biovolumes versus evaluated by (a) 2-hour moving average of the phycocyanin readings, (b) daily mean phycocyanin readings. Paired data are from the samples of all DWTPs

6.3.3 Implications for regulations and operational guidance

6.3.3.1 Ability of phycocyanin probe metrics to be used for WHO alert levels

A most important factor to consider when using the *in situ* probe is their ability to provide reliable data corresponding to threshold values used for management purposes. Regulatory authorities have issued guidelines, regulations and response frameworks in response to the CB levels in the water source. Recent CB and cyanotoxin monitoring frameworks throughout the world in drinking water supplies were summarized in Table C.5. Several studies established relationships between the CB total biovolumes and PC RFU readings, all obtained in raw water in the case of field study (Table C.6). These calculated equivalent RFU threshold values vary widely, although the monitoring probes were from the same manufacturer. The CB threshold of 0.3 mm³/L established as the WHO alert level 1 corresponds to PC readings ranging from 0.1 to 5.4 RFU; the CB threshold of 4 mm³/L (WHO alert level 2) corresponds to PC readings ranging from 1.1 to 70.9 RFU (Table C.6).

Three reasons may contribute to the large variations in the PC equivalent threshold values. Firstly, the dispersion of data points around the correlation trend line is often very large because of the errors involved in microscopic counts (McQuaid et al., 2011; Zamyadi et al., 2016a). For instance, the dispersion of data points around the correlation trendline from McQuaid et al. (2011) is up to 4 RFU. Secondly, how the outliers were interpreted is important; for instance, Almuhtaram et al. (2018) removed the outliers manually. Finally, the variability of CB species in field samples may account for the great dispersion of the biovolume when transferred to PC readings. Even from the same study, different tested strains of *Microcystis aeruginosa* could give 3.5 times different equivalent RFU values (Choo et al., 2018). In our study, the CB community displayed considerable variability in the raw water over the course of sampling (Figure 6.4), as also observed in other studies (Jalili et al., 2021). So even the site-specific conversion factor based on a dominant CB species may not avoid under or overestimations, and it is a challenge to be considered when converting and presenting the RFU units into biovolume. The need for systematic determination of the PC/cell biovolume ratio could form the basis for improved PC based alert levels.

Additionally, the limitation of probe's analytical sensitivity presents a constraint in using PC as a proxy for biovolume. Reported CB biovolume detection limit include 1.8 mm³/L for Turner Cyclops-7 probe (Cotterill et al., 2019), 1.0 mm³/L for YSI 6600 probe (McQuaid et al., 2011). (Choo et al., 2018) calculated a detection limit of 1.31 RFU for YSI EXO 2 probe based on

Microcystis aeruginosa. These detection limits are higher than the alert level 1 of 0.3 mm³/L suggested by WHO (Chorus et al., 2021b), indicating unreliable PC readings at the lower guideline threshold. In our study, the detection limit of the YSI EXO2 probe was estimated at 0.16 RFU, based on the measurements performed on dissolved PC extract. This is consistent with the result of (McQuaid et al., 2011) using YSI 6600 probe with 0.2 RFU.

WHO alert levels are determined based on toxicity risk associated with the biovolumes of presumably toxic CB in the source. Even if a strong correlation between biovolumes and PC can be established at a specific site, a significant portion of the data collected across the treatment plant is likely to show low RFU readings close or below to the detection limit. In this study, a significant fraction of PC readings in clarified, filtered, and treated water in the studied three DWTPs was lower than 0.2 RFU which corresponds to approximately 0.6 mm³/L. Operators may face limitations to determine corresponding biovolume alert levels and health advisories for CB blooms in treated water. This could be best solved by improving the detection limit of the PC probes used. Another approach is to use probe-based readings to monitor relative removals by various treatment processes, shifting from grab sample based alerts to continuous preventative process monitoring.

6.3.3.2 *In situ* based phycocyanin monitoring based framework to manage cyanobacteria in DWTPs

CB alert levels are based on total CB cell biovolumes and/or total CB cell counts in source water. These metrics are used to assess the potential health risks associated with CB and their toxins. Grab sampling, the most common way to assess the biovolumes or cell counts present at the source, cannot represent transient CB challenging periods. Indeed, CB risk levels in the drinking water source can fluctuate significantly across the day (Ma et al., 2023). At best, grab samples provide snapshots of the situation at the date and hour of sample collection. Similarly, CB removals through the DWTP processes can also vary within a day. DWTP treats surface water from sources that vary in quality (temperature, CB concentrations, turbidity, etc.) and processes are operated at varying flows to meet peak demand, a combination that can affect the efficacy of separation processes. Studies have reported CB breakthrough and accumulation inside DWTPs, including in clarified water, filtered water, surface of clarifier, surface of filter, sludge thickener and supernatant of sludge thickener (Almuhtaram et al., 2018; Jalili et al., 2021; Ma et al., 2023; Zamyadi et al., 2014; Zamyadi et al., 2019). Applying intervention threshold value only to raw

water may overlook the within-plant risk, especially the risk of sludge. As studies have addressed the risk in sludge, even in the low-risk DWTP monitored in this study (Jalili et al., 2021; Ma et al., 2023; Pestana et al., 2016).

For water quality parameters that can change rapidly, compliance with drinking water quality regulations relies on the use of proxy online parameters such as turbidity and oxidant residuals to predict pathogen removal and inactivation. Process water turbidity in DWTPs is not monitored by grab samples but must be measured on line to ensure that treatment is adjusted at all time to maintain adequate barriers for pathogen removal (United States Environmental Protection Agency (USEPA), 2006). Plant operators cannot use CB grab sampling results across the treatment plant to guide their short or medium-term treatment adjustments, because of the low sampling frequency, response delay and high costs associated with these measurements. On the other hand, a single high frequency *in situ* PC probe can monitor the situation at the water intake and across the various processes in a DWTP in near real time.

We propose implementing a more comprehensive CB removal preventative framework using probe PC measurements from source to treated water as shown in Figure 6.8. To be able to use probes to define response to alert levels, the first step is to establish a site-specific RFU equivalent to the WHO biovolume vigilance and alert levels. Then using PC probe readings for the management of CB across the DWTP, operators can interpret:

- **Levels found in the raw water at the plant intake.** CB concentrations estimated by 2h moving average RFUs serve as a general indicator of risk and a warning of potential challenges to treatment. They should be interpreted in reference to recent WHO vigilance and alert levels.
- **Short term breakthrough of CB passing through clarification and filtration.** This is best observed using 2h running average readings that provides some smoothing for sporadic extreme values, while maintaining enough sensitivity to detect lasting peaks of PC (Ma et al., 2023). The metrics can serve to identify periods of vulnerability during regular operations or point to out of the ordinary transient changes in CB across the various processes.
- **Daily total clarification and filtration CB log removals** that integrated daily fluxes of CB and normal variations in process performance caused by varying flows and other

normal treatment adjustments. This can be best observed by estimating daily mean removals from the 72 daily probe readings taken for each treatment process. Basically, daily mean values allow for the identification of days when treatment does not provide sufficient barriers for the CB loads present, or for some potential sustained changes in CB loads in the raw and treated water. As for other treatment parameters, we propose to use a site-specific log removal goal always ensuring the presence of sufficient barriers. This goal should be set based on historical data on CB occurrence in the source and be sufficient to meet the removals during possible peaks of CB during bloom developments. Combined physical process removal defines water quality entering the clear well. As lower removals are observed with low concentrations of CBs, log removals are to be validated with actual filtered water RFU values to estimate risk.

The framework is coherent with the CB management plan proposed by Erratt et al. (2022), which includes the management of the CB in the resources. Our framework is a strong component of the proposed approach by Erratt et al. (2022), since it assesses the risk inside the treatment plant, and covers the management of risk before the consumer.

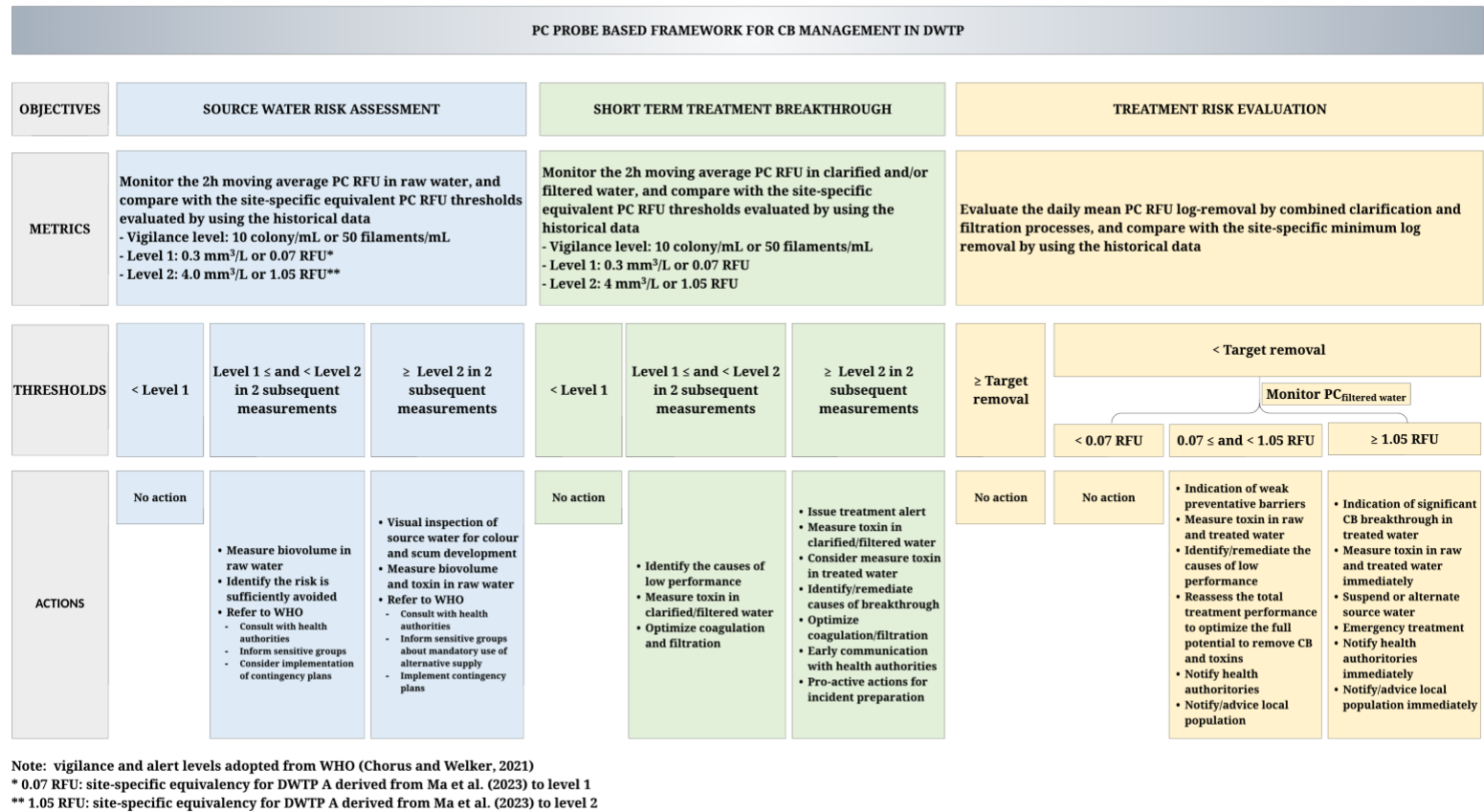


Figure 6.8 Proposed framework for the management of cyanobacterial risks at the source and across DWTPs using *in situ* phycocyanin measurements with a multi-stream probe

6.3.4 Trends of online water quality parameters across DWTP processes

Online PC probes typically offer the possibility to measure other parameters simultaneously. The interpretation of data generated by the PC probes with simultaneously measured other physio-chemical parameters in source water improves their interpretation (Bowling et al., 2013; Ma et al., 2022). Significant correlations between the physio-chemical parameters and PC readings in the raw water of the DWTPs have been reported, but no study has described the relationships between these physio-chemical parameters inside the DWTPs. A multivariate approach through PCA biplot was performed to describe how the physio-chemical parameters changed inside the DWTPs.

The probes used in this study measured PC, turbidity, conductivity, pH, DO, temperature, and Chl-a. The measured physio-chemical parameters in raw, clarified, filtered and treated water were summarized into mean, minimum and maximum values in Table C.4. A PCA biplot of the physio-chemical parameters shows the difference in the most important factor between the different types of water inside the DWTPs (Figure 6.9 and Figure C.7, C.8).

Figure 6.9a shows a clear clustering pattern based on different sites inside the DWTP A. Raw water was distantly clustered from clarified, filtered and treated water. The latter 3 types of water clustered in close proximity showing similarities in their physical-chemical characteristics driven mostly by conductivity and temperature. In particular, the loading plot shows that the positive end of PC1 (45%) was dominated by PC, turbidity, Chl-a and pH, and they were all closely associated with raw water, but not with the other types of water. The higher the value of turbidity, Chl-a and pH, the higher PC readings in raw water, thus indicating the presence of CB. This supports previous studies (McQuaid et al., 2011; Rome et al., 2021), which reported turbidity, Chl-a and pH are positively correlated in a CB bloom. For low-risk DWTPs B and C, physio-chemical properties of their raw water also allow clustering of clarified, filtered and treated water (Figure C.7a and C.8a), but not as distinctly as for high-risk DWTP A, probably reflecting the limitations of low CB cells.

As pH and conductivity are directly influenced by coagulation, the clustering of the different types of water can also be examined without these parameters. Figure 6.9b and Figure C.7b and C.8b, show that no clear association of PC, Chl-a and turbidity. Factors affecting clustering of the types of water in raw or treated water vary between DWTPs, supporting the observed lack of correlation between the removal of turbidity and CB biovolume across the treatment processes (Figure C.2).

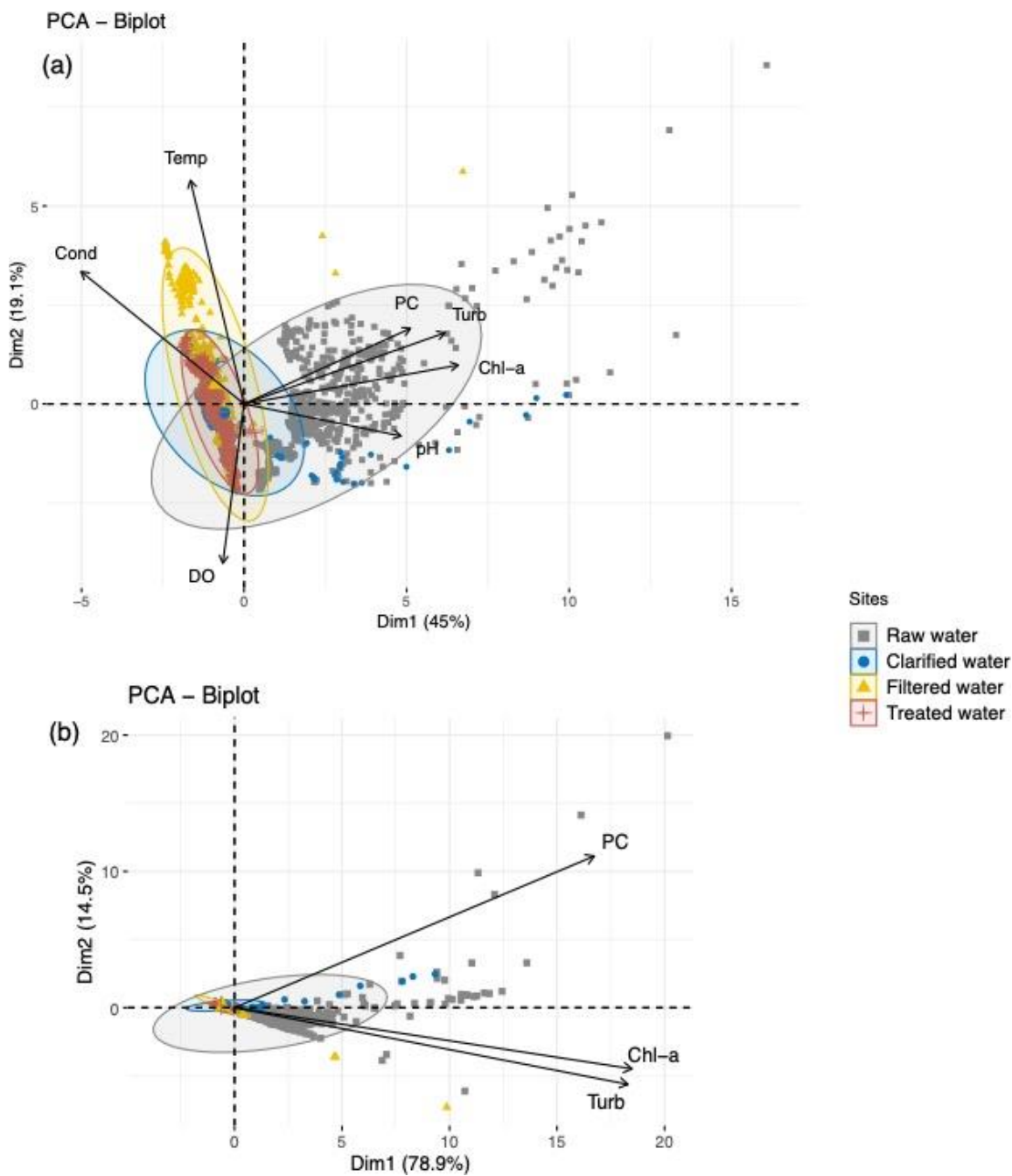


Figure 6.9 Principal component analysis (PCA) as *biplot* of the physio-chemical parameters of the raw, clarified, filtered, and treated water in DWTP A. Parameters are (a) PC = phycocyanin, Turb = turbidity, Chl-a = chlorophyll-a, DO = dissolved oxygen, Cond = conductivity, Temp = temperature and pH, (b) PC, Turb and Chl-a

6.4 Conclusion

- Treatment efficacies depend on the density of the incoming cells into the treatment processes. Higher clarification and filtration efficacies are associated with higher incoming biovolumes and are not correlated with turbidity removal efficacies.
- The treatment efficacies for removing CB can vary greatly depending on the species present. *Aphanothece clathrata brevis* was found to be least easily removed by clarification than other CB species.
- Equivalent site-specific PC RFU to the total CB biovolume is challenging to determine but can serve as a basis for using *in situ* PC probes to assess clarification and filtration removal efficacies.
- CB biovolume and PC log removal efficacies for the clarification process are well correlated. Reliance on multi-stream PC may give an instant indication of the total biovolume removal at a relatively low cost.
- The use of the proposed framework is recommended so that plant operators can monitor the CB risk breakthrough into treated water in near real-time.
- Multi-parameter measurements across the treatment processes did not show the potential for turbidity or Chl-a to serve as a proxy for CB biovolume monitoring because of clear clustering between raw and clarified/filtered/treated waters.

CHAPTER 7 GENERAL DISCUSSION

This chapter presents a global discussion on the entire results of this research project. Within this chapter, the main findings and novelties of this study will be highlighted, and the limitations of the research approach will be discussed.

7.1 Overview of the research's scope

The main objective of this research was to investigate whether the probes are reliable tools to monitor the CB level at the water intake and within the DWTPs. Figure 7.1 summarizes the research themes. The first step was to investigate the interference involved with *in situ* probe. Our laboratory study tested the influence of Suwannee River NOM and the ambient water temperature. Additionally, our field studies reveal that the correlation between probe readings and taxonomic cell counts is unreliable, and only biovolume based estimations are relevant options for CB management purposes. A simultaneous measurement of the other physical-chemical parameters by the probe may help to increase the accuracy of CB PC readings. Once the reliability of *in situ* PC probe for monitoring CB was established, we applied *in situ* PC probes to monitor the CB in raw, clarified, filtered, treated water, and the supernatant of the sludge holding tank in three full-scale DWTPs. We investigated the application and interpretation of *in situ* probes to monitor the CB risk in the DWTPs. The last part of the work focused on management. Our results on the variability of daily and hourly fluctuations of PC readings in the high CB risk intake water indicate that the sampling strategy used for monitoring of CB is a critical aspect, questioning the validity of routine grab sampling and its indication of the CB loading entering the DWTP. Also, despite the fact that the authorities have issued guidelines, and frameworks in response to the CB density, such regulations only focus on the risk at the water intake. In-plant risk is not covered by the current regulations. Moreover, converting and presenting the *in situ* PC readings into total CB biovolume which is better known to the regulators and scientific communities is still a challenge. Our findings are very important for policymakers in setting guidelines for effective CB management in water intake and with-in DWTPs.

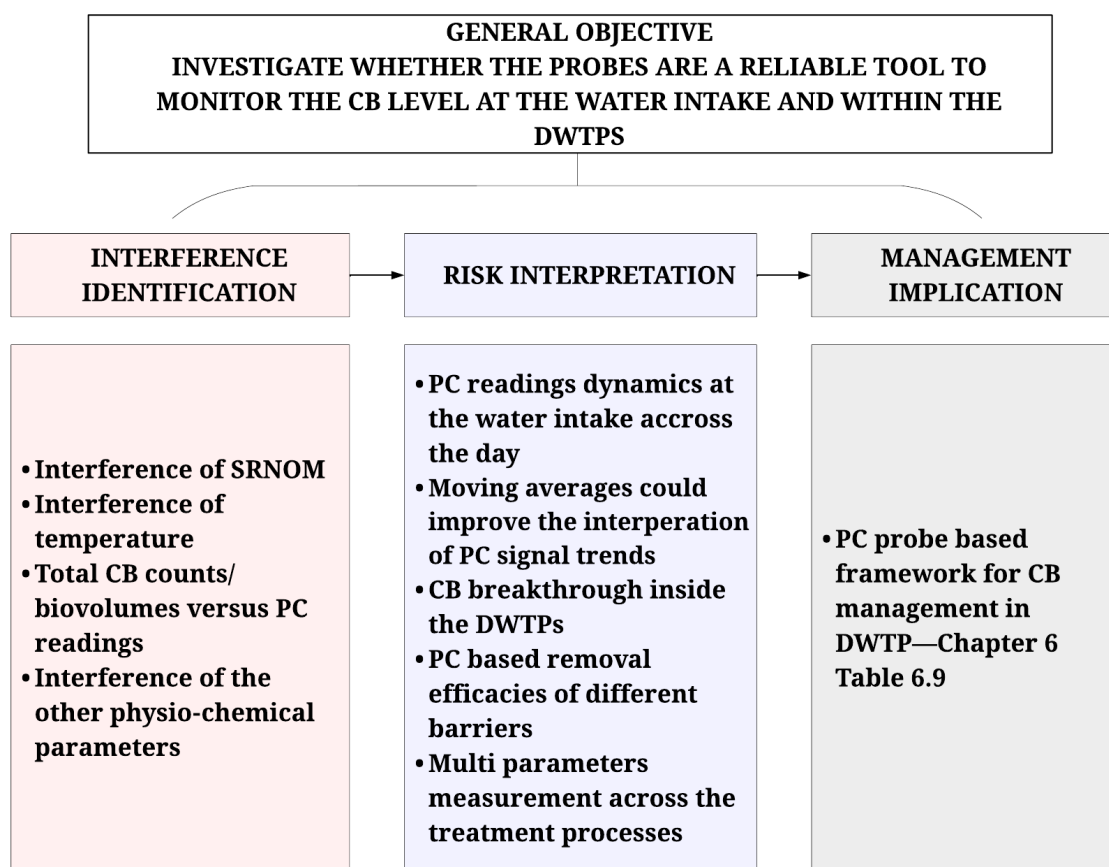


Figure 7.1 Summary of the research themes

7.2 The design and operation of multi-flow remote control data collection system

The first step of my research was to build a probe system (Figure 7.2), as no existing probe systems were available for the measurement of CB across multiple sites in DWTPs. Therefore, in collaboration with a contractor, we designed and constructed a novel probe system. Figure 7.2a shows the telemetry panel, consisting of a data logger, a modem, and switches used for controlling the electronic solenoids. Figure 7.2b presents the multi-flow probe panel, which enabled sequential measurements of raw, clarified, filtered, and treated water, regulated by the solenoids. Probe data were continuously recorded, transmitted via the modem, and downloaded remotely.

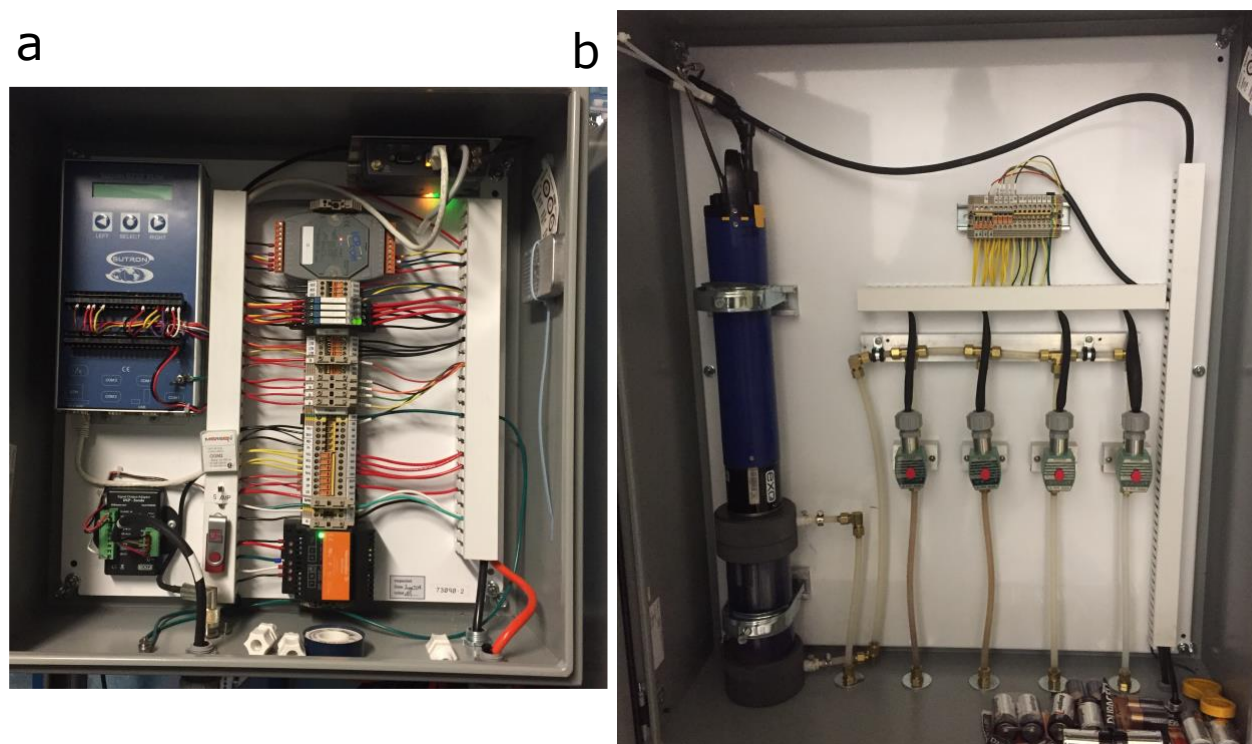


Figure 7.2 YSI EXO2 PC probe remote control and data collection system

This probe system provided reliable and good quality real-time data on CB biomass occurrence and also provided other physio-chemical characteristics in multiple sites inside the three DWTPs. Despite the success of providing sufficient data from four sites within each studied DWTP, two important questions are highlighted concerning (1) the design of the probe system, and (2) the timing of sampling to match hydraulic retention times in each treatment process.

During September and October of 2018, the solenoid controlling the flow of clarified water at Bedford DWTP malfunctioned, causing clarified water to continuously flow through the probe flow cell. Consequently, all other water types were diluted with clarified water. As a result, we had to exclude the probe data for September and October in Bedford DWTPs from our analysis. The malfunctioning of the solenoid may result from the presence of PAC in the clarified water, which obstructed the solenoid. Therefore, we recommend that future studies implement a filter to the solenoid to eliminate sediment and debris from the water, thereby preventing such issues from occurring.

Secondly, in the system, raw, clarified, filtered, and treated water went through the probe flow cell sequentially in the step of 30 minutes in each of the studied DWTP. PC readings could not be

matched to match the actual hydraulic retention times throughout the plant. However, measurements performed every 2 hours for 30 minutes provide a very solid foundation for time series for treatment performance evaluation.

7.3 Probe precision and interference

This research's results, together with insights gathered from the available literature, were investigated to discuss factors affecting PC probes' accuracy. Our study focused on NOM and water temperature. With regards to NOM, we added known concentrations of SRNOM to measure the probe response. SRNOM is a classic aromatic NOM, and has well defined spectra for excitation and emission (Yoo et al., 2016). We assessed two most commonly observed species—*Microcystis aeruginosa* and *Dolichospermum* sp.—in both ultrapure water and lake water. Lake water was from Missisquoi Bay with DOC of 6.3 mg/L, PC of 0.2 RFU. The results showed that high SRNOM content in the water could lead to an underestimation of CB PC readings. The fluorescence EEM confirmed the decreased cell fluorescence intensity caused by the addition of SRNOM. With regards to temperature, we found both cell-bound and dissolved PC readings can be significantly affected by temperature in the range of their typical application (6–33 °C), and such interference could be reduced by applying the correction algorithms. Additionally, field samples from a water intake of a DWTP were also evaluated. The correlation between probe readings and taxonomic cell counts from the field samples was found to be unreliable, and only biovolume-based estimations are a relevant option for management purposes. Our study also assessed the need to measure other physio-chemical parameters, such as Chl-a, turbidity, pH, simultaneously generated by the probe to improve the interpretation.

Our study also found a significant relationship between the PC readings and natural light intensities. This may attribute to the suppression to non-photochemical quenching (Misumi et al., 2016; Rouso et al., 2021), or attributed to the CB biomass growth during the light period. Future work may incorporate genomic analysis to have a better understanding of the behaviour of the cells under different light intensities.

Although researchers have shown the factors, for instance, water temperature, pH, and wind (Gao et al., 2018; Huisman et al., 2018; Newcombe et al., 2010; Rome et al., 2021; Zhao et al., 2014) on the formation of CB, the current study also demonstrates that the factors influencing the PC probe readings must be considered simultaneously. Applying the correction algorithms can yield high

success rates to decrease the interference. Also, it is important to highlight that based on the results of this study and the literature, the interference may vary among commercially available probes. The correction algorithms may also differ depending on the probe's manufacturer and devices.

7.4 Risk interpretation of *in situ* probe monitoring

Our results on the variability of daily and hourly fluctuations of PC readings in the high CB risk intake water indicate that the sampling strategy used for monitoring of CB is a critical aspect. This is very important as infrequent grab sampling at the source water is deployed in most field monitoring programs, which can potentially result in missing the bloom events. Hydrodynamic modelling might be helpful to discover the cyclical pattern of the CB bloom at a certain water body (Ndong et al., 2017). Also, without the input of a dynamic measurement of incoming cyanobacteria, one event of peak CB loading was not identified leading to the breakthrough of CB cells and toxins as the operators failed to timely implement treatment responses.

To compensate for this highly fluctuated PC reading, we processed the readings by moving averages. A 2-hour moving average of the PC probe readings was proposed to improve the interpretation of PC signal trends and guide treatment response, avoiding responding to peaks of a very short duration. Daily mean PC values can be used to estimate total and process specific log removals to justify treatment adjustments.

PC dynamics were affected by environmental parameters. We examined cross-correlation between the PC readings and the meteorological parameters (Figure D.1). The results show that precipitation was more strongly related with PC readings than to other meteorological parameters, displaying a high coefficient $r = 0.63$, with 0 day time lag. Light intensity and air temperature also revealed high coefficient values with $r = 0.51$ and $r = 0.49$, respectively. Wind speed and humidity showed negative relationships with PC readings with $r = -0.45$ and $r = -0.36$, respectively. In particular, correlation with air temperature had a time lag of 3 days, while humidity had time lag of 2 days. Our results suggests that PC dynamics could be predicted by the meteorological parameters. More comprehensive models show the possibility to predict the occurrence of CB blooms (reviewed by (Echard, 2021)).

The data we report were collected at a single station in the lake and therefore may not be indicative of the spatial variability of CB in all surface waters. Prediction models are often produced for a single lake or reservoir and are not easily comparable to other models for different systems (Echard,

2021), suggesting the spatial variability is large. We cannot determine the contribution of spatial variation that fit to our time series. Better integration of spatial dynamics with long-term variability is an important need for future studies of CB.

7.5 Selective removal of cyanobacterial species by treatment

CB species removal displayed considerable variability in the three studied DWTPs. Our results showed that *Aphanothece clathrata brevis* was also among the least removed species by both coagulation and filtration in all three studied DWTPs. Other studies also found *Aphanothece* was more difficult to be removed by clarification as compared to other CB genera (for instance, *Microcystis* and *Dolichospermum*) (Jalili et al., 2021; Nguyen et al., 2021; Sorlini et al., 2013; Zamyadi et al., 2013a; Zamyadi et al., 2012a). Several reasons may contribute to the lower removal of *Aphanothece*. Firstly, algal organic matter (AOM) has been considered to promote or inhibit the flocculation and coagulation of CB cells in various studies (Cai et al., 2021; Chen et al., 2009; Cheng et al., 2003; Hanumanth Rao et al., 2018; Takaara et al., 2007). The inconsistent results might be attributable to the variable concentration and characteristics of the AOM (Li et al., 2012; Pivokonsky et al., 2014). Henderson et al. (2008b) reported that AOM extracted from *Microcystis aeruginosa* was dominated by hydrophilic polysaccharides and hydrophobic proteins of low specific UV absorbance and negative zeta potentials. The hydrophobicity of AOM was attributable to the hydrophobic proteins of molecular mass greater than 500 kDa. Additionally, the charge density for the AOM, being attributable to hydrophilic and acidic carbohydrates and not hydrophobic humic acids, decreases inversely with hydrophobicity. On the other hand, very little information is available on the AOM character of the CB species *Aphanothece clathrata brevis*. One study reported that the released polysaccharide of the *Aphanothece halophytica* disturbed the flocculation of the cells with ferric chloride, by forming complexes with ferrum (Chen et al., 2009). Secondly, CB cell size affects its removal efficacy. The smaller the size of the algae species, the lower the removal efficacy (Ma et al., 2007). *Aphanothece clathrata brevis* has a small biovolume of $0.5 \mu\text{m}^3/\text{cell}$ and was mostly found to be individual cells. Its size contrasts sharply with the size of the other well-removed species, for instance, $179.6 \mu\text{m}^3/\text{cell}$ for *Dolichospermum spiroides* and $33.5 \mu\text{m}^3/\text{cell}$ for *Microcystis aeruginosa*.

7.6 Management implications

This research project provided a PC probe-based management decision framework to assist in (1) source water risk assessment, (2) short-term CB treatment breakthrough, and (3) treatment risk evaluation. The overall goal is to facilitate informed decisions based on PC readings regarding the needs. The corresponding thresholds of the metrics and actions are proposed. PC probe-based framework enables real-time treatment optimization at full-scale DWTP. No existing manuals or frameworks were available to suit such requirement.

To be able to use probes for management purposes in the drinking water treatment for the plant operator. The first step is establishing a site-specific RFU equivalent to the WHO biovolume alerts. The identification of the diversity of bloom-forming species in the source water is very important for this purpose. As for treatment evaluation, removals should be compared with the historical CB removal in the DWTP. Then, risk should be validated with the PC readings of the filtered water.

The objective of the framework is to prevent the passage of CB and their associated toxins into treated water. When considering the dynamic nature of CB loadings entering a water treatment plant, operators should be concerned about: (1) adjusting treatment processes to elevated CB concentrations to prevent breakthrough, (2) accumulation of the CB in the separation processes (clarifier, filtration, and sludge storage); (3) recycling of CB through sludge holding tank supernatant or backwash recycled flows.

7.7 The novelty of the research

The novelty of this work lies in the following aspects:

- The investigation of the impact of SRNOM and water temperature on the most frequent field applied PC probe (YSI EXO2).
- The development and the application of a probe remote control and data logging system, which allows for the multi streams measurements across the DWTP.
- The longitudinal evaluation of the CB cells and their associated toxins across various treatment processes at different sites in both high and low-risk DWTPs.
- The development of a PC probe-based framework for CB management in DWTP.

CHAPTER 8 CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

The following conclusions were drawn along the following themes:

(A) Interference affecting PC RFU readings:

- The presence of SRNOM may lead to an underestimation of the PC probe readings.
- Temperature can significantly impact the PC probe readings. Applying correction algorithms can effectively reduce such interference.
- Biovolume-based estimation is a relevant option for management purposes unlike cell counts.
- Simultaneous measurement of the physical-chemical parameters may improve the accuracy of PC readings.

(B) CB risk interpretation at the source water:

- The daily and hourly fluctuations of PC readings in the high CB risk intake water indicates that the sampling strategy which is used for monitoring of CB is critical, questioning the validity of routine grab sampling and its indication of the CB loading entering the DWTP.
- A 2-hour moving average of the PC probe readings was proposed to improve the interpretation of PC signal trends, guide treatment response, and avoid responses to peaks in very short duration.

(C) CB risk interpretation within the DWTPs:

- The *in situ* probe successfully detected high cyanobacterial biomass entering the clarification process and in the sludge supernatant of the high-risk DWTP.
- CB biomass can accumulate in the sludge of DWTPs, which were not associated with elevated MCs.
- CB treatment efficacies depend on the density of incoming cells. Higher incoming biomass leads to higher clarification and filtration efficacies, but this is not correlated with turbidity removal efficacies.
- CB removal efficacy varies significantly depending on the present species. *Aphanothece clathrata brevis* is hardly removed by clarification compared to other CB species.

- A significant relationship could be found between the CB log removal evaluated by biovolume and by PC probe. The use of multi-stream PC probe can give an instant indication of the removed total biovolume at a relatively low cost.

(D) PC probe-based management within DWTP:

- Establishing a site-specific PC RFU equivalent to the WHO biovolume alert levels is challenging, but can serve as a basis to use the probe for management purposes.
- Existing CB threshold is limited to raw water and does not account for the potential risk of CB breakthrough within the treatment plant.
- It is recommended to use the proposed PC probe-based framework to assist plant operators to minimize the risk of CB breakthrough into drinking water in near real-time. So the plant operators could react proactively.

8.2 Recommendations

Based on our findings, recommendations for further investigation are:

- The limitation of the PC probe's analytical sensitivity presents a constraint, especially in measuring filtered and treated water. Improving the probe's sensitivity could make them eligible for WHO lower alert level.
- Site specific relationships between biovolume and PC fluorescence probe reading should be evaluated by composite grab sampling.
- Validation of probe readings with taxonomic cell counts in response to high RFU readings should be done using compositive samples rather than grab samples.
- Devise and test algorithms to include the proposed metrics (correction factor for temperature, 2h and daily moving average PC readings) in the plant operation dashboard.
- Assessing whether coagulant, PAC, GAC, or other treatment processes could interfere with PC probe readings.
- Evaluating whether the presence of the AOM could interfere with PC probe readings.

REFERENCES

- Ahern, K. S., Ahern, C. R., & Udy, J. W. (2007). Nutrient additions generate prolific growth of *Lyngbya majuscula* (cyanobacteria) in field and bioassay experiments. *Harmful Algae*, 6(1), 134-151. doi:<https://doi.org/10.1016/j.hal.2006.08.004>
- Ahn, C.-Y., Joung, S.-H., Yoon, S.-K., & Oh, H.-M. (2007). Alternative alert system for cyanobacterial bloom, using phycocyanin as a level determinant. *The Journal of Microbiology*, 45(2), 98-104. Retrieved from http://cea.altiplano.uvg.edu.gt/445/1/Ahn_Alert_system_cyanobacterial_bloom.pdf
- Akyol, Ç., Ozbayram, E. G., Accoroni, S., Radini, S., Eusebi, A. L., Gorbi, S., . . . Fatone, F. (2021). Monitoring of cyanobacterial blooms and assessing polymer-enhanced microfiltration and ultrafiltration for microcystin removal in an Italian drinking water treatment plant. *Environmental Pollution*, 286, 117535. doi:<https://doi.org/10.1016/j.envpol.2021.117535>
- Al-Sammak, M. A., Hoagland, K. D., Cassada, D., & Snow, D. D. (2014). Co-occurrence of the cyanotoxins BMAA, DABA and anatoxin-a in Nebraska reservoirs, fish, and aquatic plants. *Toxins (Basel)*, 6(2), 488-508. doi:10.3390/toxins6020488
- Almuhtaram, H., Cui, Y., Zamyadi, A., & Hofmann, R. (2018). Cyanotoxins and cyanobacteria cell accumulations in drinking water treatment plants with a low risk of bloom formation at the source. *Toxins*, 10, 430. doi:10.3390/toxins10110430
- Almuhtaram, H., Kibuye, F. A., Ajjampur, S., Glover, C. M., Hofmann, R., Gaget, V., . . . Zamyadi, A. (2021). State of knowledge on early warning tools for cyanobacteria detection. *Ecological Indicators*, 133, 108442. doi:<https://doi.org/10.1016/j.ecolind.2021.108442>
- America Water Works Association (AWWA). (2010). *Algae Source to treatment. Manual of water supply practices - M57, first edition*. Retrieved from <https://www.awwa.org/store/productdetail.aspx?productid=39312113>
- Aparicio Medrano, E., Uittenbogaard, R. E., van de Wiel, B. J. H., Dionisio Pires, L. M., & Clercx, H. J. H. (2016). An alternative explanation for cyanobacterial scum formation and persistence by oxygenic photosynthesis. *Harmful Algae*, 60, 27-35. doi:<https://doi.org/10.1016/j.hal.2016.10.002>
- AquaRead. (2021). *Instruction Manual for the Aquaprobe AP-700, AP-800 & AP-2000 Multiparameter Water Quality Probe* Retrieved from Bridge House, Northdown Industrial Park, Broadstairs, Kent, England: <https://www.aquaread.com/downloads/manuals/10401-00870-rev-u-ap-700-ap-800-ap-2000-instruction-manual.pdf>
- Armbruster, D. A., & Pry, T. (2008). Limit of blank, limit of detection and limit of quantitation. *The Clinical biochemist. Reviews*, 29 Suppl 1(Suppl 1), S49-S52. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/18852857>
- Asai, R., Horiguchi, Y., Yoshida, A., McNiven, S., Tahira, P., Ikebukuro, K., . . . Karube, I. (2001). Detection of phycobilin pigments and their seasonal change in lake Kasumigaura using a sensitive in situ fluorometric sensor. *Analytical Letters*, 34(14), 2521-2533. Retrieved from <http://www.informaworld.com/10.1081/AL-100107533>

- Bastien, C., Cardin, R., Veilleux, E., Deblois, C., Warren, A., & Laurion, I. (2011). Performance evaluation of phycocyanin probes for the monitoring of cyanobacteria. *Journal of Environmental Monitoring*, 13(1), 110-118. Retrieved from <http://pubs.rsc.org/en/content/articlepdf/2011/em/c0em00366b>
- Beaulieu, M., Pick, F., & Gregory-Eaves, I. (2013). Nutrients and water temperature are significant predictors of cyanobacterial biomass in a 1147 lakes data set. *Limnology and Oceanography*, 58(5), 1736-1746. doi:10.4319/lo.2013.58.5.1736
- Bennett, L. (2017). *Algae, cyanobacteria blooms, and climate change*. Retrieved from Washington, DC, USA: <http://climate.org/algae-cyanobacteria-blooms-and-climate-change/>
- Berry, M. A., Davis, T. W., Cory, R. M., Duhaime, M. B., Johengen, T. H., Kling, G. W., . . . Denef, V. J. (2017). Cyanobacterial harmful algal blooms are a biological disturbance to Western Lake Erie bacterial communities. *Environmental Microbiology*, 19(3), 1149-1162. doi:10.1111/1462-2920.13640
- Bertani, I., Steger, C. E., Obenour, D. R., Fahnenstiel, G. L., Bridgeman, T. B., Johengen, T. H., . . . Scavia, D. (2017). Tracking cyanobacteria blooms: Do different monitoring approaches tell the same story? *Science of The Total Environment*, 575, 294-308. doi:<https://doi.org/10.1016/j.scitotenv.2016.10.023>
- Bertone, E., Burford, M. A., & Hamilton, D. P. (2018). Fluorescence probes for real-time remote cyanobacteria monitoring: A review of challenges and opportunities. *Water Research*, 141, 152-162. doi:10.1016/j.watres.2018.05.001
- Bertone, E., Chuang, A., Burford, M. A., & Hamilton, D. P. (2019). In-situ fluorescence monitoring of cyanobacteria: Laboratory-based quantification of species-specific measurement accuracy. *Harmful Algae*, 87, 101625. doi:10.1016/j.hal.2019.101625
- Beutler, M., Wiltshire, K. H., Arp, M., Kruse, J., Reineke, C., Moldaenke, C., & Hansen, U. P. (2003). A reduced model of the fluorescence from the cyanobacterial photosynthetic apparatus designed for the in situ detection of cyanobacteria. *Biochimica and Biophysica Acta*, 1604(1), 33-46. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0005272803000227>
- Bharti, R., & Grimm, D. G. (2021). Current challenges and best-practice protocols for microbiome analysis. *Brief Bioinform*, 22(1), 178-193. doi:10.1093/bib/bbz155
- Binding, C. E., Greenberg, T. A., McCullough, G., Watson, S. B., & Page, E. (2018). An analysis of satellite-derived chlorophyll and algal bloom indices on Lake Winnipeg. *Journal of Great Lakes Research*, 44(3), 436-446. doi:<https://doi.org/10.1016/j.jglr.2018.04.001>
- Bishop, S. L., Kerkovius, J. K., Menard, F., & Murch, S. J. (2018). N-β-methylamino-l-alanine and its naturally occurring isomers in cyanobacterial blooms in Lake Winnipeg. *Neurotox Research*, 33(1), 133-142. doi:10.1007/s12640-017-9820-z
- Bowling, L. C., Blais, S., & Sinotte, M. (2015). Heterogeneous spatial and temporal cyanobacterial distributions in Missisquoi Bay, Lake Champlain: An analysis of a 9 year data set. *Journal of Great Lakes Research*, 41(0), 164-179. doi:10.1016/j.jglr.2014.12.012
- Bowling, L. C., Merrick, C., Swann, J., Green, D., Smith, G., & Neilan, B. A. (2013). Effects of hydrology and river management on the distribution, abundance and persistence of

- cyanobacterial blooms in the Murray River, Australia. *Harmful Algae*, 30, 27-36. doi:10.1016/j.hal.2013.08.002
- Bowling, L. C., Zamyadi, A., & Henderson, R. K. (2016). Assessment of in situ fluorometry to measure cyanobacterial presence in water bodies with diverse cyanobacterial populations. *Water Research*, 105, 22-33. doi:10.1016/j.watres.2016.08.051
- Brient, L., Lengronne, M., Bertrand, E., Rolland, D., Sipel, A., Steinmann, D., . . . Bormans, M. (2008). A phycocyanin probe as a tool for monitoring cyanobacteria in freshwater bodies. *Journal of Environmental Monitoring*, 10(2), 248-255. Retrieved from <http://www.rsc.org/ej/EM/2008/b714238b.pdf>
- Bryant, D. A. (1982). Phycoerythrocyanin and phycoerythrin: Properties and occurrence in cyanobacteria. *Microbiology*, 128(4), 835-844. Retrieved from <https://mic.microbiologyresearch.org/content/journal/micro/10.1099/00221287-128-4-835>
- Bryant, D. A. (1986). The cyanobacterial photosynthetic apparatus: comparisn to those of higher plants and photosynthetic bacteria. *Canadian Bulletin of Fisheries and Aquatic Sciences*, 214, 423-500.
- Bunting, L., Leavitt, P. R., Simpson, G. L., Wissel, B., Laird, K. R., Cumming, B. F., . . . Engstrom, D. R. (2016). Increased variability and sudden ecosystem state change in Lake Winnipeg, Canada, caused by 20th century agriculture. *Limnology and Oceanography*, 61(6), 2090-2107. doi:<https://doi.org/10.1002/lno.10355>
- Buratti, F. M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., & Funari, E. (2017). Cyanotoxins: Producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Archives of Toxicology*, 91(3), 1049-1130. doi:10.1007/s00204-016-1913-6
- Bushon, R. N., Brady, A. M., Likirdopulos, C. A., & Cireddu, J. V. (2009). Rapid detection of *Escherichia coli* and enterococci in recreational water using an immunomagnetic separation/adenosine triphosphate technique. *Journal of Applied Microbiology*, 106(2), 432-441. doi:10.1111/j.1365-2672.2008.04011.x
- Cai, Q., Song, K., Tian, C., Wu, X., Li, Y., Huang, Y., . . . Xiao, B. (2021). Harvesting of Microcystis from waterbody by flocculation and filtration: the essential role of extracellular organic matters. *Journal of Water Process Engineering*, 41, 102053. doi:<https://doi.org/10.1016/j.jwpe.2021.102053>
- Carey, C. C., Ibelings, B. W., Hoffmann, E. P., Hamilton, D. P., & Brookes, J. D. (2012). Ecophysiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Research*, 46(5), 1394-1407. doi:10.1016/j.watres.2011.12.016
- Carmichael, W. W., & Boyer, G. L. (2016). Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. *Harmful Algae*, 54, 194-212. doi:10.1016/j.hal.2016.02.002
- Carr, N. G., & Whitton, B. A. (1982). *The biology of cyanobacteria*: University of California Press.
- Catherine, Q., Susanna, W., Isidora, E.-S., Mark, H., Aurélie, V., & Jean-François, H. (2013). A review of current knowledge on toxic benthic freshwater cyanobacteria – Ecology, toxin

- production and risk management. *Water Research*, 47(15), 5464-5479. doi:<https://doi.org/10.1016/j.watres.2013.06.042>
- Chaffin, J. D., Mishra, S., Kane, D. D., Bade, D. L., Stanislawczyk, K., Slodysko, K. N., . . . Fox, E. L. (2019). Cyanobacterial blooms in the central basin of Lake Erie: Potentials for cyanotoxins and environmental drivers. *Journal of Great Lakes Research*, 45(2), 277-289. doi:10.1016/j.jglr.2018.12.006
- Chang, D.-W., Hobson, P., Burch, M., & Lin, T.-F. (2012). Measurement of cyanobacteria using in-vivo fluoroscopy - Effect of cyanobacterial species, pigments, and colonies. *Water Research*, 46(16), 5037-5048. doi:10.1016/j.watres.2012.06.050
- Chen, L., Li, P., Liu, Z., & Jiao, Q. (2009). The released polysaccharide of the cyanobacterium *Aphanothece halophytica* inhibits flocculation of the alga with ferric chloride. *Journal of Applied Phycology*, 21(3), 327-331. doi:10.1007/s10811-008-9371-z
- Cheng, W. P., & Chi, F.-H. (2003). Influence of eutrophication on the coagulation efficiency in reservoir water. *Chemosphere*, 53(7), 773-778. Retrieved from http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6V74-49BS7VK-3-G&_cdi=5832&_user=2101137&_pii=S0045653503005101&_origin=browse&_zone=rslt_list_item&_coverDate=11%2F30%2F2003&_sk=999469992&wchp=dGLbVlz-zSkWA&md5=45c226d432d519f13b94c3fb77a1b318&ie=/sdarticle.pdf
- Chernoff, N., Hill, D. J., Diggs, D. L., Faison, B. D., Francis, B. M., Lang, J. R., . . . Lugo, J. N. (2017). A critical review of the postulated role of the non-essential amino acid, β -N-methylamino-L-alanine, in neurodegenerative disease in humans. *Journal of Toxicology and Environmental Health, Part B*, 20(4), 183-229. doi:10.1080/10937404.2017.1297592
- Chiu, Y. T., Chen, Y. H., Wang, T. S., Yen, H. K., & Lin, T. F. (2017). A qPCR-based tool to diagnose the presence of harmful cyanobacteria and cyanotoxins in drinking water sources. *International Journal of Environmental Research and Public Health*, 14(5). doi:10.3390/ijerph14050547
- Choo, F., Zamyadi, A., Newton, K., Newcombe, G., Bowling, L., Stuetzb, R., & Henderson, R. K. (2018). Performance evaluation of in situ fluorometers for real-time cyanobacterial monitoring. *H2 Open Journal*, 1(1), 26-46. doi:10.2166/h2oj.2018.009
- Choo, F., Zamyadi, A., Stuetz, R. M., Newcombe, G., Newton, K., & Henderson, R. K. (2019). Enhanced real-time cyanobacterial fluorescence monitoring through chlorophyll-a interference compensation corrections. *Water Research*, 148, 86-96. doi:10.1016/j.watres.2018.10.034
- Chorus, I., & Bartram, J. (1999). *Toxic cyanobacteria in water : A guide to their public health consequences, monitoring, and management*. London; New York: WHO.
- Chorus, I., Fastner, J., & Welker, M. (2021a). Cyanobacteria and Cyanotoxins in a Changing Environment: Concepts, Controversies, Challenges. *Water*, 13(18). doi:10.3390/w13182463
- Chorus, I., & Welker, M. (2021b). *Toxic cyanobacteria in water : A guide to their public health consequences, monitoring and management*: Taylor & Francis.

- Christiansen, G., Molitor, C., Philmus, B., & Kurmayer, R. (2008). Nontoxic strains of cyanobacteria are the result of major gene deletion events induced by a transposable element. *Molecular Biology and Evolution*, 25(8), 1695-1704. doi:10.1093/molbev/msn120
- Cirés, S., & Ballot, A. (2016). A review of the phylogeny, ecology and toxin production of bloom-forming *Aphanizomenon* spp. and related species within the Nostocales (cyanobacteria). *Harmful Algae*, 54, 21-43. doi:10.1016/j.hal.2015.09.007
- Cohen, A., Sendersky, E., Carmeli, S., & Schwarz, R. (2014). Collapsing aged culture of the cyanobacterium *Synechococcus elongatus* produces compound (s) toxic to photosynthetic organisms. *PloS one*, 9(6), e100747. doi:10.1371/journal.pone.0100747
- Cotterill, V., Hamilton, D. P., Puddick, J., Suren, A., & Wood, S. A. (2019). Phycocyanin sensors as an early warning system for cyanobacteria blooms concentrations: a case study in the Rotorua lakes. *New Zealand Journal of Marine and Freshwater Research*, 53(4), 555-570. doi:10.1080/00288330.2019.1617322
- Courtois, S., Steinmann, D., Cajon, A., & van der Linden, L. (2017). Continuous monitoring of cyanobacterial blooms: benefits and conditions for using fluorescence probes. *Revue des sciences de l'eau / Journal of Water Science*, 30(2), 149-155. doi:10.7202/1042923ar
- De Oliveira, F. G., Bertone, E., Stewart, A. R., Awad, J., Holland, A., O'Halloran, K., & Bird, S. (2018). Multi-parameter compensation method for accurate in situ fluorescent dissolved organic matter monitoring and properties characterization. *Water*, 10(9). doi:10.3390/w10091146
- Dervaux, J., Mejean, A., & Brunet, P. (2015). Irreversible Collective Migration of Cyanobacteria in Eutrophic Conditions. *PloS one*, 10(3), e0120906. doi:10.1371/journal.pone.0120906
- Donkor, V., & Häder, D.-P. (1996). Effects of ultraviolet irradiation on photosynthetic pigments in some filamentous cyanobacteria. *Aquatic Microbial Ecology*, 11, 143-149. Retrieved from <http://www.int-res.com/articles/ame/11/a011p143.pdf>
- Downing, B. D., Pellerin, B. A., Bergamaschi, B. A., Saraceno, J. F., & Kraus, T. E. C. (2012). Seeing the light: The effects of particles, dissolved materials, and temperature on in situ measurements of DOM fluorescence in rivers and streams. *Limnology and Oceanography: Methods*, 10, 767-775. doi:10.4319/lom.2012.10.767
- Dreyfus, J., Monrolin, Y., Pestana, C. J., Reeve, P. J., Sawade, E., Newton, K., . . . Newcombe, G. (2016). Identification and assessment of water quality risks associated with sludge supernatant recycling in the presence of cyanobacteria. *Journal of Water Supply: Research and Technology - Aqua*, 65(6), 441-452. doi:10.2166/aqua.2016.030
- Echard, J. S. (2021). *A Review of Harmful Algal Bloom Prediction Models for Lakes and Reservoirs*. (Master of Science (MS)). Utah State University,
- Elliott, J. A. (2012). Is the future blue-green? A review of the current model predictions of how climate change could affect pelagic freshwater cyanobacteria. *Water Research*, 46(5), 1364-1371. doi:https://doi.org/10.1016/j.watres.2011.12.018
- Ellis, D. (2009). *Guide d'intervention pour les propriétaires, les exploitants ou les concepteurs de stations de production d'eau potable municipales aux prises avec une problématique de*

fleurs d'eau de cyanobactéries. Retrieved from <http://www.mddelcc.gouv.qc.ca/eau/algues-bv/guide-mun.pdf>

- Erratt, K. J., Creed, I. F., & Trick, C. G. (2022). Harmonizing science and management options to reduce risks of cyanobacteria. *Harmful Algae*, 116, 102264. doi:<https://doi.org/10.1016/j.hal.2022.102264>
- Fayad, P. B., Roy-Lachapelle, A., Vo Duy, S., Prévost, M., & Sauvé, S. (2015). On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry for the analysis of cyanotoxins in algal blooms. *Toxicon*, 108, 167-175. doi:[10.1016/j.toxicon.2015.10.010](https://doi.org/10.1016/j.toxicon.2015.10.010)
- Fogg, G. E., Stewart, W. D. P., Fay, P., & Waslsby, A. E. (1973). *The blue-green algae*. San, Diego, California, USA: Academic Press, Inc.
- Fortin, N., Munoz-Ramos, V., Bird, D., Levesque, B., Whyte, L. G., & Greer, C. W. (2015). Toxic cyanobacterial bloom triggers in Missisquoi bay, lake Champlain, as determined by next-generation sequencing and quantitative PCR. *Life (Basel)*, 5(2), 1346-1380. doi:[10.3390/life5021346](https://doi.org/10.3390/life5021346)
- Gagné, F., Gélinas, M., Fortier, M., & Fournier, M. (2018). The effects of cyanobacterial blooms on the immune system of *Elliptio complanata* in urban and agricultural areas in the Yamaska River watershed. *Invertebrate Survival Journal*, 15(1), 39-51. Retrieved from <http://espace.inrs.ca/id/eprint/7448/>
- Gao, J., Zhu, J., Wang, M., & Dong, W. (2018). Dominance and growth factors of *pseudanabaena* sp. In drinking water source reservoirs, Southern China. *Sustainability*, 10(11). doi:[10.3390/su10113936](https://doi.org/10.3390/su10113936)
- Genzoli, L., & Kann, J. (2016). *Evaluation of phycocyanin probes as a monitoring tool for toxigenic cyanobacteria in the Klamath River below Iron Gate Dam*. Retrieved from <http://dx.doi.org/10.13140/RG.2.2.23897.31841>
- Government of Québec. (2022). *Chapter Q-2, r. 40. Regulation respecting the quality of drinking water, Environmental quality act*. Retrieved from <https://www.legisquebec.gouv.qc.ca/en/pdf/cr/Q-2,%20R.%2040.pdf>
- Govindjee, & Shevela, D. (2011). Adventures with cyanobacteria: a personal perspective. *Front Plant Sci*, 2, 28. doi:[10.3389/fpls.2011.00028](https://doi.org/10.3389/fpls.2011.00028)
- Graham, J. L., Loftin, K. A., Meyer, M. T., & Ziegler, A. C. (2010). Cyanotoxin mixtures and taste-and-odor compounds in Cyanobacterial blooms from the Midwestern United States. *Environmental Science & Technology*, 44(19), 7361-7368. doi:[10.1021/es1008938](https://doi.org/10.1021/es1008938)
- Grattan, L. M., Holobaugh, S., & Morris, J. G. (2016). Harmful algal blooms and public health. *Harmful Algae*, 57, 2-8. doi:[10.1016/j.hal.2016.05.003](https://doi.org/10.1016/j.hal.2016.05.003)
- Greenstein, K. E., & Wert, E. C. (2019). Using rapid quantification of adenosine triphosphate (ATP) as an indicator for early detection and treatment of cyanobacterial blooms. *Water Research*, 154, 171-179. doi:<https://doi.org/10.1016/j.watres.2019.02.005>
- Gregor, J., & Maršálek, B. (2004). Freshwater phytoplankton quantification by chlorophyll a: a comparative study of in vitro, in vivo and in situ methods. *Water Research*, 38(3), 517-522. doi:<https://doi.org/10.1016/j.watres.2003.10.033>

- Gregor, J., Marsalek, B., & Sipkova, H. (2007). Detection and estimation of potentially toxic cyanobacteria in raw water at the drinking water treatment plant by in vivo fluorescence method. *Water Research*, 41(1), 228-234. doi:<https://doi.org/10.1016/j.watres.2006.08.011>
- Griffith, A. W., & Gobler, C. J. (2020). Harmful algal blooms: A climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae*, 91, 101590. doi:<https://doi.org/10.1016/j.hal.2019.03.008>
- Grigoryeva, N. (2020). Studying Cyanobacteria by Means of Fluorescence Methods: A Review. In *Fluorescence Methods for Investigation of Living Cells and Microorganisms* (pp. 3-33).
- Guo, D., Robinson, C., & Herrera, J. E. (2016). Mechanism of dissolution of minium (Pb3O4) in water under depleting chlorine conditions. *Corrosion Science*, 103, 42-49. doi:[10.1016/j.corsci.2015.10.042](https://doi.org/10.1016/j.corsci.2015.10.042)
- Haas, C. N., & Trussell, R. R. (1998). Frameworks for assessing reliability of multiple, independent barriers in potable water reuse. *Water Science and Technology*, 38(6), 1-8. doi:[https://doi.org/10.1016/S0273-1223\(98\)00561-7](https://doi.org/10.1016/S0273-1223(98)00561-7)
- Hanumanth Rao, N. R., Yap, R., Whittaker, M., Stuetz, R. M., Jefferson, B., Peirson, W. L., . . . Henderson, R. K. (2018). The role of algal organic matter in the separation of algae and cyanobacteria using the novel “Posi” - Dissolved air flotation process. *Water Research*, 130, 20-30. doi:<https://doi.org/10.1016/j.watres.2017.11.049>
- Harke, M. J., Steffen, M. M., Gobler, C. J., Otten, T. G., Wilhelm, S. W., Wood, S. A., & Paerl, H. W. (2016). A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. *Harmful Algae*, 54, 4-20. doi:[10.1016/j.hal.2015.12.007](https://doi.org/10.1016/j.hal.2015.12.007)
- Hawkins, P. R., Holliday, J., Kathuria, A., & Bowling, L. (2005). Change in cyanobacterial biovolume due to preservation by Lugol's Iodine. *Harmful Algae*, 4(6), 1033-1043. doi:<https://doi.org/10.1016/j.hal.2005.03.001>
- Health Canada. (2007). *Guidelines for Canadian drinking water quality: summary table*. Retrieved from Ottawa, Ontario, Canada: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/doc_sup-appui/sum_guide-res_recom/index_e.html
- Health Canada. (2021). *Guidelines for Canadian drinking water quality: Guideline technical document. Cyanobacterial toxins*. Retrieved from Ottawa, ON, CANADA: <https://www.canada.ca/en/health-canada/services/publications/healthy-living/guidelines-canadian-drinking-water-quality-guideline-technical-document-cyanobacterial-toxins-document.html>
- Henderson, R., Parsons, S. A., & Jefferson, B. (2008a). The impact of algal properties and pre-oxidation on solid-liquid separation of algae. *Water Research*, 42(8-9), 1827-1845. Retrieved from <http://dx.doi.org/10.1016/j.watres.2007.11.039>
- Henderson, R. K., Baker, A., Parsons, S. A., & Jefferson, B. (2008b). Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. *Water Research*, 42(13), 3435-3445. Retrieved from <http://dx.doi.org/10.1016/j.watres.2007.10.032>
- Ho, L., Sawade, E., & Newcombe, G. (2012). Biological treatment options for cyanobacteria metabolite removal – A review. *Water Research*, 46(5), 1536-1548. doi:[10.1016/j.watres.2011.11.018](https://doi.org/10.1016/j.watres.2011.11.018)

- Hodges, C. M. (2016). *A validation study of phycocyanin sensors for monitoring cyanobacteria in cultures and field samples*. (Master of Science (Research) (MSc(Research)) Masters). University of Waikato, Hamilton, New Zealand. Retrieved from <https://hdl.handle.net/10289/10639>
- Hodges, C. M., Wood, S. A., Puddick, J., McBride, C. G., & Hamilton, D. P. (2018). Sensor manufacturer, temperature, and cyanobacteria morphology affect phycocyanin fluorescence measurements. *Environmental Science and Pollution Research*, 25(2), 1-10. doi:10.1007/s11356-017-0473-5
- Huang, J., Wang, J., & Xu, H. (2014). The circadian rhythms of photosynthesis, ATP content and cell division in *Microcystis aeruginosa* PCC7820. *Acta Physiologiae Plantarum*, 36(12), 3315-3323. doi:10.1007/s11738-014-1699-1
- Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., & Visser, P. M. (2018). Cyanobacterial blooms. *Nature Reviews Microbiology*, 16(8), 471-483. doi:10.1038/s41579-018-0040-1
- Izydorczyk, K., Carpentier, C., Mrowczynski, J., Wagenvoort, A., Jurczak, T., & Tarczynska, M. (2009). Establishment of an alert level framework for cyanobacteria in drinking water resources by using the algae online analyser for monitoring cyanobacterial chlorophyll a. *Water Research*, 43(4), 989-996. Retrieved from http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6V73-4V4KPP1-2&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=9cabdff66ef9b91b8038c17116c4579d
- Izydorczyk, K., Tarczynska, M., Jurczak, T., Mrowczynski, J., & Zalewski, M. (2005). Measurement of phycocyanin fluorescence as an online early warning system for cyanobacteria in reservoir intake water. *Environmental Toxicology*, 20(4), 425-430. Retrieved from <http://www3.interscience.wiley.com/cgi-bin/fulltext/110561573/PDFSTART>
- J. Alex, E. (2012). Is the future blue-green? A review of the current model predictions of how climate change could affect pelagic freshwater cyanobacteria. *Water Research*, 46(5), 1364-1371. doi:10.1016/j.watres.2011.12.018
- Jalili, F., Trigui, H., Guerra Maldonado, J. F., Dorner, S., Zamyadi, A., Shapiro, B. J., . . . Prévost, M. (2021). Can cyanobacterial diversity in the source predict the diversity in sludge and the risk of toxin release in a drinking water treatment plant? *Toxins*, 13(1), 25. Retrieved from <https://www.mdpi.com/2072-6651/13/1/25>
- Jalili, F., Trigui, H., Maldonado, J. F., Dorner, S., Zamyadi, A., Shapiro, B. J., . . . Prévost, M. (2022). Impact of Stagnation on the Diversity of Cyanobacteria in Drinking Water Treatment Plant Sludge. *Toxins*, 14(11). doi:10.3390/toxins14110749
- Jankowiak, J., Hattenrath-Lehmann, T., Kramer, B. J., Ladds, M., & Gobler, C. J. (2019). Deciphering the effects of nitrogen, phosphorus, and temperature on cyanobacterial bloom intensification, diversity, and toxicity in western Lake Erie. *Limnology and Oceanography*, 64(3), 1347-1370. doi:10.1002/lno.11120

- John, N., Koehler, A. V., Ansell, B. R. E., Baker, L., Crosbie, N. D., & Jex, A. R. (2018). An improved method for PCR-based detection and routine monitoring of geosmin-producing cyanobacterial blooms. *Water Research*, 136, 34-40. doi:10.1016/j.watres.2018.02.041
- Kasinak, J.-M. E., Holt, B. M., Chislock, M. F., & Wilson, A. E. (2015). Benchtop fluorometry of phycocyanin as a rapid approach for estimating cyanobacterial biovolume. *Journal of Plankton Research*, 37(1), 248-257. doi:10.1093/plankt/fbu096
- Kim, K. T., & Park, Y.-G. (2021). Geosmin and 2-MIB Removal by Full-Scale Drinking Water Treatment Processes in the Republic of Korea. *Water*, 13(5). doi:10.3390/w13050628
- Kong, Y., Lou, I., Zhang, Y., Lou, C. U., & Mok, K. M. (2014). Using an online phycocyanin fluorescence probe for rapid monitoring of cyanobacteria in Macau freshwater reservoir. *Hydrobiologia*, 741(1), 33-49. doi:10.1007/s10750-013-1759-3
- Korak, J. A., Wert, E. C., & Rosario-Ortiz, F. L. (2015). Evaluating fluorescence spectroscopy as a tool to characterize cyanobacteria intracellular organic matter upon simulated release and oxidation in natural water. *Water Research*, 68, 432-443.
- Kring, S., E. Figary, S., Boyer, G., Watson, S., & Twiss, M. (2014). Rapid in situ measures of phytoplankton communities using the bbe FluoroProbe: Evaluation of spectral calibration, instrument intercompatibility, and performance range. *Journal canadien des sciences halieutiques et aquatiques*, 71, 1087-1095. doi:10.1139/cjfas-2013-0599
- Le, K. T., Goitom, E., Trigui, H., Sauvé, S., Prévost, M., & Dorner, S. (2021). The Effects of Ferric Sulfate (Fe₂(SO₄)₃) on the Removal of Cyanobacteria and Cyanotoxins: A Mesocosm Experiment. *Toxins*, 13(11). doi:10.3390/toxins13110753
- Ledreux, A., Thomazeau, S., Catherine, A., Duval, C., Yepremian, C., Marie, A., & Bernard, C. (2010). Evidence for saxitoxins production by the cyanobacterium *Aphanizomenon gracile* in a French recreational water body. *Harmful Algae*, 10(1), 88-97. doi:https://doi.org/10.1016/j.hal.2010.07.004
- Lee, T., Tsuzuki, M., Takeuchi, T., Yokoyama, K., & Karube, I. (1994). In vivo fluorometric method for early detection of cyanobacterial waterblooms. *Journal of Applied Phycology*, 6(5-6), 489-495. Retrieved from <http://www.springerlink.com/content/r13k01x47302277k/fulltext.pdf>
- Li, L., Gao, N., Deng, Y., Yao, J., & Zhang, K. (2012). Characterization of intracellular & extracellular algae organic matters (AOM) of *Microcystic aeruginosa* and formation of AOM-associated disinfection byproducts and odor & taste compounds. *Water Research*, 46(4), 1233-1240. doi:10.1016/j.watres.2011.12.026
- Liu, Y.-X., Qin, Y., Chen, T., Lu, M., Qian, X., Guo, X., & Bai, Y. (2021). A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein & Cell*, 12(5), 315-330. doi:10.1007/s13238-020-00724-8
- Lou, I., Han, B., & Zhang, W. (2017). *Advances in monitoring and modelling algal blooms in freshwater reservoirs: General principles and a case study of Macau*.
- Lund, J. W. G. (1959). A simple counting chamber for Nannoplankton. *Limnology and Oceanography*, 4(1), 57-65. doi:10.4319/lo.1959.4.1.0057

- Ma, J., Lei, G., & Fang, J. (2007). Effect of algae species population structure on their removal by coagulation and filtration processes – a case study. *Journal of Water Supply: Research and Technology—AQUA*, 56(1). doi:10.2166/aqua.2007.062
- Ma, L., Guerra Maldonado, J. F., Zamyadi, A., Dorner, S., & Prévost, M. (2023). Monitoring of cyanobacterial breakthrough and accumulation by in situ phycocyanin probe system within full-scale treatment plants [Manuscript accepted for publication]. *Environmental Monitoring and Assessment*.
- Ma, L., Moradinejad, S., Guerra Maldonado, J. F., Zamyadi, A., Dorner, S., & Prévost, M. (2022). Factors Affecting the Interpretation of Online Phycocyanin Fluorescence to Manage Cyanobacteria in Drinking Water Sources. *Water*, 14(22). doi:10.3390/w14223749
- Ma, R., Fan, L., Bi, Y., & Hu, Z. (2015). Effects of light intensity and quality on phycobiliprotein accumulation in the cyanobacterium *Nostoc sphaeroides* Kützinger. *Biotechnology letters*, 37. doi:10.1007/s10529-015-1831-3
- Macário, I. P. E., Castro, B. B., Nunes, M. I. S., Antunes, S. C., Pizarro, C., Coelho, C., . . . de Figueiredo, D. R. (2015). New insights towards the establishment of phycocyanin concentration thresholds considering species-specific variability of bloom-forming cyanobacteria. *Hydrobiologia*, 757(1), 155-165. doi:10.1007/s10750-015-2248-7
- Maghsoudi, E., Fortin, N., Greer, C., Maynard, C., Page, A., Duy, S. V., . . . Dorner, S. (2016). Cyanotoxin degradation activity and mlr gene expression profiles of a *Sphingopyxis* sp. isolated from Lake Champlain, Canada. *Environmental Science: Processes & Impacts*, 18(11), 1417-1426. doi:10.1039/C6EM00001K
- Magrann, T., Howard, M. D. A., Sutula, M., Boskovic, D. S., Hayes, W., & Dunbar, S. G. (2015). Screening assessment of cyanobacteria and cyanotoxins in Southern California lentic habitats. *Environmental Management and Sustainable Development*, 4(2), 91-111. doi:10.5296/emsd.v4i2.8036
- McKindles, K. M., Zimba, P. V., Chiu, A. S., Watson, S. B., Gutierrez, D. B., Westrick, J., . . . Davis, T. W. (2019). A multiplex analysis of potentially toxic cyanobacteria in Lake Winnipeg during the 2013 bloom season. *Toxins*, 11(10), 587. doi:10.3390/toxins11100587
- McQuaid, N., Zamyadi, A., Prévost, M., Bird, D. F., & Dorner, S. (2011). Use of in vivo phycocyanin fluorescence to monitor potential microcystin-producing cyanobacterial biovolume in a drinking water source. *Journal of Environmental Monitoring*, 13(2), 455-463. doi:10.1039/C0EM00163E
- Merel, S., Walker, D., Chicana, R., Snyder, S., Baures, E., & Thomas, O. (2013). State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environment International*, 59, 303-327. doi:10.1016/j.envint.2013.06.013
- Meriluoto, J., Blaha, L., Bojadzija, G., Bormans, M., Brient, L., Codd, G. A., . . . Svirčev, Z. (2017). Toxic cyanobacteria and cyanotoxins in European waters – recent progress achieved through the CYANOCOST Action and challenges for further research. *Advances in Oceanography and Limnology*, 8(1). doi:10.4081/aiol.2017.6429
- Metcalf, J. S., Banack, S. A., Lindsay, J., Morrison, L. F., Cox, P. A., & Codd, G. A. (2008). Co-occurrence of β -N-methylamino-l-alanine, a neurotoxic amino acid with other

- cyanobacterial toxins in British waterbodies, 1990–2004. *Environmental Microbiology*, 10(3), 702-708. doi:<https://doi.org/10.1111/j.1462-2920.2007.01492.x>
- Mhlanga, L., Day, J., Cronberg, G., Chimbari, M., Siziba, N., & Annadotter, H. (2006). Cyanobacteria and cyanotoxins in the source water from Lake Chivero, Harare, Zimbabwe, and the presence of cyanotoxins in drinking water. *African Journal of Aquatic Science*, 31(2), 165-173. doi:10.2989/16085910609503888
- Miller, T. R., Beversdorf, L., Chaston, S. D., & McMahon, K. D. (2013). Spatiotemporal molecular analysis of cyanobacteria blooms reveals Microcystis--Aphanizomenon interactions. *PloS one*, 8(9), e74933-e74933. doi:10.1371/journal.pone.0074933
- Ministry of Health. (2020). Cyanobacterial compliance. In *Guidelines for drinking-water quality management for New Zealand* (pp. 59).
- Ministry of Health. (2022). *Drinking-water standards for New Zealand*. Wellington, New Zealand: Government of New Zealand Retrieved from <https://www.legislation.govt.nz/regulation/public/2022/0168/latest/whole.html>
- Misumi, M., Katoh, H., Tomo, T., & Sonoike, K. (2016). Relationship between photochemical quenching and non-photochemical quenching in six species of cyanobacteria reveals species difference in redox state and species commonality in energy dissipation. *Plant and Cell Physiology*, 57(7), 1510-1517. doi:10.1093/pcp/pcv185
- Mohamed, Z. (2016). Breakthrough of *Oscillatoria limnetica* and microcystin toxins into drinking water treatment plants – examples from the Nile River, Egypt. *Water SA*, 42(1), 161. doi:10.4314/wsa.v42i1.16
- Molica, R. J. R., Oliveira, E. J. A., Carvalho, P. V. V. C., Costa, A. N. S. F., Cunha, M. C. C., Melo, G. L., & Azevedo, S. M. F. O. (2005). Occurrence of saxitoxins and an anatoxin-a(s)-like anticholinesterase in a Brazilian drinking water supply. *Harmful Algae*, 4(4), 743-753. doi:10.1016/j.hal.2004.11.001
- Moradinejad, S., Glover, C., Mailly, J., Zadfatollah-Seighalani, T., Peldszus, S., Barbeau, B., . . . Zamyadi, A. (2019). Using advanced spectroscopy and organic matter characterization to evaluate the impact of oxidation on cyanobacteria. *Toxins*, 11(5). doi:10.3390/toxins11050278
- Moradinejad, S., Trigui, H., Guerra Maldonado, J. F., Shapiro, J., Terrat, Y., Zamyadi, A., . . . Prévost, M. (2020). Diversity assessment of toxic cyanobacterial blooms during oxidation. *Journal Toxins*, 12(11). doi:10.3390/toxins12110728
- Moradinejad, S., Trigui, H., Maldonado, J. F. G., Shapiro, B. J., Terrat, Y., Sauvé, S., . . . Prévost, M. (2021). Metagenomic study to evaluate functional capacity of a cyanobacterial bloom during oxidation. *Chemical Engineering Journal Advances*, 8, 100151. doi:10.1016/j.cej.2021.100151
- Moreira, C., Gomes, C., Vasconcelos, V., & Antunes, A. (2020). Cyanotoxins Occurrence in Portugal: A New Report on Their Recent Multiplication. *Toxins*, 12(3). doi:10.3390/toxins12030154

- Mulders, K. J. M., Lamers, P. P., Martens, D. E., & Wijffels, R. H. (2014). Phototrophic pigment production with microalgae: Biological constraints and opportunities. *Journal of phycology*, 50(2), 229-242. doi:10.1111/jpy.12173
- Munoz, G., Vo Duy, S., Roy-Lachapelle, A., Husk, B., & Sauve, S. (2017). Analysis of individual and total microcystins in surface water by on-line preconcentration and desalting coupled to liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*, 1516, 9-20. doi:10.1016/j.chroma.2017.07.096
- Ndong, M., Bird, D., Nguyen Quang, T., Kahawita, R., Hamilton, D., de Boutray, M. L., . . . Dorner, S. (2017). A novel Eulerian approach for modelling cyanobacteria movement: Thin layer formation and recurrent risk to drinking water intakes. *Water Research*, 127, 191-203. doi:10.1016/j.watres.2017.10.021
- Ndong, M., Bird, D., Nguyen-Quang, T., de Boutray, M. L., Zamyadi, A., Vincon-Leite, B., . . . Dorner, S. (2014). Estimating the risk of cyanobacterial occurrence using an index integrating meteorological factors: Application to drinking water production. *Water Research*, 56, 98-108. doi:10.1016/j.watres.2014.02.023
- Newcombe, G. (2009). *International guidance manual for the management of toxic cyanobacteria*. Retrieved from London, United Kingdom:
- Newcombe, G., House, J., Ho, L., Baker, P., & Burch, M. (2010). *Management strategies for cyanobacteria (blue-green algae): a guide for water utilities*. Retrieved from Adelaide, South Australia:
- Nguyen, K. T., Goitom, E., Trigui, H., Sauvé, S., Prévost, M., & Dorner, S. (2021). The Effects of Ferric Sulfate ($\text{Fe}_2(\text{SO}_4)_3$) on the Removal of Cyanobacteria and Cyanotoxins: A Mesocosm Experiment. *Toxins*, 1-17. doi:10.3390/toxins13110753
- NHMRC. (2022). Australian drinking water guidelines 6. National Water Quality Management Strategy. In (Vol. Version 3.8 Updated September 2022). Canberra: Commonwealth of Australia.
- NOAA. (2018). Experimental Lake Erie Harmful Algal Bloom (HAB) Tracker. Retrieved from https://www.glerl.noaa.gov/res/HABs_and_Hypoxia/habTracker.html
- Nwankwegu, A. S., Li, Y., Huang, Y., Wei, J., Norgbey, E., Sarpong, L., . . . Wang, K. (2019). Harmful algal blooms under changing climate and constantly increasing anthropogenic actions: the review of management implications. *3 Biotech*, 9(12), 449. doi:10.1007/s13205-019-1976-1
- O'Neil, J., Davis, T., Burford, M., & Gobler, C. (2013). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, 14. doi:10.1016/j.hal.2011.10.027
- Ohio EPA. (2022). *Public water system. Harmful algal bloom. Response strategy*. Retrieved from <https://epa.ohio.gov/static/Portals/28/documents/habs/PWS-HAB-Strategy.pdf>
- Pachauri, R. K., Intergovernmental Panel on Climate Change (IPCC), & Working Group Impacts. (2015). *Climate change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland: IPCC.

- Pacheco, A. B., Guedes, I. A., & Azevedo, S. M. (2016). Is qPCR a reliable indicator of cyanotoxin risk in freshwater? *Toxins (Basel)*, 8(6). doi:10.3390/toxins8060172
- Paerl, H. W., Havens, K. E., Hall, N. S., Otten, T. G., Zhu, M., Xu, H., . . . Qin, B. (2019). Mitigating a global expansion of toxic cyanobacterial blooms: Confounding effects and challenges posed by climate change. *Marine and Freshwater Research*, 71(5). doi:10.1071/MF18392
- Paerl, H. W., & Paul, V. J. (2012). Climate change: Links to global expansion of harmful cyanobacteria. *Water Research*, 46(5), 1349-1363. doi:10.1016/j.watres.2011.08.002
- Pazouki, P. (2016). *Cyanobacteria in surface and bank filtered drinking water sources: Application of phycocyanin probes for monitoring blooms*. (M.Sc.A). Polytechnique Montréal, Montréal, QC, Canada. Retrieved from <https://publications.polymtl.ca/2172/>
- Pazouki, P., Dorner, S., Bouchard, R., & Zamyadi, A. (2014, Oct. 17). *Applying an online multi-probe to monitor the potentially toxic cyanobacteria in Lake Erie*. Paper presented at the 29th Eastern Canadian Symposium on Water Quality Research, Montréal, QC, Canada.
- Pazouki, P., Prévost, M., McQuaid, N., Barbeau, B., de Boutray, M.-L., Zamyadi, A., & Dorner, S. (2016). Breakthrough of cyanobacteria in bank filtration. *Water Research*, 102, 170-179. doi:<http://dx.doi.org/10.1016/j.watres.2016.06.037>
- Pestana, C. J., Reeve, P. J., Sawade, E., Voldoire, C. F., Newton, K., Praptiwi, R., . . . Newcombe, G. (2016). Fate of cyanobacteria in drinking water treatment plant lagoon supernatant and sludge. *Science of The Total Environment*, 565, 1192-1200. doi:10.1016/j.scitotenv.2016.05.173
- Pham, T.-L., Shimizu, K., Dao, T.-S., Hong-Do, L.-C., & Utsumi, M. (2015). Microcystin uptake and biochemical responses in the freshwater clam *Corbicula leana* P. exposed to toxic and non-toxic *Microcystis aeruginosa*: Evidence of tolerance to cyanotoxins. *Toxicology Reports*, 2, 88-98. doi:<https://doi.org/10.1016/j.toxrep.2015.01.012>
- Pires, M. D. (2010). *Evaluation of fluorometers for the in situ monitoring of chlorophyll and/or cyanobacteria*. Retrieved from http://publications.deltares.nl/1203593_000.pdf
- Pivokonsky, M., Safarikova, J., Baresova, M., Pivokonska, L., & Kopecka, I. (2014). A comparison of the character of algal extracellular versus cellular organic matter produced by cyanobacterium, diatom and green alga. *Water Research*, 51(0), 37-46. doi:10.1016/j.watres.2013.12.022
- Planas, D., Desrosiers, M., Groulx, S. R., Paquet, S., & Carignan, R. (2000). Pelagic and benthic algal responses in eastern Canadian Boreal Shield lakes following harvesting and wildfires. *Canadian Journal of Fisheries and Aquatic Sciences*, 57, 136-145. doi:10.1139/cjfas-57-S2-136
- Prescott, L. M., Harley, J. P., & Klein, D. A. (2005). *Microbiology (Sixth Edition)*. New York, NY, USA: The McGraw Hill Companies Inc.
- Puschner, B., Bautista, A. C., & Wong, C. (2017). Debromoaplysiatoxin as the Causative Agent of Dermatitis in a Dog after Exposure to Freshwater in California. *Frontiers in Veterinary Science*, 4. Retrieved from <https://www.frontiersin.org/articles/10.3389/fvets.2017.00050>

- Qi, L., Hu, C., Visser, P. M., & Ma, R. (2018a). Diurnal changes of cyanobacteria blooms in Taihu Lake as derived from GOCI observations. *Limnology and Oceanography*, 63(4), 1711-1726. doi:<https://doi.org/10.1002/lno.10802>
- Qi, Z., Zheng, F., Guo, D., Zhang, T., Shao, Y., Yu, T., . . . Maier, H. R. (2018b). A comprehensive framework to evaluate hydraulic and water quality impacts of pipe breaks on water distribution systems. *Water Resources Research*, 54(10), 8174-8195. doi:10.1029/2018wr022736
- Qin, B., Yang, G., Ma, J., Wu, T., Li, W., Liu, L., . . . Zhou, J. (2018). Spatiotemporal changes of cyanobacterial bloom in large shallow eutrophic Lake Taihu, China. *Frontiers in Microbiology*, 9, 451. doi:10.3389/fmicb.2018.00451
- Qin, B., Zhu, G., Gao, G., Zhang, Y., Li, W., Paerl, H. W., & Carmichael, W. W. (2010). A drinking water crisis in Lake Taihu, China: Linkage to climatic variability and lake management. *Environ Manage*, 45(1), 105-112. doi:10.1007/s00267-009-9393-6
- Qu, F., Liang, H., Wang, Z., Wang, H., Yu, H., & Li, G. (2012). Ultrafiltration membrane fouling by extracellular organic matters (EOM) of *Microcystis aeruginosa* in stationary phase: Influences of interfacial characteristics of foulants and fouling mechanisms. *Water Research*, 46(5), 1490-1500. doi:10.1016/j.watres.2011.11.051
- Remmal, Y., Hudon, C., Hamilton, P. B., Rondeau, M., & Gagnon, P. (2017). Forecasting the magnitude and composition of phytoplankton blooms in a eutrophic lowland river (Rivière Yamaska, Que., Canada). *Canadian Journal of Fisheries and Aquatic Sciences*, 74(8), 1298-1311. doi:10.1139/cjfas-2016-0305
- Richardson, J., Feuchtmayr, H., Miller, C., Hunter, P. D., Maberly, S. C., & Carvalho, L. (2019). Response of cyanobacteria and phytoplankton abundance to warming, extreme rainfall events and nutrient enrichment. *Global change biology*, 25(10), 3365-3380. doi:10.1111/gcb.14701
- Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. I., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology*, 111(1), 1-61. doi:10.1099/00221287-111-1-1
- Romanis, C. S., Pearson, L. A., & Neilan, B. A. (2021). Cyanobacterial blooms in wastewater treatment facilities: Significance and emerging monitoring strategies. *Journal of Microbiological Methods*, 180, 106123. doi:10.1016/j.mimet.2020.106123
- Rome, M., Beighley, R. E., & Faber, T. (2021). Sensor-based detection of algal blooms for public health advisories and long-term monitoring. *Science of The Total Environment*, 767, 144984. doi:10.1016/j.scitotenv.2021.144984
- Rousso, B. Z., Bertone, E., Stewart, R., Aguiar, A., Chuang, A., Hamilton, D. P., & Burford, M. A. (2022). Chlorophyll and phycocyanin in-situ fluorescence in mixed cyanobacterial species assemblages: Effects of morphology, cell size and growth phase. *Water Research*, 212, 118127. doi:<https://doi.org/10.1016/j.watres.2022.118127>
- Rousso, B. Z., Bertone, E., Stewart, R., & Hamilton, D. P. (2020). A systematic literature review of forecasting and predictive models for cyanobacteria blooms in freshwater lakes. *Water Research*, 182, 115959. doi:<https://doi.org/10.1016/j.watres.2020.115959>

- Rousso, B. Z., Bertone, E., Stewart, R. A., Rinke, K., & Hamilton, D. P. (2021). Light-induced fluorescence quenching leads to errors in sensor measurements of phytoplankton chlorophyll and phycocyanin. *Water Research*, 198, 117133. doi:10.1016/j.watres.2021.117133
- Roy-Lachapelle, A., Duy, S. V., Munoz, G., Dinh, Q. T., Bahl, E., Simon, D. F., & Sauvé, S. (2019). Analysis of multiclass cyanotoxins (microcystins, anabaenopeptins, cylindrospermopsin and anatoxins) in lake waters using on-line SPE liquid chromatography high-resolution Orbitrap mass spectrometry. *Analytical Methods*. doi:10.1039/c9ay01132c
- Sabart, M., Crenn, K., Perrière, F., Abila, A., Lereboure, M., Colombet, J., . . . Latour, D. (2015). Co-occurrence of microcystin and anatoxin-a in the freshwater lake Aydat (France): Analytical and molecular approaches during a three-year survey. *Harmful Algae*, 48, 12-20. doi:10.1016/j.hal.2015.06.007
- Saha, R., Liu, D., Hoynes-O'Connor, A., Liberton, M., Yu, J., Bhattacharyya-Pakrasi, M., . . . Pakrasi Himadri, B. (2016). Diurnal Regulation of Cellular Processes in the Cyanobacterium *Synechocystis* sp. Strain PCC 6803: Insights from Transcriptomic, Fluxomic, and Physiological Analyses. *mBio*, 7(3), e00464-00416. doi:10.1128/mBio.00464-16
- Sanseverino, I., Conduto António, D., Loos, R., & Lettieri, T. (2017). *Cyanotoxins: Methods and approaches for their analysis and detection* (EUR 28624;). Retrieved from <http://publications.jrc.ec.europa.eu/repository/bitstream/JRC106478/kjna28624enn.pdf>
- Sarada, R., Pillai, M. G., & Ravishankar, G. A. (1999). Phycocyanin from spirulina sp: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. *Process Biochemistry*, 34(8), 795-801. Retrieved from <http://www.sciencedirect.com/science/article/B6THB-3XJTT46-4/2/beeba2eab97403b7e9944d5871d6b6f6>
- Sellner, K. G., Doucette, G. J., & Kirkpatrick, G. J. (2003). Harmful algal blooms: causes, impacts and detection. *Journal of Industrial Microbiology and Biotechnology*, 30(7), 383-406. Retrieved from <http://www.springerlink.com/content/ptybc0qg8y4klr5c/fulltext.pdf>
- Serrano-Silva, N., & Calderón-Ezquerro, M. C. (2018). Metagenomic survey of bacterial diversity in the atmosphere of Mexico City using different sampling methods. *Environmental Pollution*, 235, 20-29. doi:10.1016/j.envpol.2017.12.035
- So, P. T. C., & Dong, C. Y. (2001). Fluorescence spectrophotometry. *e LS*. Retrieved from <http://web.mit.edu/solab/Documents/Assets/So-Fluorescence%20spectrophotometry.pdf>
- Sobiechowska-Sasim, M., Ston-Egiert, J., & Kosakowska, A. (2014). Quantitative analysis of extracted phycobilin pigments in cyanobacteria - an assessment of spectrophotometric and spectrofluorometric methods. *Journal of Applied Phycology*, 26(5), 2065-2074. doi:10.1007/s10811-014-0244-3
- Song, K., Li, L., Tedesco, L. P., Clercin, N., Hall, R., Li, S., . . . Sun, Y. (2013). Remote estimation of phycocyanin (PC) for inland waters coupled with YSI PC fluorescence probe. *Environmental Science and Pollution Research*, 20(8), 5330-5340. doi:10.1007/s11356-013-1527-y

- Sorlini, S., Gialdini, F., & Collivignarelli, C. (2013). Removal of cyanobacterial cells and Microcystin-LR from drinking water using a hollow fiber microfiltration pilot plant. *Desalination*, 309, 106-112. doi:<https://doi.org/10.1016/j.desal.2012.09.028>
- Sotero-Santos, R. B., Carvalho, E. G., Dellamano-Oliveira, M. J., & Rocha, O. (2008). Occurrence and toxicity of an *Anabaena* bloom in a tropical reservoir (Southeast Brazil). *Harmful Algae*, 7(5), 590-598. doi:<https://doi.org/10.1016/j.hal.2007.12.017>
- Srivastava, A., Singh, S., Ahn, C.-Y., Oh, H.-M., & Asthana, R. K. (2013). Monitoring approaches for a toxic cyanobacterial Bloom. *Environmental Science & Technology*, 47(16), 8999-9013. doi:10.1021/es401245k
- Stedmon, C., & Bro, R. (2008). Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial: Fluorescence-PARAFAC analysis of DOM. *Limnology and Oceanography: Methods*, 6, 572-579. doi:10.4319/lom.2008.6.572b
- Steffen, M. M., Davis, T. W., McKay, R. M. L., Bullerjahn, G. S., Krausfeldt, L. E., Stough, J. M. A., . . . Wilhelm, S. W. (2017). Ecophysiological examination of the Lake Erie *Microcystis* bloom in 2014: Linkages between biology and the water supply shutdown of Toledo, OH. *Environmental Science & Technology*, 51(12), 6745-6755. doi:10.1021/acs.est.7b00856
- Sun, F., Pei, H.-Y., Hu, W.-R., & Ma, C.-X. (2012). The lysis of *Microcystis aeruginosa* in $AlCl_3$ coagulation and sedimentation processes. *Chemical Engineering Journal*, 193-194, 196-202. doi:10.1016/j.cej.2012.04.043
- Svircev, Z., Lalic, D., Bojadzija Savic, G., Tokodi, N., Drobac Backovic, D., Chen, L., . . . Codd, G. A. (2019). Global geographical and historical overview of cyanotoxin distribution and cyanobacterial poisonings. *Archives of Toxicology*, 93(9), 2429-2481. doi:10.1007/s00204-019-02524-4
- Svrcek, C., & Smith, D. W. (2004). Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. *Journal of Environmental Engineering and Science*, 3(3), 155-185. doi:10.1139/s04-010
- Symes, E., & Van Ogtrop, F. (2016). Determining the Efficacy of a Submersible in situ Fluorometric Device for Cyanobacteria Monitoring Coalesced with Total Suspended Solids Characteristic of Lowland Reservoirs. *River Research and Applications*, 32, 1632-1641. doi:10.1002/rra.2993
- Szlag, D. C., Sinclair, J. L., Southwell, B., & Westrick, J. A. (2015). Cyanobacteria and Cyanotoxins Occurrence and Removal from Five High-Risk Conventional Treatment Drinking Water Plants. *Toxins*, 7(6), 2198-2220. doi:10.3390/toxins7062198
- Takaara, T., Sano, D., Konno, H., & Omura, T. (2007). Cellular proteins of *Microcystis aeruginosa* inhibiting coagulation with polyaluminum chloride. *Water Research*, 41(8), 1653-1658. Retrieved from <http://www.sciencedirect.com/science/article/pii/S0043135407000735>
- Tan, B., Ng, C., Nshimiyimana, J., Loh, L.-L., Gin, K., & Thompson, J. (2015). Next-generation sequencing (NGS) for assessment of microbial water quality: current progress, challenges, and future opportunities. *Frontiers in Microbiology*, 6(1027). doi:10.3389/fmicb.2015.01027

- Tanabe, Y., Kaya, K., & Watanabe, M. M. (2004). Evidence for recombination in the microcystin synthetase (mcy) genes of toxic cyanobacteria *Microcystis* spp. *Journal of Molecular Evolution*, 58(6), 633-641. doi:10.1007/s00239-004-2583-1
- Te, S. H., Chen, E. Y., & Gin, K. Y.-H. (2015). Comparison of quantitative PCR and droplet digital PCR multiplex assays for two genera of bloom-forming cyanobacteria, *Cylindrospermopsis* and *Microcystis*. *Applied and environmental microbiology*, 81(15), 5203-5211. doi:10.1128/AEM.00931-15
- Thimijan, R., & Heins, R. (1983). Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. *Hortic Sci*, 18, 818-822.
- Thomson-Laing, G., Puddick, J., & Wood, S. A. (2020). Predicting cyanobacterial biovolumes from phycocyanin fluorescence using a handheld fluorometer in the field. *Harmful Algae*, 97, 101869. doi:10.1016/j.hal.2020.101869
- Trios Optical Sensor. (2009). *MicroFlu Manual (Version 2.0)*. Retrieved from Germany: http://www.trios.de/downloads/Brochures%20-%20English/microFlu_en.pdf
- Trios Optical Sensor. (2021). *MicroFlu V2*. Retrieved from Germany: <https://www.trios.de/en/microflu-v2.html>
- Tromas, N., Fortin, N., Bedrani, L., Terrat, Y., Cardoso, P., Bird, D., . . . Shapiro, B. J. (2017). Characterising and predicting cyanobacterial blooms in an 8-year amplicon sequencing time course. *ISME Journal*, 11(8), 1746-1763. doi:10.1038/ismej.2017.58
- Turner Designs. (2017). *CyanoFluor handheld HAB indicator user manual*. Retrieved from San Jose, CA, USA: <http://docs.turnerdesigns.com/t2/doc/manuals/998-8701.pdf>
- Turner Designs. (2018). *Optical Specification Guide: AquaFluor Handheld Fluorometer/Turbidimeter*. Retrieved from San Jose, CA, USA: <http://docs.turnerdesigns.com/t2/doc/spec-guides/998-8081.pdf>
- Turner Designs. (2019). *Optical Specification Guide: Trilogy Laboratory Fluorometer*. Retrieved from San Jose, CA, USA: <http://docs.turnerdesigns.com/t2/doc/spec-guides/998-7281.pdf>
- Turner Designs. (2020a). *Optical Specification Guide: C3 and C6P Submersible Fluorometers*. Retrieved from San Jose, CA, USA: <http://docs.turnerdesigns.com/t2/doc/spec-guides/998-2381.pdf>
- Turner Designs. (2020b). *Optical Specification Guide: Cyclops Submersible Sensors*. Retrieved from San Jose, CA, USA: <http://docs.turnerdesigns.com/t2/doc/spec-guides/998-2181.pdf>
- United States Environmental Protection Agency (USEPA). (2006). *Federal Register. Part II. National primary drinking water regulations: stage 2 disinfectants and disinfection byproducts rule; final rule*. Retrieved from Washington, DC, USA: <http://www.biovir.com/Images/pdf057.pdf>
- United States Environmental Protection Agency (USEPA). (2015). *Recommendations for public water systems to manage cyanotoxins in drinking water* (EPA 815-R-15-010). Retrieved from
- Vareli, K., Zarali, E., Zacharioudakis, G. S. A., Vagenas, G., Varelis, V., Pilidis, G., . . . Sainis, I. (2012). Microcystin producing cyanobacterial communities in Amvrakikos Gulf (Mediterranean Sea, NW Greece) and toxin accumulation in mussels (*Mytilus*

- galloprovincialis). *Harmful Algae*, 15, 109-118. doi:<https://doi.org/10.1016/j.hal.2011.12.005>
- Visser, P. M., Verspagen, J. M. H., Sandrini, G., Stal, L. J., Matthijs, H. C. P., Davis, T. W., . . . Huisman, J. (2016). How rising CO₂ and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae*, 54, 145-159. doi:10.1016/j.hal.2015.12.006
- Walker, S. R., Lund, J. C., Schumacher, D. G., Brakhage, P. A., McManus, B. C., Miller, J. D., . . . Gitelson, A. A. (2008). *Nebraska experience (Chapter 6)*. Retrieved from New York, USA: http://www.epa.gov/cyano_habs_symposium/monograph/Ch06.pdf
- Watras, C. J., Morrison, K. A., Rubsam, J. L., Hanson, P. C., Watras, A. J., LaLiberte, G. D., & Milewski, P. (2017). A temperature compensation method for chlorophyll and phycocyanin fluorescence sensors in freshwater. *Limnology and Oceanography: Methods*, 15(7), 642-652. doi:10.1002/lom3.10188
- Wauthy, M., Rautio, M., Christoffersen, K. S., Forsström, L., Laurion, I., Mariash, H. L., . . . Vincent, W. F. (2018). Increasing dominance of terrigenous organic matter in circumpolar freshwaters due to permafrost thaw. *Limnology and Oceanography Letters*, 3(3), 186-198. doi:10.1002/lol2.10063
- Wells, M. L., Trainer, V. L., Smayda, T. J., Karlson, B. S. O., Trick, C. G., Kudela, R. M., . . . Cochlan, W. P. (2015). Harmful algal blooms and climate change: Learning from the past and present to forecast the future. *Harmful Algae*, 49, 68-93. doi:10.1016/j.hal.2015.07.009
- Whitton, B. A. (2012). *Ecology of cyanobacteria II: Their diversity in space and time*: Springer Science & Business Media.
- Wicaksono, H., Satyantini, W., & Masithah, E. (2019). The spectrum of light and nutrients required to increase the production of phycocyanin *Spirulina platensis*. *IOP Conference Series: Earth and Environmental Science*, 236, 012008. doi:10.1088/1755-1315/236/1/012008
- Wilmotte, A. (1994). Molecular evolution and taxonomy of the cyanobacteria. In *The molecular biology of cyanobacteria* (pp. 1-25): Springer.
- Winter, J. G., DeSellas, A. M., Fletcher, R., Heintsch, L., Morley, A., Nakamoto, L., & Utsumi, K. (2011). Algal blooms in Ontario, Canada: Increases in reports since 1994. *Lake and Reservoir Management*, 27(2), 107-114. doi:10.1080/07438141.2011.557765
- Ye, J., Du, Y., Wang, L., Qian, J., Chen, J., Wu, Q., & Hu, X. (2017). Toxin release of cyanobacterium *Microcystis aeruginosa* after exposure to typical tetracycline antibiotic contaminants. *Toxins*, 9(2), 53. doi:10.3390/toxins9020053
- Yilmaz, M., & Phlips, E. J. (2011). Diversity of and selection acting on cylindrospermopsin *cyrB* gene adenylation domain sequences in Florida. *Applied and Environmental Microbiology*, 77(7), 2502-2507. doi:10.1128/AEM.02252-10
- Yoo, S. M., & Lee, S. Y. (2016). Optical biosensors for the detection of pathogenic microorganisms. *Trends Biotechnol*, 34(1), 7-25. doi:10.1016/j.tibtech.2015.09.012
- YSI Incorporated. (2006). *6-Series: multiparameter water quality sondes (User manual)*. Yellow Springs, Ohio, USA.
- YSI Incorporated. (2019). *EXO user manual*. Yellow Springs, OH, USA.

- Zamyadi, A. (2011). *The value of in vivo monitoring and chlorination for the control of toxic Cyanobacteria in drinking water production* (Ph.D. Ph.D.). Ecole Polytechnique de Montréal, Montréal, QC, Canada. Retrieved from <http://publications.polymtl.ca/618/>
- Zamyadi, A., Choo, F., Newcombe, G., Stuetz, R., & Henderson, R. K. (2016a). A review of monitoring technologies for real-time management of cyanobacteria: Recent advances and future direction. *TrAC Trends in Analytical Chemistry*, 85, 83-96. doi:10.1016/j.trac.2016.06.023
- Zamyadi, A., Coral, L. A., Barbeau, B., Dorner, S., Lapolli, F. R., & Prévost, M. (2015). Fate of toxic cyanobacterial genera from natural bloom events during ozonation. *Water Research*, 73, 204-215. doi:10.1016/j.watres.2015.01.029
- Zamyadi, A., Dorner, S., Ellis, D., Bolduc, A., Bastien, C., & Prévost, M. (2013a). Species-dependence of cyanobacteria removal efficiency by different drinking water treatment processes. *Water Research*, 47(8), 2689–2700. doi:10.1016/j.watres.2013.02.040
- Zamyadi, A., Dorner, S., Ndong, M., Bolduc, A., Bastien, C., & Prévost, M. (2014). Application of *in vivo* measurements for the management of cyanobacterial cell breakthrough into drinking water treatment plants. *Environmental Science: Processes & Impacts*, 16, 313-323. doi:10.1039/C3EM00603D
- Zamyadi, A., Dorner, S., Ndong, M., Ellis, D., Bolduc, A., Bastien, C., & Prévost, M. (2013b). Low-risk cyanobacterial bloom sources: Cell accumulation within full-scale treatment plants. *Journal American Water Works Association*, 102(11), E651-E663. doi:10.5942/jawwa.2013.105.0141
- Zamyadi, A., Henderson, R. K., Stuetz, R., Newcombe, G., Newtown, K., & Gladman, B. (2016b). Cyanobacterial management in full-scale water treatment and recycling processes: Reactive dosing following intensive monitoring. *Environmental Science: Water Research & Technology*, 2(2), 362-375. doi:10.1039/C5EW00269A
- Zamyadi, A., MacLeod, S., Fan, Y., McQuaid, N., Dorner, S., Sauvé, S., & Prévost, M. (2012a). Toxic cyanobacterial breakthrough and accumulation in a drinking water plant: A monitoring and treatment challenge. *Water Research*, 46(5), 1511-1523. doi:10.1016/j.watres.2011.11.012
- Zamyadi, A., McQuaid, N., Dorner, S., Bird, D. F., Burch, M., Baker, P., . . . Prévost, M. (2012b). Cyanobacterial detection using *in vivo* fluorescence probes: Managing interferences for improved decision-making. *Journal American Water Works Association*, 104(8), E466-E479. doi:10.5942/jawwa.2012.104.0114
- Zamyadi, A., McQuaid, N., Prévost, M., & Dorner, S. (2012c). Monitoring of potentially toxic cyanobacteria using an online multi-probe in drinking water sources. *Journal of Environmental Monitoring*, 14(2), 579-588. doi:10.1039/C1EM10819K
- Zamyadi, A., Romanis, C., Mills, T., Neilan, B., Choo, F., Coral, L. A., . . . Henderson, R. K. (2019). Diagnosing water treatment critical control points for cyanobacterial removal: Exploring benefits of combined microscopy, next-generation sequencing, and cell integrity methods. *Water Research*, 152, 96-105. doi:10.1016/j.watres.2019.01.002

- Zhang, X.-J., Chen, C., Ding, J.-Q., Hou, A., Li, Y., Niu, Z.-B., . . . Laws, E. A. (2010). The 2007 water crisis in Wuxi, China: Analysis of the origin. *Journal of Hazardous Materials*, 182(1-3), 130-135. doi:<https://doi.org/10.1016/j.jhazmat.2010.06.006>
- Zhao, L., & Huang, W. (2014). Models for identifying significant environmental factors associated with cyanobacterial bloom occurrence and for predicting cyanobacterial blooms. *Journal of Great Lakes Research*, 40(2), 265-273. doi:<https://doi.org/10.1016/j.jglr.2014.02.011>
- Zhu, C., Zhang, J., Wang, X., Yang, Y., Chen, N., Lu, Z., . . . Chen, T. (2021). Responses of cyanobacterial aggregate microbial communities to algal blooms. *Water Research*, 196, 117014. doi:[10.1016/j.watres.2021.117014](https://doi.org/10.1016/j.watres.2021.117014)
- Ziegmann, M., Abert, M., Muller, M., & Frimmel, F. H. (2010). Use of fluorescence fingerprints for the estimation of bloom formation and toxin production of *Microcystis aeruginosa*. *Water Research*, 44(1), 195-204. doi:[10.1016/j.watres.2009.09.035](https://doi.org/10.1016/j.watres.2009.09.035)

APPENDIX A SUPPLEMENTARY INFORMATION, ARTICLE 1

Journal: *Water*

Title: Factors affecting the interpretation of online phycocyanin fluorescence to manage cyanobacteria in drinking water sources

Authors: Liya Ma, Saber Moradinejad, Juan Francisco Guerra Maldonado, Arash Zamyadi, Sarah Dorner and Michèle Prévost

Table A.1 Correlations between the *in situ* PC fluorescence probe readings and microscopic cell counts, biovolume estimation, and extracted pigment concentration

Correlation	Experimental Result	Probe model	Reference
Probe measured PC ($\mu\text{g/L}$) and microscopic CB cell counts (cells/mL)	$R^2 = 0.99$ for laboratory cultured <i>Microcystis aeruginosa</i> $R^2 = 0.745$ for field collected samples	TriOS microFlu	(Bastien et al., 2011)
	$R^2 \geq 0.9954$ for laboratory cultured 4 different unicellular species $R^2 = -0.93$ and 0.73 for laboratory cultured mixed species $R^2 = 0.71$ for field collected samples	TriOS microFlu	(Kong et al., 2014)
	$R^2 \geq 0.88$ for laboratory cultured 3 different unicellular specie $R^2 \geq 0.90$ when a unicellular species is mixed with a microalga <i>Pseudokirchneriella subcapitata</i> .	Fluo-Imager Fluorometer	(Macário et al., 2015)
	$R^2 = 0.994$ for laboratory cultured <i>Planktothrix agardhii</i> $R^2 = 0.997$ for laboratory cultured <i>Lemmermanniella</i> sp. $R^2 = 0.729$ for field collected samples	TriOS microFlu	(Brient et al., 2008)
Probe measured PC (RFU) and microscopic CB cell counts (cells/mL)	$R^2 = 0.96$ for field collected samples	YSI 6600	(Zamyadi et al., 2014)
Probe measured PC (cells/mL) and microscopic CB cell counts (cells/mL)	$R^2 > 0.993$ for laboratory cultured <i>Microcystis aeruginosa</i> $R^2 = 0.705$ for field collected samples	YSI 6600	(Bastien et al., 2011)
	$R^2 = 0.95$ for laboratory cultured <i>Microcystis aeruginosa</i>	Eureka Manta II	(Symes et al., 2016)
Probe measured PC ($\mu\text{g/L}$) and microscopic CB biovolume (mm^3/L)	$R^2 > 0.85$ for laboratory cultured unicellular <i>Microcystis aeruginosa</i> and 3 filamentous species The relationship is weak for laboratory cultured colonial strain of <i>Microcystis aeruginosa</i> No significant relation for field collected samples	Turner Cyclops-7, Eureka Manta II, TriLux, YSI EXO	(Hodges et al., 2018)
	$R^2 = 0.43$ and 0.69 for field collected samples	Turner Cyclops-7	(Cotterill et al., 2019)
	$R^2 \geq 0.99$ for laboratory cultured 4 different unicellular species $R^2 \geq 0.97$ for laboratory cultured mixed species $R^2 = 0.77$ for field collected samples	TriOS microFlu	(Kong et al., 2014)
	$R^2 = 0.829$ for field collected samples	TriOS microFlu	(Bastien et al., 2011)
	$R^2 = 0.43$ and 0.63 for field collected samples	Turner Cyclops-7	(Cotterill et al., 2019)

Table A.1 Correlations between the *in situ* PC fluorescence probe readings and microscopic cell counts, biovolume estimation, and extracted pigment concentration (cont'd)

Correlation	Experimental Result	Probe model	Reference
	$R^2 \geq 0.98$ for laboratory cultured 3 different unicellular species $R^2 \geq 0.86$ when a unicellular species is mixed with a microalga <i>Pseudokirchneriella subcapitata</i> $R^2 \geq 0.93$ when in the mixtures of two cyanobacteria species	Fluo-Imager Fluorometer	(Macário et al., 2015)
	$R^2 \geq 0.88$ for laboratory cultured 12 species $R^2 \geq 0.78$ for field collected samples	Turner CyanoFluor	(Thomson-Laing et al., 2020)
	Linear signal for laboratory cultured <i>Planktothrix agardhii</i> and <i>Lemmermanniella</i> sp	TriOS microFlu	(Brient et al., 2008)
Probe measured PC (RFU) and microscopic CB cell biovolume (mm^3/L)	$R^2 = 0.68$ for field collected samples	YSI 6600	(McQuaid et al., 2011)
	$R^2 \geq 0.92$ for laboratory cultured mono cell culture <i>Microcystis aeruginosa</i> , <i>Dolichospermum circinale</i> , <i>Cylindrospermopsis raciborskii</i> and <i>Microcystis flos-aquae</i>	YSI EXO2, YSI 6600, bbe Moldanke Algaetorch, TriOS microFlu	(Choo et al., 2018)
	$R^2 \geq 0.73$ for field collected samples	YSI EXO2	(Almuhtaram et al., 2018)
	$R^2 > 0.996$ for laboratory cultured <i>Microcystis aeruginosa</i> $R^2 = 0.87$ for field collect samples	YSI 606131, YSI 6600, TriOS microFlu, bbe Moldanke Fluoroprobe	(Zamyadi et al., 2012b)
	$R^2 = 0.77$ and 0.72 in two filed locations, but $R^2 = 0.19$ in the third field location	YSI EXO2	(Bowling et al., 2016)
	$R^2 = 0.70$ for field collected samples	YSI EXO2	(Zamyadi et al., 2016b)
	$0.71 \leq R^2 \leq 0.81$ for laboratory cultured 4 species	YSI EXO2	(Bertone et al., 2019)
Probe measured PC (cells/mL) and microscopic CB cell biovolume (mm^3/L)	$R^2 = 0.815$ for field collected samples	YSI 6600	(Bastien et al., 2011)
	$R^2 = 0.60$ for laboratory cultured <i>Microcystis aeruginosa</i>	Eureka Manta II	(Symes et al., 2016)
Probe measured PC ($\mu\text{g}/\text{L}$) and laboratory extracted PC concentration ($\mu\text{g}/\text{L}$)	$R^2 > 0.70$ for field collected samples	Turner Cyclops-7, YSI EXO	(Hodges et al., 2018)
Probe measured PC (RFU) and laboratory extracted PC concentration ($\mu\text{g}/\text{L}$)	$R^2 = 0.55, 0.93$ and 0.96 for laboratory cultured 3 species	YSI EXO2	(Bertone et al., 2019)
	$R^2 = 0.54$ for mixed laboratory cultured 4 species $R^2 = 0.84, 0.81, 0.82$ and 0.92 for laboratory cultured 4 species	YSI EXO2	(Rousso et al., 2022)
	$R^2 = 0.96$ for laboratory cultured <i>Microcystis aeruginosa</i> $R^2 = 0.79$ for field collected samples	YSI 6600	(Zamyadi et al., 2012c)
	$R^2 = 0.86$ for field collected samples	YSI 6600	(Song et al., 2013)

Table A.2 Main sources of interference associated with the *in situ* PC fluorescence probe

Source	Interference and relative error of PC content	Reference
Varying cell growth phase	Maximum 125% higher PC observed during logarithmic phase comparing with the lag and stationary phases in the study of <i>Microcystis aeruginosa</i> and <i>Dolichospermum circinalis</i>	(Chang et al., 2012)
	Phycocyanin dominates in the decay phase in the study of <i>Microcystis aeruginosa</i>	(Ziegmann et al., 2010)
	Decrease as <i>Aphanocapsa</i> sp. and <i>Sphaerospermopsis</i> sp. reached stationary phase, but increased for <i>Microcystis</i> sp For <i>Raphidiopsis raciborskii</i> , the content varies throughout the growth phases	(Bertone et al., 2019)
	<i>Microcystis aeruginosa</i> has consistently higher PC in the exponential phase compared to stationary and/or late stationary phases <i>Raphidiopsis raciborskii</i> shows decreases in fluorescence per unit biomass between exponential and late stationary phases <i>Dolichospermum circinale</i> overall increases PC fluorescence per unit biomass and per unit cell, but not vary significantly between growth phases <i>Aphanocapsa</i> sp. PC fluorescence does not vary significantly between growth phases	(Rousso et al., 2022)
Varying CB cell size, biovolume and cellular agglomeration	Weaker relationships for colonial <i>Microcystis</i> in comparison to single-celled and filamentous species	(Hodges et al., 2018)
	Maximum 88% PC increase after the disaggregation of the colonies	(Chang et al., 2012)
	Wide ranges of CB biovolume, agglomeration and PC cell quota observed	(McQuaid et al., 2011)
	Much weaker relationship between PC fluorescence probe readings and laboratory measured CB biovolume when picoplanktonic sized taxa contributed > 50% of the CB biovolume	(Bowling et al., 2016)
	93% and 226% error observed in the two strains of <i>Dolichospermum flos-aquae</i> , due to the filamentous nature and lack of probe fingerprinting	(Kring et al., 2014)
	20–90% of error	(Zamyadi et al., 2016a)
	Sample lysis (by sonication) had no effect on the strength of the relationships between PC and CB biovolume for the laboratory-cultured 12 CB species measured by handheld CyanoFluor fluorometer	(Thomson-Laing et al., 2020)
Prior light exposure	Maximum 59% underestimation during $354 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ (artificial light) compared to those in the dark	(Rousso et al., 2021)
	Maximum 25% underestimation during $1200 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ (artificial light) compared to values in grow culture	(Misumi et al., 2016)
	Prior light exposure of up to 7 h under natural light had no effect on the <i>Microcystis aeruginosa</i> PC fluorescence monitored by the YSI 606131 probe	(Zamyadi et al., 2012b)
	No significance difference was observed when <i>Planktothrix agardhii</i> exposed to $0\text{--}1900 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ of natural light for 30 min Three times more fluorescence was observed when <i>Planktothrix agardhii</i> exposed to $0\text{--}150 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ of artificial light for 30 min	(Brient et al., 2008)
	An increase in the fluorescence signal following exposure to ambient light compared to dimmed ambient light	(Hodges, 2016)
Probe calibration algorithm	If cell concentrations are ≥ 4 times of the calibration range, error is $\geq 20\%$; if cell concentrations are ≤ 0.1 times of the calibration range, error is 50 to 90%	(Chang et al., 2012)
	The calibration with cyanobacteria significantly improved the accuracy beyond 5000 cells/mL by comparison to the manufacturer's calibration for YSI 6600 probe	(Bastien et al., 2011)

Table A.2 Main sources of interference associated with the *in situ* PC fluorescence probe (cont'd)

Source	Interference and relative error of PC content	Reference
Water quality		
Presence of chlorophyll-a	Maximum 200% underestimation, and maximum 405% overestimation	(Choo et al., 2018)
	Positive correlation ($R^2 > 0.8$, $p < 0.05$)	(Choo et al., 2019)
	Maximum 600% error	(Zamyadi et al., 2016a)
	Linear relationship between cells and chlorophyll-a, at approximately 52.4 CB cells/ μ g- chlorophyll-a	(Chang et al., 2012)
Water turbidity	Maximum 49% underestimation due to the presence of mineral turbidity	(Zamyadi et al., 2012b)
	Turbidity >50 NTU can make probe measurement ineffective	(Bowling et al., 2013)
	The addition of mineral turbidity to the <i>Planktothrix agardhii</i> solution reduced the fluorescence intensity by 12.28% at 0.1 g/L for particle size of 0.1 μ m, and 5.87% at 0.1 μ g/L for a grain size of 0.316 μ m	(Brient et al., 2008)
	Lower accuracy results were observed when mineral turbidity is over 50 NTU, resulting in false positives	(Symes et al., 2016)
	Linear relationship between cells and mineral kaolinite turbidity, at approximately 15.5 CB cells/NTU	(Chang et al., 2012)
Water temperature	<i>Microcystis aeruginosa</i> was spiked in Milli-Q water to reach the concentration of high (828,000 cells/mL), medium (562,000 cells/ mL), and low (181,000 cells/mL), then being assessed at the water temperature of 4, 13.8, 17, and 23.5 °C: Turner Cyclops-7 T926 and Eureka Manta II: PC fluorescence readings at 4 °C is higher from any other temperature treatments. Maximum 50% higher comparing at 23.5 °C for higher and low cell concentration; not significant different among other temperatures Turner Cyclops-7 T927 and TriLux: no significant PC fluorescence difference with the changing of water temperature	(Hodges et al., 2018)
	Turner Cyclops-7 chlorophyll fluorometer and Turner Cyclops-7 PC fluorometer: Whole lake water and PC extract: fluorescence signals for chlorophyll and PC are linear functions of temperature over the range 5–30 °C. Equation is derived to compensate for the error; Reversibility of temperature quench during a sequence of heating and cooling. Laboratory cultured species: fluorescence signals over the temperature range 5–30 °C are linear functions for chlorophyll in the green algae (<i>Scenedesmus dimorphus</i>), and exponential functions for PC in the cyanobacteria (<i>Synechococcus leopoliensis</i>). However, anomalous response found for other two green algae (<i>Scenedesmus acutus</i> , and <i>Selenastrum minutum</i>), where fluorescence inhibited when temperature below 20 °C	(Watras et al., 2017)
Presence of dissolved organic matter (DOM)	<i>Microcystis</i> sp. And <i>Raphidiopsis raciborskii</i> were added to concentrated DOM from 2 different lakes water. DOM was measured by YSI EXO2 fDOM sensor, PC was measured by YSI EXO2 PC probe Maximum 14% underestimation when concentrated DOM was added	(Bertone et al., 2019)
	When 3 different cell species are spiked into the river water (2.65 mg/L DOC), more than an order of magnitude PC fluorescence intensity decreasing measured by fluorescence excitation-emission matrices (EEMs); while when adding extracted PC pigment, EEMs didn't change	(Korak et al., 2015)

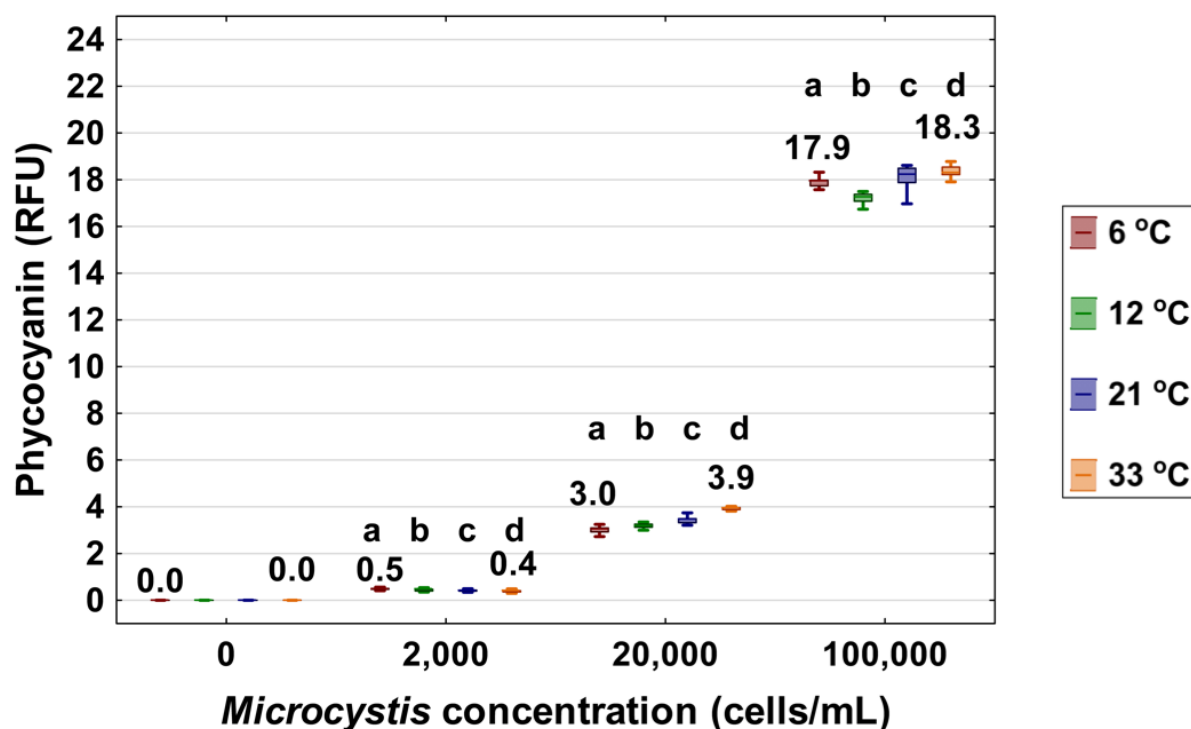


Figure A.1 PC readings (RFU) with the YSI EXO2 probe of the 0, 2000, 20,000, and 100,000 cells/mL of *Microcystis aeruginosa* strain CPCC 300 suspended in 6, 12, 21, 33 °C of ultrapure water. The probe measured each of the sample at a 250 ms interval for 10 min. The temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation 4.2. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$)

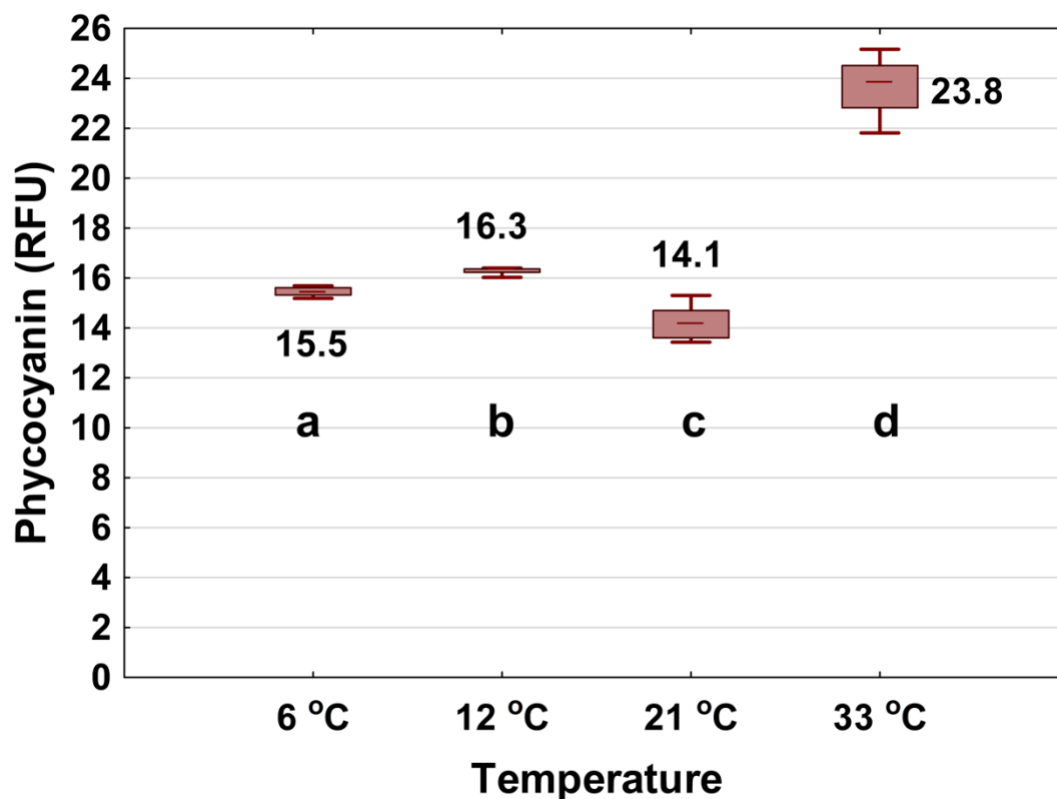


Figure A.2 PC readings (RFU) using the YSI EXO2 probe of 1 mg/L of extracted PC pigment in the 6, 12, 21, 33 °C of ultrapure water. All samples were tested over the period of 10 min. The probe measured each of the samples at a 250 ms interval for 10 min. The temperature interference was removed by adjusting the raw data to a reference temperature of 21 °C using Equation 4.2. The bottom and top of each box represent the 25th–75th percentiles, respectively. The whiskers represent the minimum and maximum values. The line within each box corresponds to the median value. Different letters represent significant differences between treatment comparisons (Kruskal–Wallis followed by Dunn’s post hoc test, $p \leq 0.05$)

APPENDIX B SUPPLEMENTARY INFORMATION, ARTICLE 2

Journal: *Environmental Monitoring and Assessment*

Title: Monitoring of cyanobacterial breakthrough and accumulation by *in situ* phycocyanin probe system within full-scale treatment plants

Authors: Liya Ma, Juan Francisco Guerra Maldonado, Arash Zamyadi, Sarah Dorner, and Michèle Prévost



Figure B.1 The location of the studied three DWTPs: A and B are located in southern Quebec, and C is located in southwest Ontario

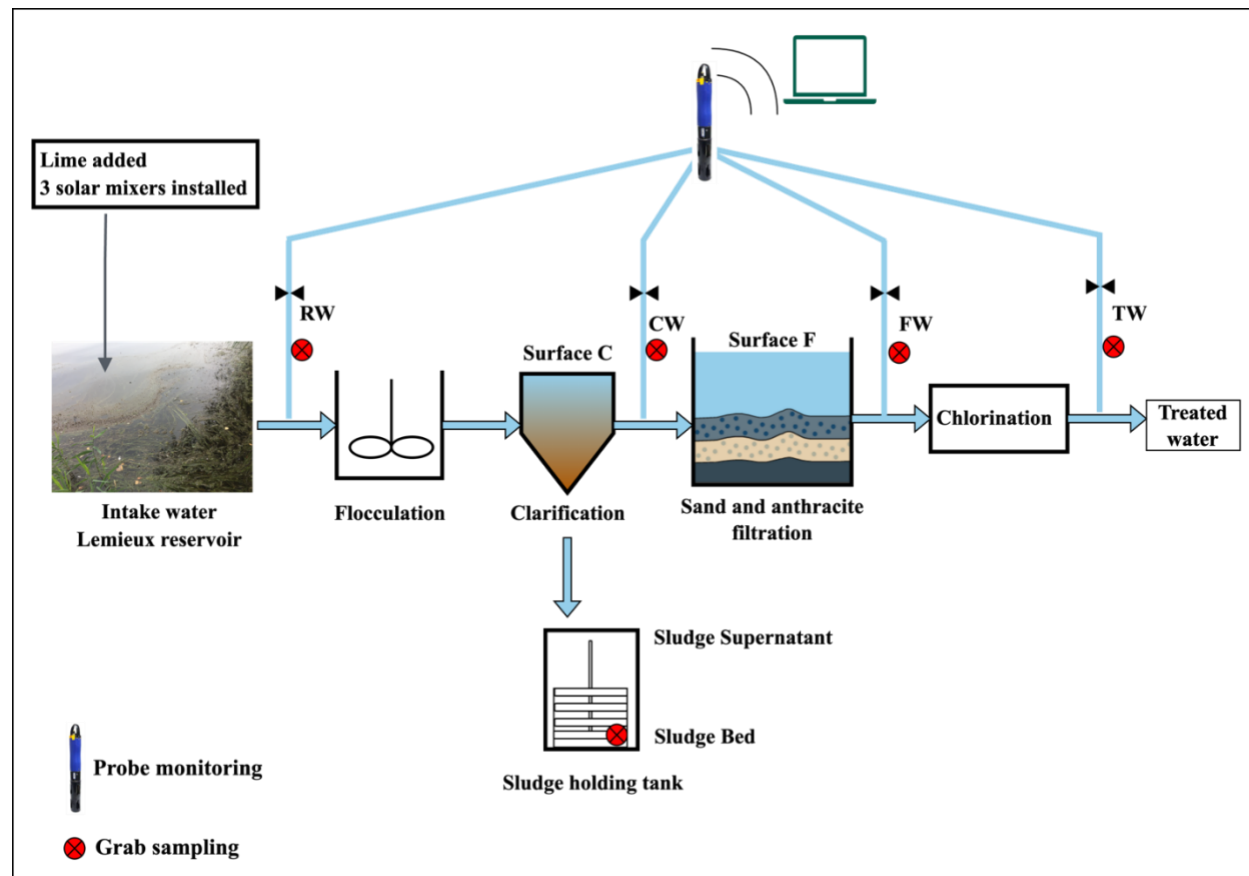


Figure B.2 Schematic of the treatment processes of DWTP B, grab sampling and probe monitoring locations. The water intake is from Lemieux reservoir. Sampling points are indicated by: raw water (RW), clarified water (CW), filtered water (FW), treated water (TW), and sludge bed of sludge holding tank (Sludge Bed)

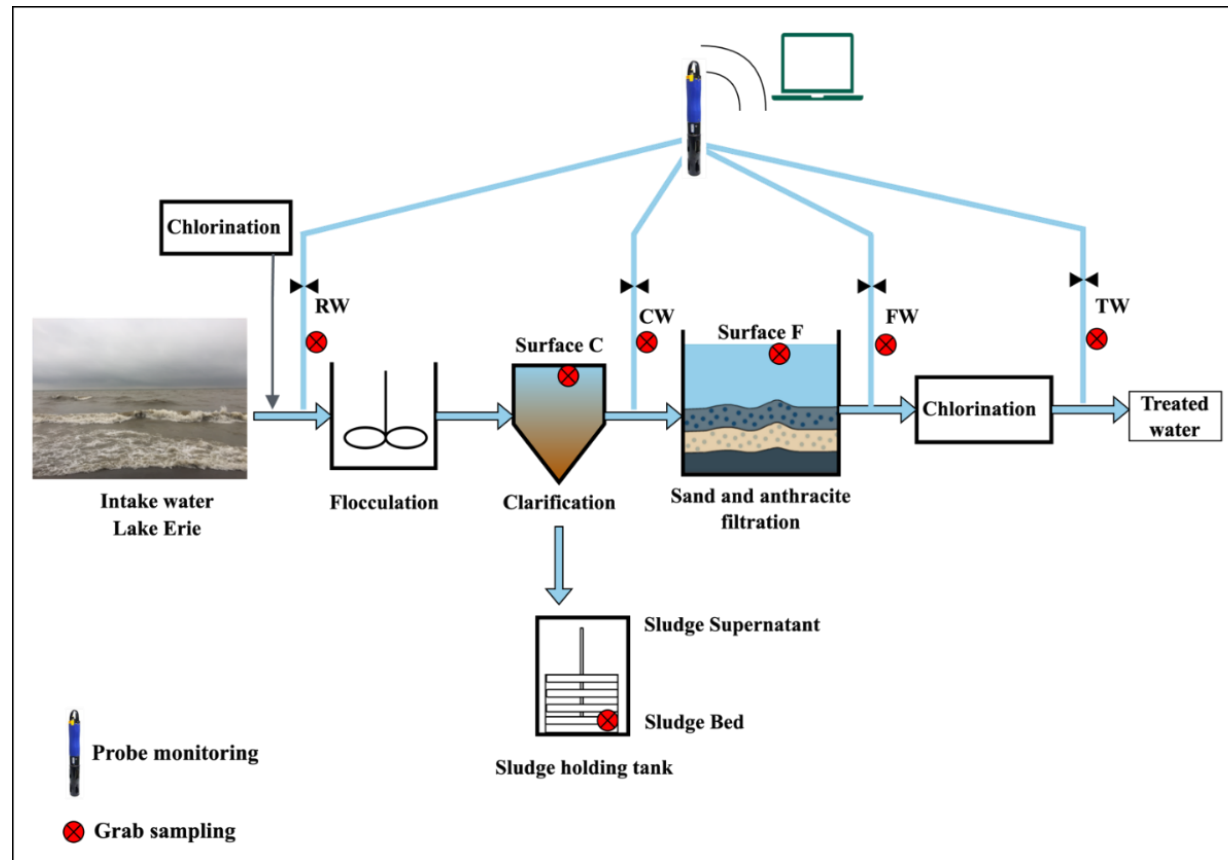


Figure B.3 Schematic of the treatment processes of DWTP C, grab sampling and probe monitoring locations. The water intake is from Lake Erie. Sampling points are indicated by: raw water (RW), clarified water (CW), filtered water (FW), treated water (TW), surface of clarifier (Surface C), surface of filter (Surface F), and sludge bed of sludge holding tank (Sludge Bed)

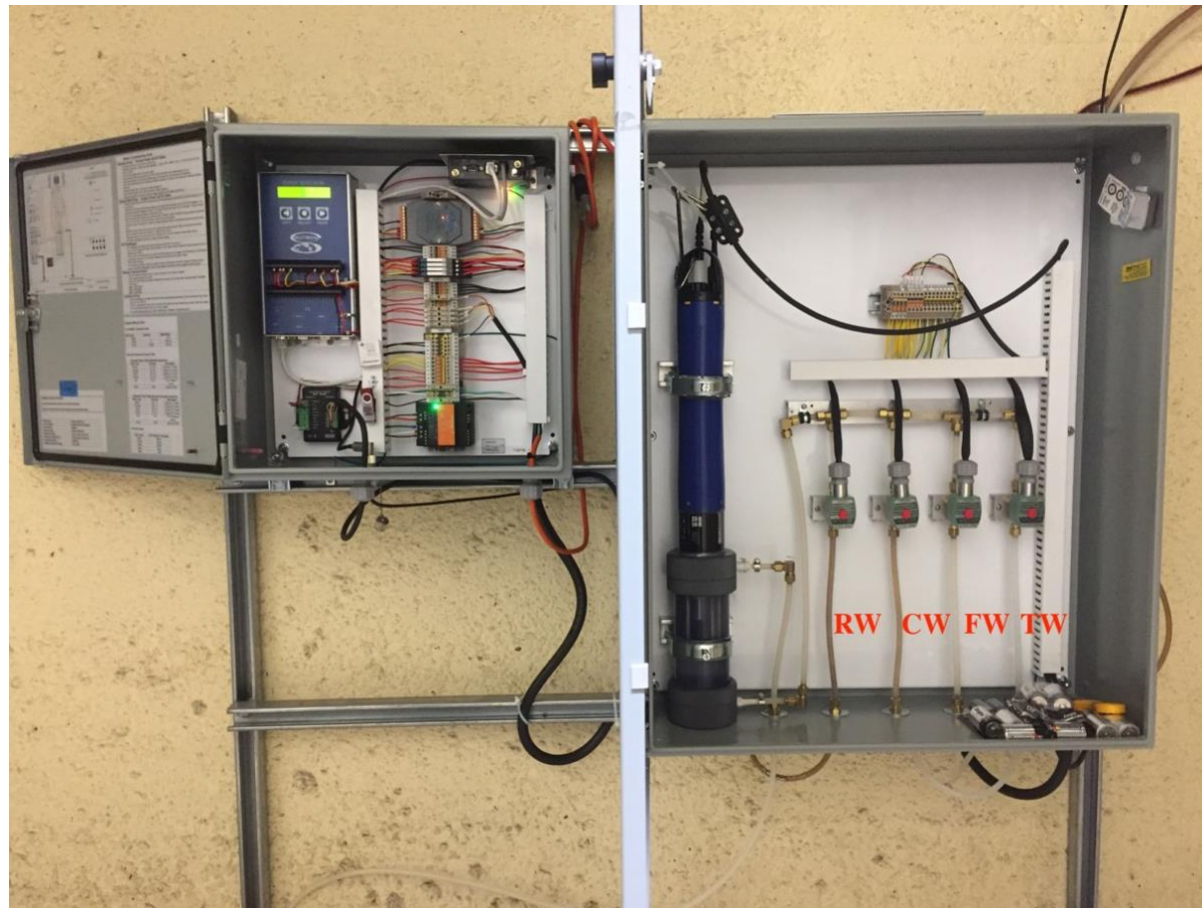


Figure B.4 Example of YSI EXO2 probe data collection system at the studied three DWTPs. Sampling points are indicated by: raw water (RW), clarified water (CW), filtered water (FW) and treated water (TW)

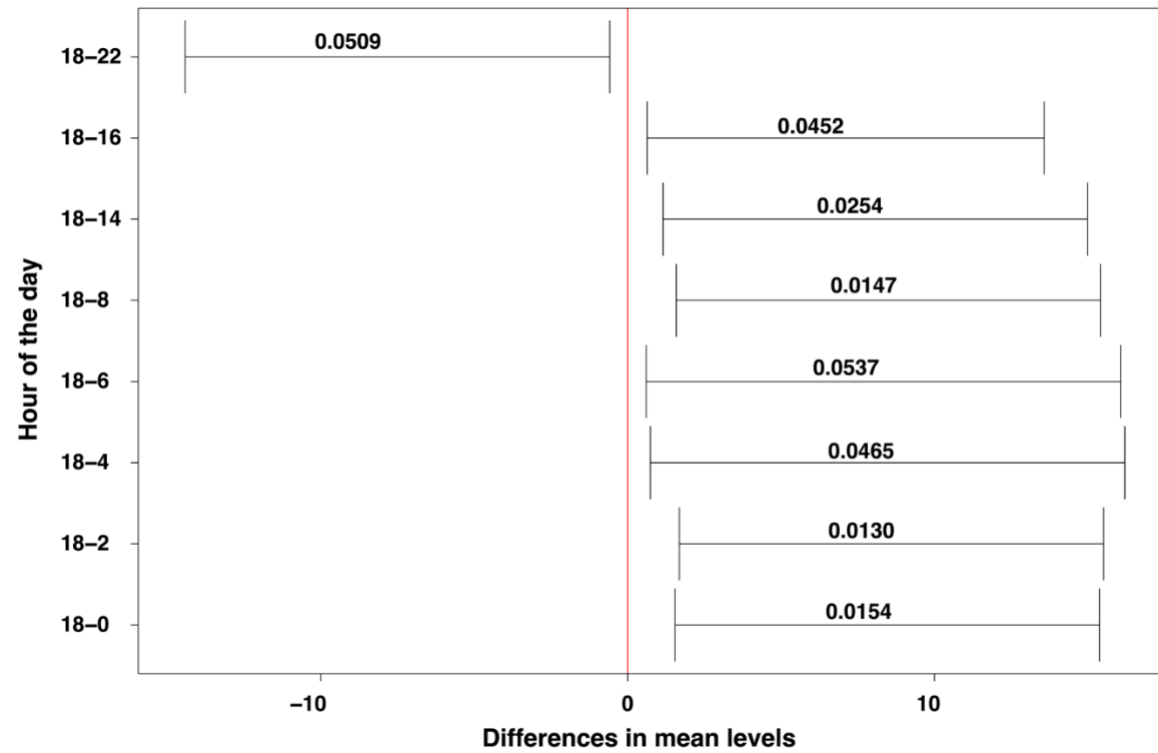


Figure B.5 Post-hoc analysis (Tukey's HSD) of the phycocyanin RFU readings during July following ANOVA with group variables of 18 h and the other measured times (significance level $\alpha = 0.1$). Numbers in the graph indicate the adjusted p value

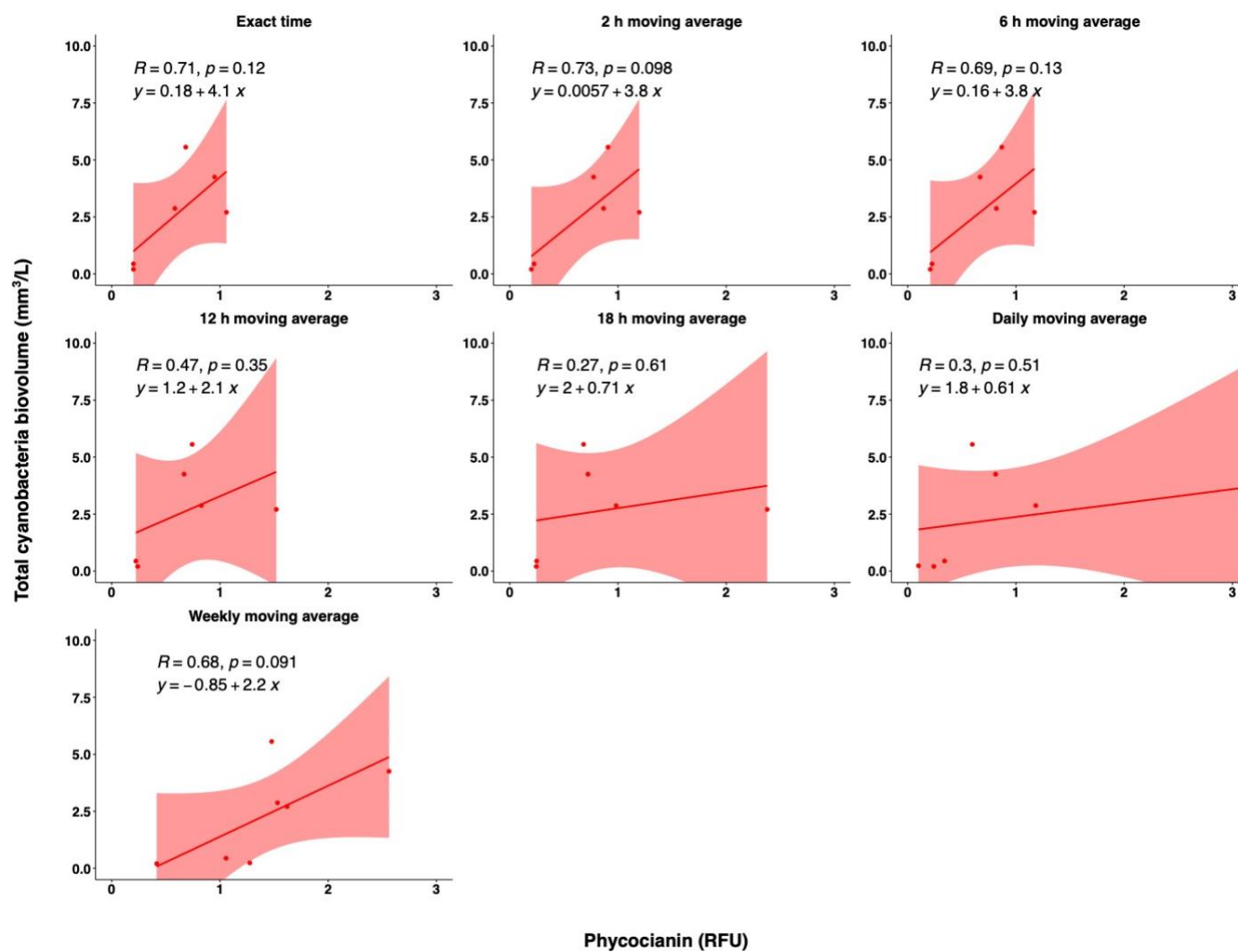


Figure B.6 Correlations between phycocyanin (RFU) YSI EXO2 probe readings computed by different time series analysis (exact time, 2, 6, 12 and 18-h moving averages, daily moving average and weekly moving average) and total cyanobacterial biovolume (mm³/L) from grab samples of the raw water of DWTP A

Table B.1 Details of treatment process characteristics of the studied three DWTPs

Parameters	DWTP A	DWTP B	DWTP C
Source water entering the plant intake	Missisquoi Bay	Yamaska Reservoir	Lake Erie
Hydraulic retention time	Sludge blanket clarifier: 1.2–2.9 h Filter: 0.7–1.6 h	Sludge blanket clarifier: 1.43–2.54 h Filter: 0.28–0.56 h	Clarifier: 1.7 h (average) Filter: 0.1 h (average)
Pre-treatment prior to coagulation	N/A	Lime: 5.7–13.08 mg/L	0.3–0.5 mg/L total chlorine as residuals
Coagulant	0.08 L PAX/L (average)	ALS-2260 (Alufer) and PIX-312 (Ferric Sulfate): 42.64–83.65 µg/L	Hyperion 1090: 15–20 mg/L
Polymer	N/A	Superfloc C-492PWG: 0.12 mg/L	Magnafloc LT 22: 0.1–0.15 mg/L
PAC (powdered activated carbon)	3.7 mg/L (average)	9.95–14.94 mg/L	3–4 mg/L
Post chlorination	0.04–3.08 mg/L total chlorine as residuals	NaOCl: 0.99–1.06 mg/L	1.7 mg/L total chlorine as residuals

APPENDIX C SUPPLEMENTARY INFORMATION, ARTICLE 3

Journal: Submitted to *Journal of Environmental Management*

Title: A framework to use *in vivo* phycocyanin readings for cyanobacterial risk assessment within drinking water treatment plants

Authors: Liya Ma, Sarah Dorner, and Michèle Prévost

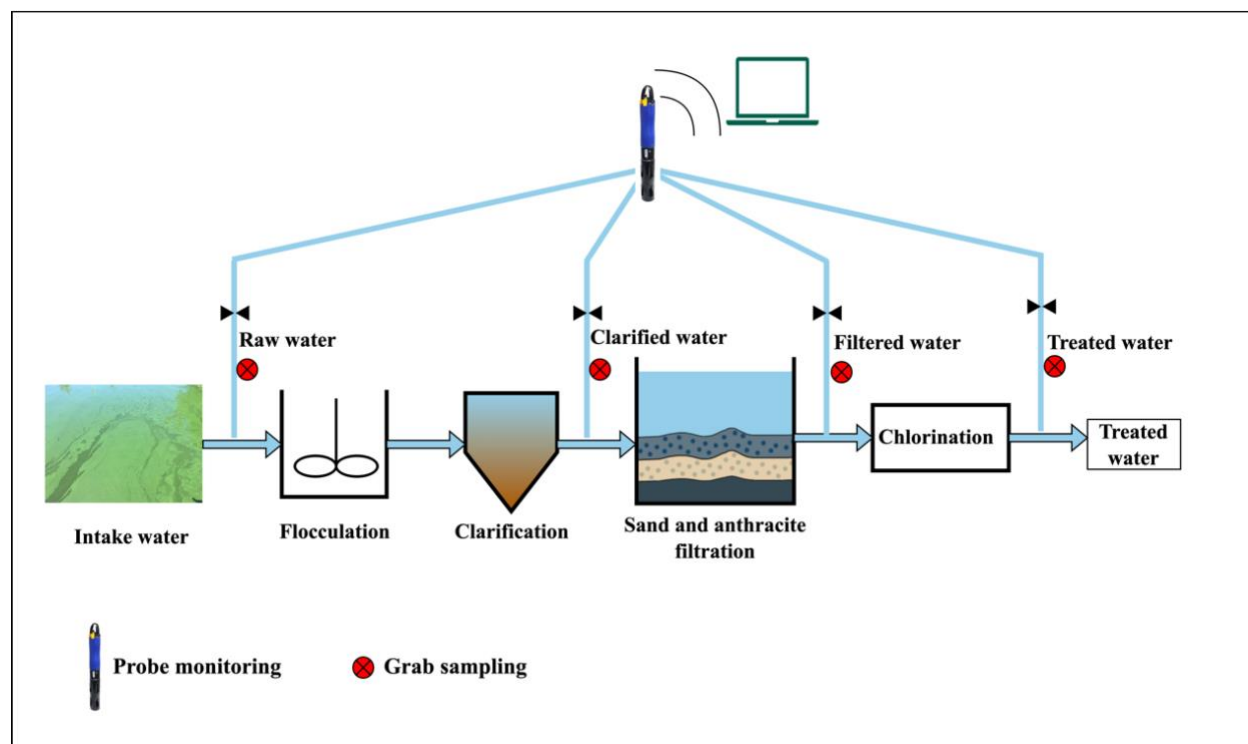


Figure C.1 Schematic of the treatment processes of the studied three DWTPs, grab sampling and probe monitoring locations

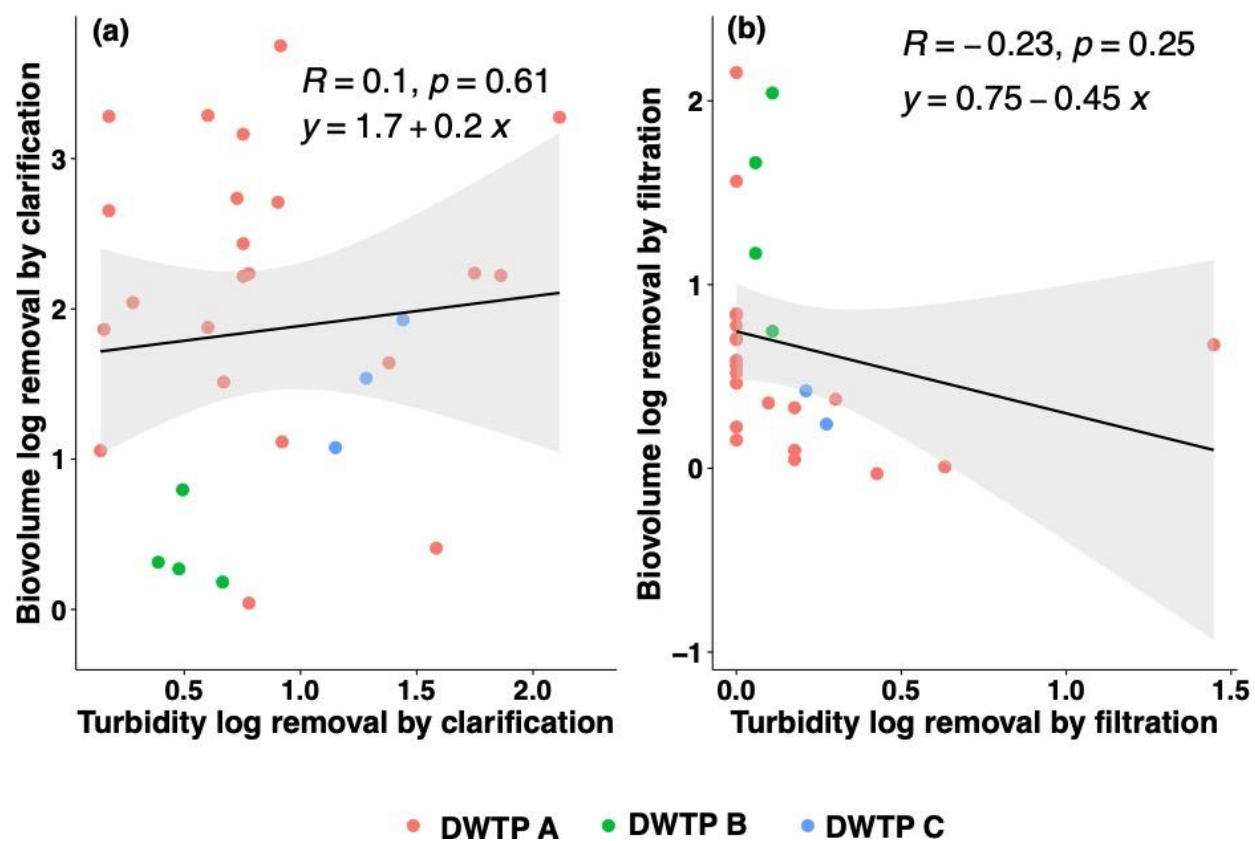


Figure C.2 Log removal of total cyanobacterial cell biovolume (mm³/L) versus log removal of turbidity (NTU) by (a) clarification, and (b) filtration

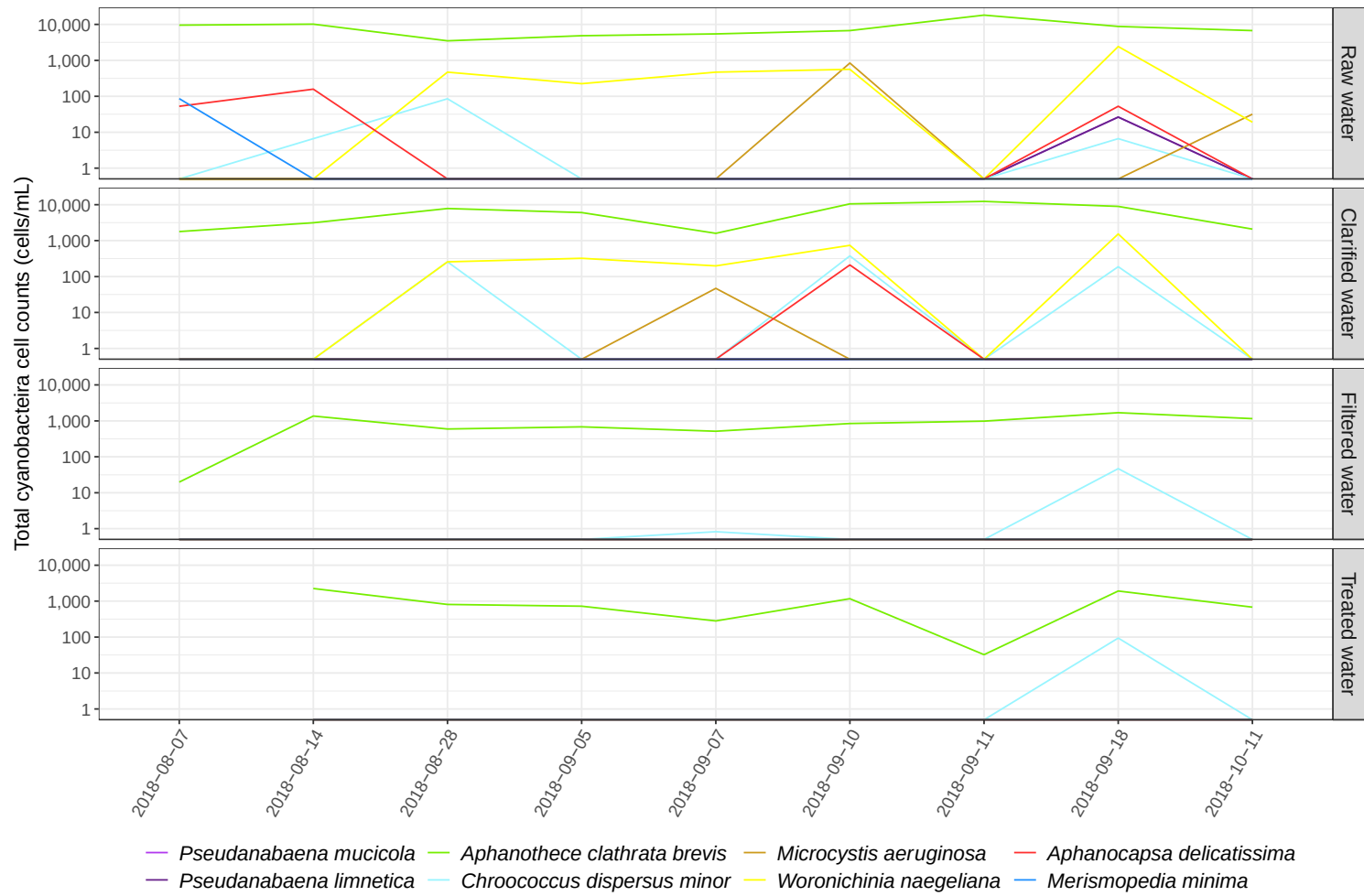


Figure C.3 Cyanobacteria speciation evaluated as cell counts (cells/mL) in the raw, clarified, filtered, and treated water in DWTP B

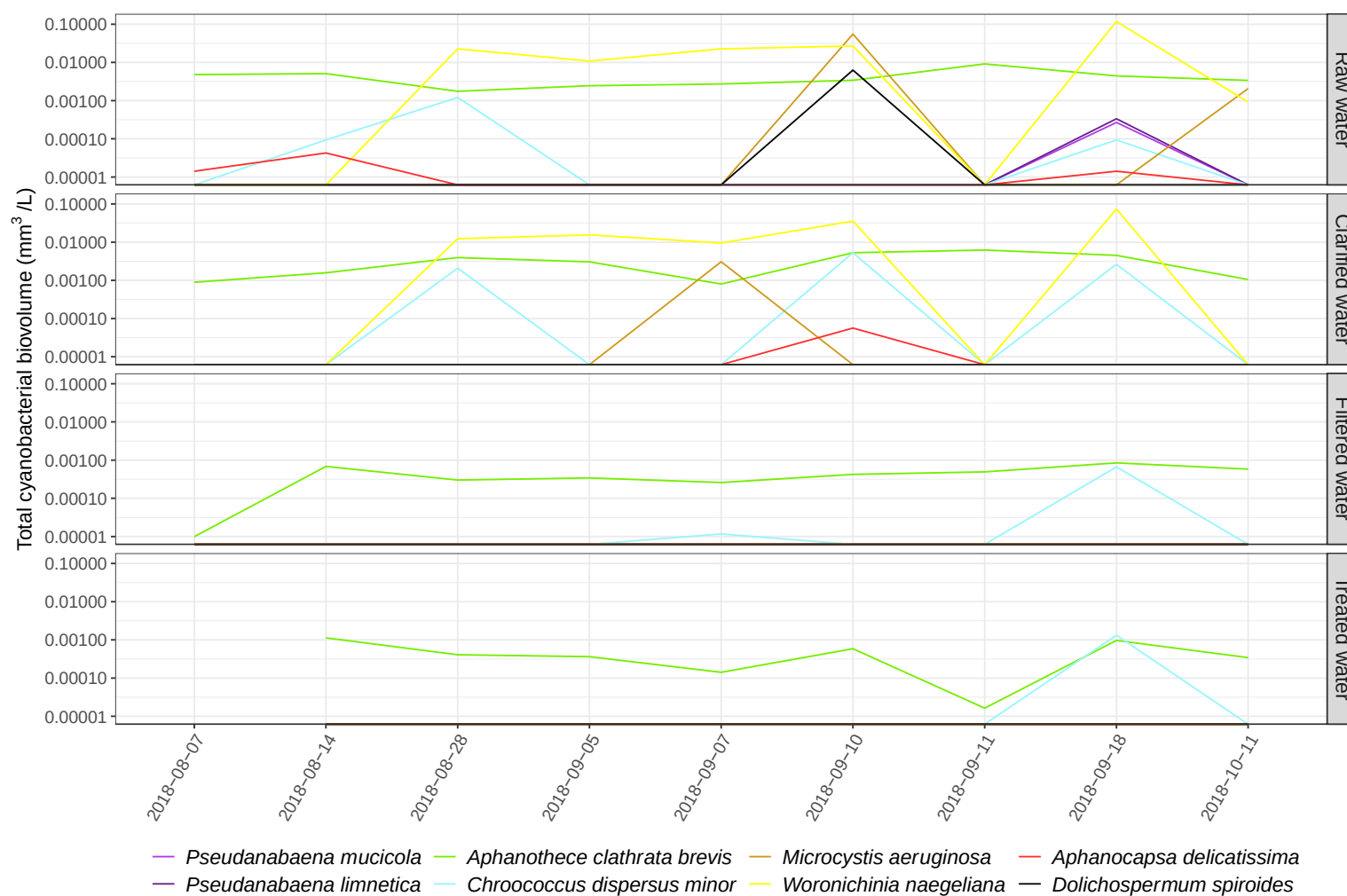


Figure C.4 Cyanobacteria speciation evaluated as cell biovolumes (mm^3/L) in the raw, clarified, filtered, and treated water in DWTP B

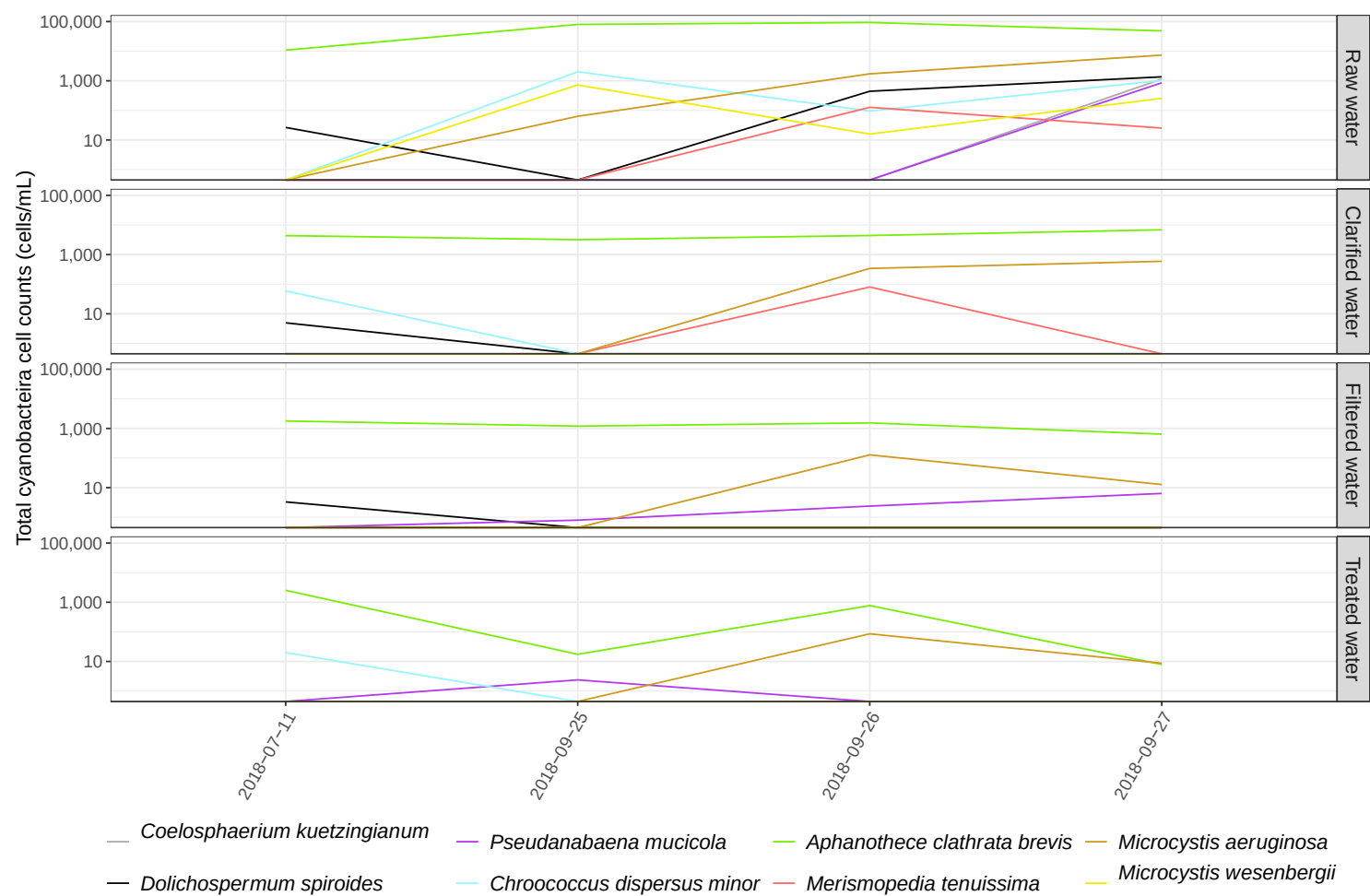


Figure C.5 Cyanobacteria speciation evaluated as cell counts (cells/mL) in the raw, clarified, filtered, and treated water in DWTP C

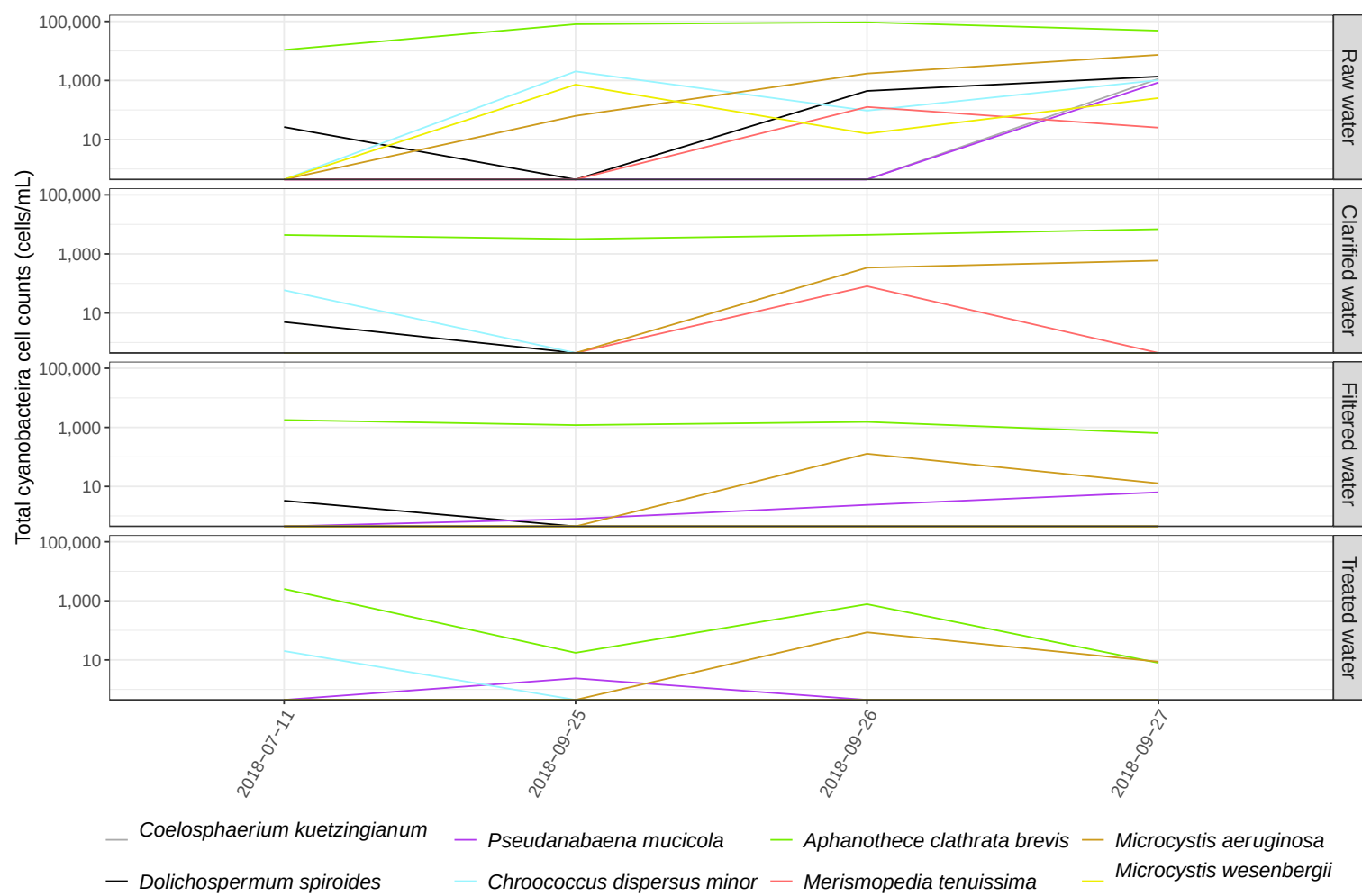


Figure C.6 Cyanobacteria speciation evaluated as cell biovolumes (mm^3/L) in the raw, clarified, filtered, and treated water in DWTP C

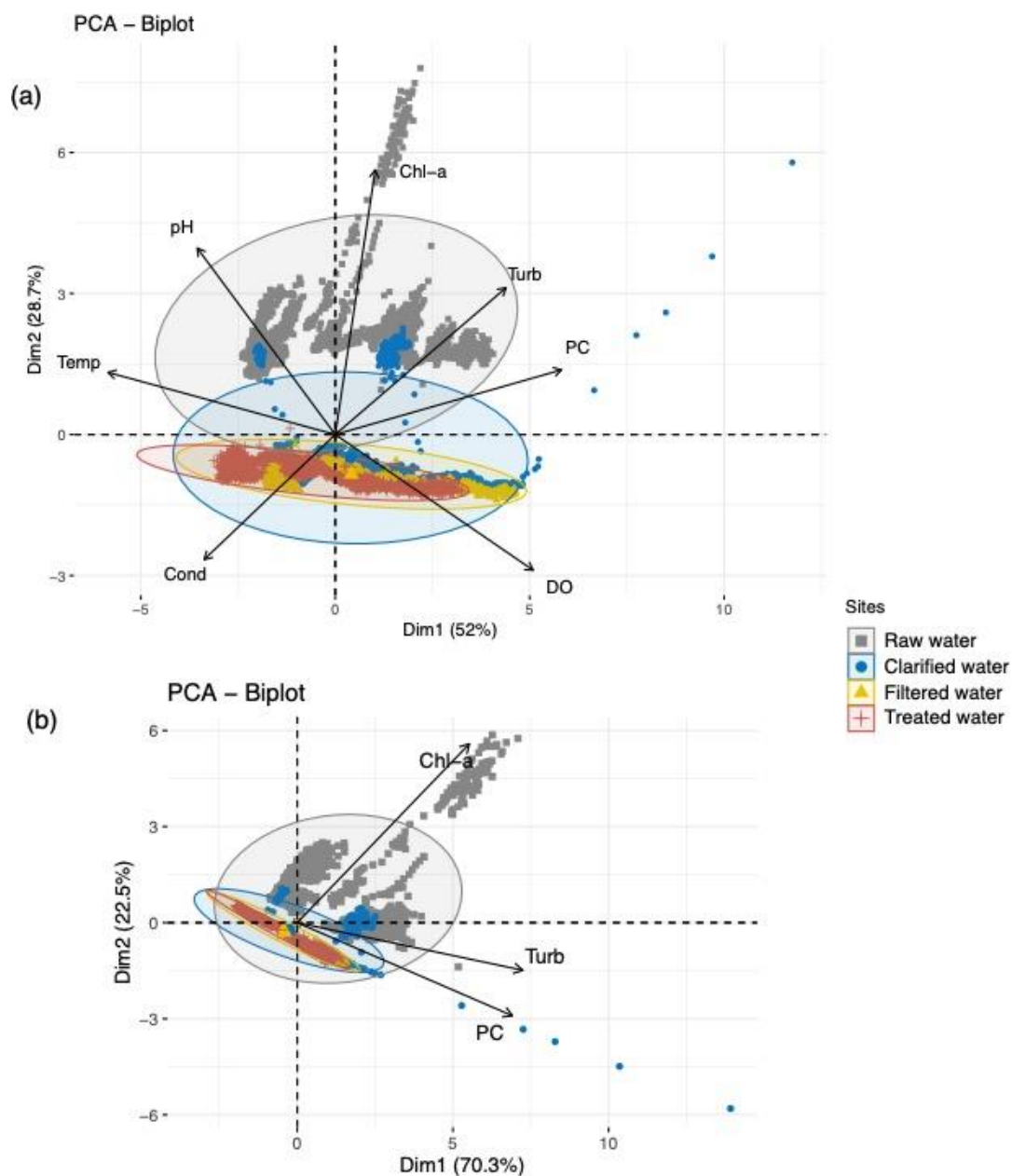


Figure C.7 Principal component analysis (PCA) as *biplot* of the physio-chemical parameters of the raw, clarified, filtered, and treated water in DWTP B. Parameters are (a) PC = phycocyanin, Turb = turbidity, Chl-a = chlorophyll-a, DO = dissolved oxygen, Cond = conductivity, Temp = temperature and pH, (b) PC, Turb and Chl-a

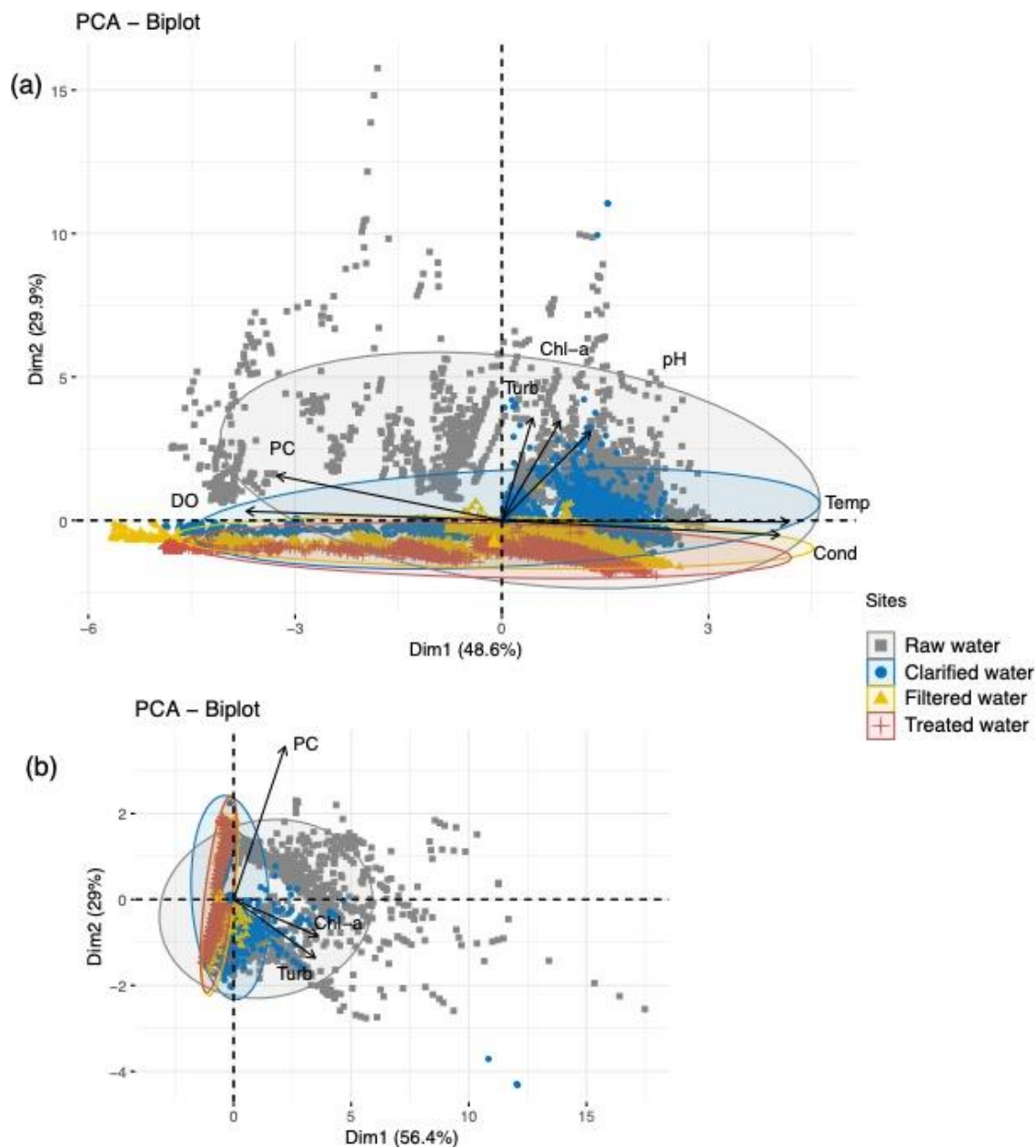


Figure C.8 Principal component analysis (PCA) as *biplot* of the physio-chemical parameters of the raw, clarified, filtered, and treated water in DWTP C. Parameters are (a) PC = phycocyanin, Turb = turbidity, Chl-a = chlorophyll-a, DO = dissolved oxygen, Cond = conductivity, Temp = temperature and pH, (b) PC, Turb and Chl-a

Table C.1 Log removal of the most abundant cyanobacterial species (cell counts and biovolumes) by clarification, filtration, and post-chlorination of DWTP A

Species		Clarification		Filtration		Post-chlorination	
		mean	SD	mean	SD	mean	SD
Cell counts (cells/mL)	<i>Aphanothece clathrata brevis</i>	1.2	0.5	0.2	0.3	0.0	0.2
	<i>Aphanocapsa delicatissima</i>	2.7	NA	NA	NA	NA	NA
	<i>Microcystis aeruginosa</i>	3.0	0.7	0.6	NA	0.0	NA
	<i>Dolichospermum spiroides</i>	2.6	0.6	-0.3	0.3	NA	NA
	<i>Pseudanabaena mucicola</i>	1.0	1.1	-0.1	0.6	-0.1	0.3
	<i>Chroococcus dispersus minor</i>	∞	NA	NA	NA	NA	NA
	<i>Aphanocapsa holsatica</i>	∞	NA	NA	NA	NA	NA
	<i>Chroococcus minimus</i>	∞	NA	NA	NA	NA	NA
Biovolume (mm ³ /L)	<i>Dolichospermum spiroides</i>	2.6	0.6	-0.3	0.3	NA	NA
	<i>Microcystis aeruginosa</i>	3.0	0.7	0.6	NA	0.0	NA
	<i>Microcystis wesenbergii</i>	∞	NA	NA	NA	NA	NA
	<i>Dolichospermum circinalis</i>	∞	NA	NA	NA	NA	NA
	<i>Aphanothece clathrata brevis</i>	1.2	0.5	0.2	0.3	0.0	0.2
	<i>Chroococcus dispersus minor</i>	∞	NA	NA	NA	NA	NA
	<i>Pseudanabaena mucicola</i>	1.0	1.1	-0.1	0.6	-0.1	0.3
	<i>Merismopedia punctata</i>	∞	NA	NA	NA	NA	NA

Table C.2 Log removal of the most abundant cyanobacterial species (cell counts and biovolumes) by clarification, filtration, and post-chlorination of DWTP B

Species		Clarification		Filtration		Post-chlorination	
		mean	SD	mean	SD	mean	SD
Cell counts (cells/mL)	<i>Aphanothece clathrata brevis</i>	0.2	0.4	0.9	0.5	0.2	0.6
	<i>Woronichinia naegeliana</i>	0.1	0.2	NA	NA	NA	NA
	<i>Microcystis aeruginosa</i>	∞	NA	NA	NA	NA	NA
	<i>Aphanocapsa delicatissima</i>	∞	NA	NA	NA	NA	NA
	<i>Chroococcus dispersus minor</i>	∞	NA	NA	NA	NA	NA
	<i>Merismopedia minima</i>	∞	NA	NA	NA	NA	NA
	<i>Pseudanabaena mucicola</i>	∞	NA	NA	NA	NA	NA
	<i>Pseudanabaena limnetica</i>	∞	NA	NA	NA	NA	NA
Biovolume (mm ³ /L)	<i>Woronichinia naegeliana</i>	0.1	0.2	NA	NA	NA	NA
	<i>Microcystis aeruginosa</i>	∞	NA	NA	NA	NA	NA
	<i>Aphanothece clathrata brevis</i>	0.2	0.4	0.9	0.5	0.2	0.6
	<i>Dolichospermum spiroides</i>	∞	NA	NA	NA	NA	NA
	<i>Chroococcus dispersus minor</i>	∞	NA	NA	NA	NA	NA
	<i>Pseudanabaena limnetica</i>	∞	NA	NA	NA	NA	NA
	<i>Pseudanabaena mucicola</i>	∞	NA	NA	NA	NA	NA
	<i>Aphanocapsa delicatissima</i>	∞	NA	NA	NA	NA	NA

Table C.3 Log removal of the most abundant cyanobacterial species (cell counts and biovolumes) by clarification, filtration, and post-chlorination of DWTP C

Species		Clarification		Filtration		Post-chlorination	
		mean	SD	mean	SD	mean	SD
Cell counts (cells/mL)	<i>Aphanothece clathrata brevis</i>	1.0	0.5	0.6	0.3	1.0	1.1
	<i>Microcystis aeruginosa</i>	0.9	0.3	1.0	0.9	0.2	0.0
	<i>Chroococcus dispersus minor</i>	∞	NA	NA	NA	NA	NA
	<i>Dolichospermum spiroides</i>	0.7	NA	0.2	NA	NA	NA
	<i>Coelosphaerium kuetzingianum</i>	∞	NA	NA	NA	NA	NA
	<i>Microcystis wesenbergii</i>	∞	NA	NA	NA	NA	NA
	<i>Pseudanabaena mucicola</i>	∞	NA	NA	NA	NA	NA
	<i>Merismopedia tenuissima</i>	0.2	NA	NA	NA	NA	NA
Biovolume (mm ³ /L)	<i>Microcystis aeruginosa</i>	1.0	0.5	0.9	1.1	0.3	0.2
	<i>Dolichospermum spiroides</i>	0.7	NA	0.2	NA	NA	NA
	<i>Aphanothece clathrata brevis</i>	1.0	0.5	0.6	0.3	1.0	1.1
	<i>Microcystis wesenbergii</i>	∞	NA	NA	NA	NA	NA
	<i>Chroococcus dispersus minor</i>	∞	NA	NA	NA	NA	NA
	<i>Coelosphaerium kuetzingianum</i>	∞	NA	NA	NA	NA	NA
	<i>Pseudanabaena mucicola</i>	∞	NA	NA	NA	NA	NA
	<i>Aphanizomenon flos-aquae</i>	∞	NA	NA	NA	NA	NA

Table C.4 Physicochemical variables for the raw water, clarified water, filtered water, and treated water of the studied three DWTPs

DWTPs	Parameters	Units	Raw Water			Clarified Water			Filtered Water			Treated Water		
			Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
DWTP A	PC	RFU	1.1	0.0	38.2	0.1	0.0	10.1	0.0	0.0	0.1	0.0	0.0	0.1
	Chl-a	RFU	1.3	0.2	4.5	0.1	0.0	2.8	0.0	0.0	0.3	0.0	0.0	0.3
	DO	mg/L	5.8	0.5	14.5	7.1	5.0	13.4	6.6	3.0	11.3	7.3	5.7	8.6
	TDS	mg/L	91.0	84.0	106.0	106.8	86.3	132.9	107.6	98.5	133.2	116.9	102.0	149.7
	Temperature	°C	25.7	23.0	28.2	26.4	23.2	28.7	27.6	23.1	33.0	26.1	23.1	28.0
	Turbidity	NTU	7.3	1.6	63.6	0.6	0.0	25.2	0.7	0.0	77.3	0.4	0.0	4.7
	Conductivity	µS/cm	141.8	132.0	165.5	168.6	135.3	210.4	173.8	153.0	226.0	183.9	153.0	237.3
	pH		7.3	6.5	9.4	6.6	6.2	9.3	6.5	6.2	7.1	6.9	6.4	8.1
DWTP B	PC	RFU	0.1	0.0	0.4	0.1	0.0	0.4	0.1	0.0	0.4	0.1	0.0	0.3
	Chl-a	RFU	0.5	0.2	2.3	0.0	0.0	0.5	0.0	0.0	0.1	0.0	0.0	0.1
	DO	mg/L	6.9	5.0	10.8	8.2	5.8	11.8	8.5	6.3	11.8	8.1	6.7	11.3
	TDS	mg/L	110.3	98.8	126.7	119.8	105.0	135.2	125.0	115.0	139.3	153.4	140.9	167.0
	Temperature	°C	22.8	12.8	27.8	21.0	9.6	26.1	20.6	9.5	26.1	21.9	12.4	26.5
	Turbidity	NTU	1.3	0.0	7.5	0.4	0.0	20.2	0.2	0.0	1.0	0.2	0.0	2.0
	Conductivity	µS/cm	163.5	141.8	175.5	169.2	143.4	182.4	175.1	146.2	205.0	221.6	182.0	243.1
	pH		7.1	6.1	8.1	5.5	4.6	7.9	5.3	4.6	6.5	6.4	5.4	7.9
DWTP C	PC	RFU	0.0	0.0	0.3	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.1
	Chl-a	RFU	0.2	0.0	2.4	0.0	0.0	1.1	0.0	0.0	0.2	0.0	0.1	0.1
	DO	mg/L	8.5	3.9	11.1	8.6	5.0	11.2	8.9	5.1	13.7	8.6	11.2	11.2
	TDS	mg/L	153.6	145.0	165.0	155.0	146.3	166.0	156.1	148.0	166.0	156.6	166.0	166.0
	Temperature	°C	22.4	12.0	26.4	21.9	11.3	26.0	22.1	11.7	26.0	21.6	25.6	25.6
	Turbidity	NTU	9.7	1.3	98.8	3.0	0.0	78.3	1.0	0.0	15.9	0.5	8.1	8.1
	Conductivity	µS/cm	224.6	172.3	253.0	224.5	173.3	252.0	226.7	176.7	253.8	225.4	252.0	252.0
	pH		8.4	7.8	8.9	8.2	7.7	8.6	7.9	7.5	8.2	7.7	7.9	7.9

Table C.5 Alert level frames, standards and guideline values of countries and regions for cyanobacteria and cyanotoxin in drinking water supplies

Cell counts and/or cell biovolume guidelines	Cyanotoxin guidelines (value in µg/L)	References
WHO		
Vigilance Level in raw water: ≥ 10 colony/mL or ≥ 50 filaments/mL Alert level 1 in raw water: ≥ 0.3 mm ³ /L total CB biovolume Alert Level 2 in raw water: ≥ 4 mm ³ /L total CB biovolume	Alert level 1 in raw water: Microcystin: 1, or Cylindrospermopsin: 0.7, or Anatoxin: 3, or Saxitoxins: 0.3 Alert level 2 in raw water: Microcystin: 12, or Cylindrospermopsin: 3, or Anatoxin: 30, or Saxitoxins: 3	(Chorus et al., 2021b)
USEPA		
Not specified	Microcystin: Bottle-fed infants and pre-school children: 0.3 School-age children and adults: 1.6 Cylindrospermopsin: Bottle-fed infants and pre-school children: 0.7 School-age children and adults: 3.0	(United States Environmental Protection Agency (USEPA), 2015)
Ohio EPA		
Not specified	Microcystins: Do not drink for children under 6 and sensitive populations: 0.3 Do not drink for children 6 and older and adults: 1.6 Cylindrospermopsin: Do not drink for children under 6 and sensitive populations: 0.7 Do not drink for children 6 and older and adults: 3.0 Anatoxin-a: Do not drink for children under 6 and sensitive populations: 0.3 Do not drink for children 6 and older and adults: 1.6 Saxitoxins: Do not drink for children under 6 and sensitive populations: 0.3 Do not drink for children 6 and older and adults: 1.6	(Ohio EPA, 2022)

Table C.5 Alert level frames, standards and guideline values of countries and regions for cyanobacteria and cyanotoxin in drinking water supplies (cont'd)

Cell counts and/or cell biovolume guidelines	Cyanotoxin guidelines (value in µg/L)	References
Australia—NHMRC		
Initial notification in raw water: ≥ 4500 cells/mL (0.2 mm³/L) <i>Cylindrospermopsis raciborskii</i> or ≥ 2000 cells/mL (0.2 mm³/L) <i>Microcystis aeruginosa</i> or ≥ 12,000 cells/ mL (2.7 mm³/L) <i>Nodularia spumigena</i> or ≥ 6,000 cells/ mL (1.5 mm³/L) <i>Dolichospermum circinalis</i>	Cylindrospermopsin: 1 Microcystin: 1.3	(NHMRC, 2022)
Alert in raw water: ≥ 15,500 cells/mL (0.6 mm³/L) <i>Cylindrospermopsis raciborskii</i> or ≥ 6500 cells/mL (0.6 mm³/L) <i>Microcystis aeruginosa</i> or ≥ 40,000 cells/ mL (9.1 mm³/L) <i>Nodularia spumigena</i> or ≥ 20,000 cells/ mL (5 mm³/L) <i>Dolichospermum circinalis</i>	Saxitoxins: 3 Nodularin: 1.3	
New Zealand Ministry of Health		
Vigilance Level in raw water: ≥ 50 cells/mL (excluding picocyanobacteria).		(Ministry of Health, 2020; Ministry of Health, 2022)
Alert level 1 in raw water: ≥ 75 cells/mL for potential cylindrospermopsin producers 		

Table C.6 Comparison of equivalent PC RFU to WHO alert levels (Chorus et al., 2021b)

R ² value	Equation Y: biovolume (mm ³ /L) X: PC (RFU)	Equivalent RFU to WHO 2021 Alert levels		Probe model	Scale of study (dominant species)	Reference
		Alert level 1 ≥ 0.3 mm ³ /L	Alert level 2 ≥ 4 mm ³ /L			
R ² = 0.53	Y = 3.8X + 0.0057	0.07 RFU	1.05 RFU	YSI EXO2	Field (<i>Dolichospermum spiroides</i>)	(Ma et al., 2023)
R ² = 0.46	Y = 1.16X - 1.78	1.8 RFU	5.0 RFU	YSI 6600	Field (<i>Microcystis</i> spp.)	(McQuaid et al., 2011)
R ² ≥ 0.94	Y = 1.1X Y = 3.8X	0.27 RFU 0.07 RFU	3.6 RFU 1.05 RFU	YSI EXO2	Lab (2 strains of <i>M. aeruginosa</i>)	(Choo et al., 2018)
R ² = 0.73	Y = 0.0564X - 0.0025	5.4 RFU	70.9 RFU	YSI EXO2	Field (<i>Microcystis</i> sp.)	(Almuhtaram et al., 2018)
R ² = 0.999	Y = 0.5X	0.6 RFU	8 RFU	YSI 6600	Lab (<i>M. aeruginosa</i>)	(Zamyadi et al., 2012b)
R ² = 0.77 R ² = 0.72 R ² = 0.19	Log Y = 2.0446 log X - 0.3592 Log Y = 1.8458 log X - 0.2318 Log Y = 0.6743 log X + 0.3804	0.83 RFU 0.70 RFU 0.05 RFU	2.95 RFU 2.83 RFU 2.13 RFU	YSI EXO2	3 field locations (mixed species)	(Bowling et al., 2016)
R ² = 0.70	Y = 0.068X	4.4 RFU	58.8 RFU	YSI EXO2	Field (<i>Pseudanabaena</i> sp.)	(Zamyadi et al., 2016b)
0.71 ≤ R ² ≤ 0.81	No equation provided	NA	NA	YSI EXO2	Lab (4 species)	(Bertone et al., 2019)

APPENDIX D SUPPLEMENTARY INFORMATION

We calculated cross-correlation functions of daily mean PC readings and meteorological parameters of eastward wind speed (m/s), relative humidity at 2 meters (%), PAR (W/m²), precipitation (mm/day), and air temperature at 2 meters (°C) of the source water for DWTP A during July and August 2018.

Data for meteorological parameters were downloaded from the NASA POWER using the nasapower package in R (version 4.2.1), and were obtained for the source water for DWTP A (latitude 45.039469, longitude -73.078182). Cross-correlations were computed using the CCF function of the base package of R. The cross-correlation measured the similarity of the first time series and the shifted copies of the second one. The time lag between measurements was estimated by selecting the highest cross-correlation coefficient.

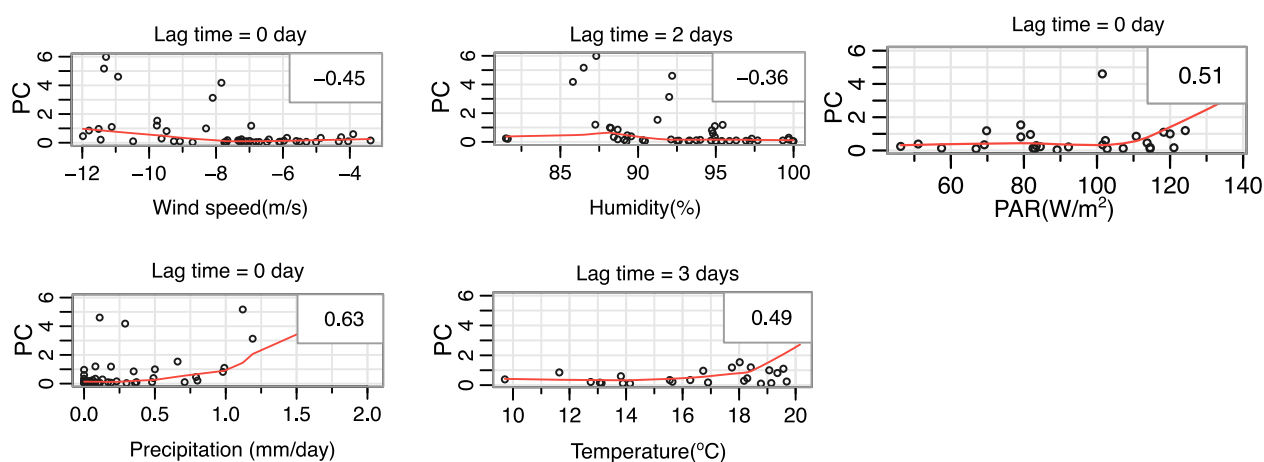


Figure D.1 Cross-correlation between the meteorological parameters and the phycocyanin probe readings at the intake water of DWTP A during July and August of 2018