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Breakthrough of cyanobacteria in bank filtration

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ABSTRACT

The removal of cyanobacteria cells in well water following bank filtration was investigated from a source water consisting of two artificial lakes (A and B). Phycocyanin probes used to monitor cyanobacteria in the source and in filtered well water showed an increase of fluorescence values demonstrating a progressive seasonal growth of cyanobacteria in the source water that were correlated with cyanobacterial biovolumes from taxonomic counts ($r = 0.59$, $p < 0.00001$). A strong correlation was observed between the cyanobacterial concentrations in the lake water and in the well water as measured by the phycocyanin probe ($p < 0.001$, $0.73 \leq r^2 \leq 0.94$). Log removals from bank filtration estimated from taxonomic counts ranged from $0.96 \pm (0.5)$ and varied according to the species of cyanobacteria. Of cyanobacteria that passed through bank filtration, smaller cells were significantly more frequent in well water samples ($p < 0.05$) than larger cells. Travel times from the lakes to the wells were estimated as 2 days for Lake B and 10 days for Lake A. Cyanobacterial species in the wells were most closely related to species found in Lake B. Thus, a travel time of less than 1 week permitted the breakthrough of cyanobacteria to wells. Winter samples demonstrated that cyanobacteria accumulate within bank filters, leading to continued passage of cells beyond the bloom season. Although no concentrations of total microcystin-LR were above detection limits in filtered well water, there is concern that cyanobacterial cells that reach the wells have the potential to contain intracellular toxins.

1. Introduction

Cyanobacteria are prokaryote photosynthetic microorganisms that are of concern because of their ability to produce toxins and taste and odour compounds as well as disrupt drinking water treatment (Scott and Marcarelli, 2012; Zamyadi et al., 2013). The increasing proliferation of cyanobacteria is linked to the eutrophication of water bodies, notably from nitrogen and phosphorus concentrations (Taranu et al., 2015; Whitton and Potts, 2012). Cyanotoxins are most likely to occur following the accumulation of high densities of cyanobacteria in the form of blooms (Rastogi et al., 2014), with the upregulation of toxin production genes occurring when cells reach high numbers (Wood et al., 2011). Several species of cyanobacteria produce a wide range of toxic compounds with many reviews available on cyanotoxin occurrences worldwide (e.g. Gkelis and Zaoutsos, 2014; Rastogi et al., 2014). Given the increasing frequency of cyanobacteria blooms in fresh waters, there is a need for risk management strategies for drinking water suppliers to meet drinking water guidelines (e.g. Chorus, 2005) for the protection of public health (Ibelings et al., 2014; Otten and Paerl, 2015).

In regions with surficial geology appropriate for bank filtration, this technique can be an effective means of improving water quality and controlling a variety of contaminants through natural physical, chemical, and biological processes that occur during ground passage (Tufenkji et al., 2002). Table S1 (Supplementary Information) provides an overview of selected studies on the removal of algae, microbial indicators and cyanotoxins by bank filtration. Although cyanotoxins have been measured in bank filtered water with coccoid cells and filamentous cyanobacteria cell fragments (Lahti et al., 2001), other studies have not reported any cyanobacteria cells in bank filtered water with the exception of Rachman et al. (2014) in one well system with potential direct hydraulic connections to the water source. Previous research on the effectiveness of

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bank filtration for cyanobacterial removal focused on the physiochemical parameters involved in filtration, including sorption (Romero et al., 2014) or the importance of the colmation layer in the removal of cells (Harvey et al., 2015). The removal of cyanobacteria through drinking water treatment processes has shown that some species of cyanobacteria are more likely than others to pass through conventional sand filters and could result in the release of intracellular toxins into treated drinking water (Zamyadi et al., 2012c, 2013). It is unknown whether similar patterns of removal as a function of cyanobacterial species would occur in full-scale bank filtration as no studies have consistently observed the passage of cells (Supplementary Information Table S1). Furthermore, in stratified lakes, different species of cyanobacteria can be present at different depths. It is unknown whether cyanobacteria passing through bank filtration are typically benthic or planktonic species, or whether some species are more effectively removed than others.

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

2. Materials and methods

2.1. Study site

The study site is a bank filtration system located in Southern Quebec (Canada) near the Lake of Two Mountains. The bank filtration system consists of eight wells that pump water through the bank from two artificial lakes, A and B (Fig. 1). The lakes are not treated to control cyanobacterial blooms. An approximately 85 m wide bank separates the two lakes within which are located the wells. In order to supply the population with drinking water, seven of the eight wells (with the 8th as a stand-by pump) produce a mean daily flow rates of 8100 m^3/day. The distance of Lake A to the well field is approximately 64 m and 26 m for Lake B. Lake B was created as a result of many years of sand quarrying, and Lake A was created immediately after Lake B and remains an active sand quarry. Both lakes have a maximum depth of greater than 10 m (Richard et al., 2010).

The aquifer reservoir supplying the wells receives lateral inflows of groundwater from Lakes A and B. The local geology of the aquifer is alluvial sand filling a palaeo valley carved in the clays of the Champlain Sea (Richard et al., 2010). The aquifer section has a maximum thickness of 25–26 m, with a thickness of 21 m near the production wells. The aquifer sand is characterized by textures of medium to fine sands, with gravel in some locations. The sand bank filter materials vary from grain sizes of 0.08–2.5 mm from samples collected near the wells. Three classes of sands are present around the wells: a) a yellow sand with a low percent of silt is present in the first 6 m layer, b) a middle layer consisting of 18 m of fine beige sands, and c) the bottom layer (<2 m) consisting of fine sand and silt. The uniformity coefficient is 1.9–2.5. The mean hydraulic conductivity is 2.7×10^{-1} cm/s (Richard et al., 2010).

Results from a detailed hydrogeological model of the system were available as the model was used in the permitting process for the municipal wells (Richard et al., 2010). The regional water balance and the effects of municipal well pumping were estimated using MODFLOW-2000 (Harbaugh et al., 2000), a groundwater model under steady state and transient conditions. Measurements of water levels were conducted at 8 production wells, 9 piezometers, both Lakes A and B to calibrate the transient model. The regional water balance demonstrated that an unnamed creek acts as a regional drain entering and leaving Lake A. Lake A receives inflow from the alluvium as well as runoff from the unnamed creek that drains a predominantly agricultural watershed. No stream flows enter Lake B, although a housing development along its shoreline has limited the availability of continuous buffer strips that could mitigate lawn fertilizer loads to the lake. The vertical infiltration recharge rate to the aquifer is 310 mm/year. The horizontal velocities calculated by the model were 0.35 m/d from the edge of Lake A and 7.8 m/d from Lake B. The travel time from Lake B was 2 days and 10 days for Lake A. Results from the transient model estimate that approximately 80% of the water supply comes from the southwest (Lake A) and 20% from the northeast (Lake B) (Richard et al., 2010).

2.2. Water and sediment sampling and analysis

Lake and well water monitoring consisted of measurements conducted with: 1) an *in situ* YSI multi-parameter probe model YSI 6600 V2-4 (YSI, Yellow Spring, Ohio, USA) and 2) grab samples for phytoplankton taxonomic counts, cyanotoxin and nutrient concentrations. The *in situ* multi-probe measured phycocyanin (PC) (Relative Fluorescence Units (RFU)), Chlorophyll *a* (Chl *a,* µg/l or RFU), temperature (°C), specific electrical conductivity (mS), turbidity (NTU), DO (mg/l), and pH. A description of the probe and its use including calibration is provided in McQuaid et al. (2011).

The phosphorus and nitrogen concentrations (total phosphorus, orthophosphate and Total Kjeldahl Nitrogen (TKN)) from two measurement points in Lake A (A2 and A4), one measurement point in Lake B (B1) and well water were performed twice during the sampling period. Orthophosphates, total phosphorus, and Total Kjeldahl Nitrogen (TKN) were analyzed as per Standard Methods (APHA et al., 2012).

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

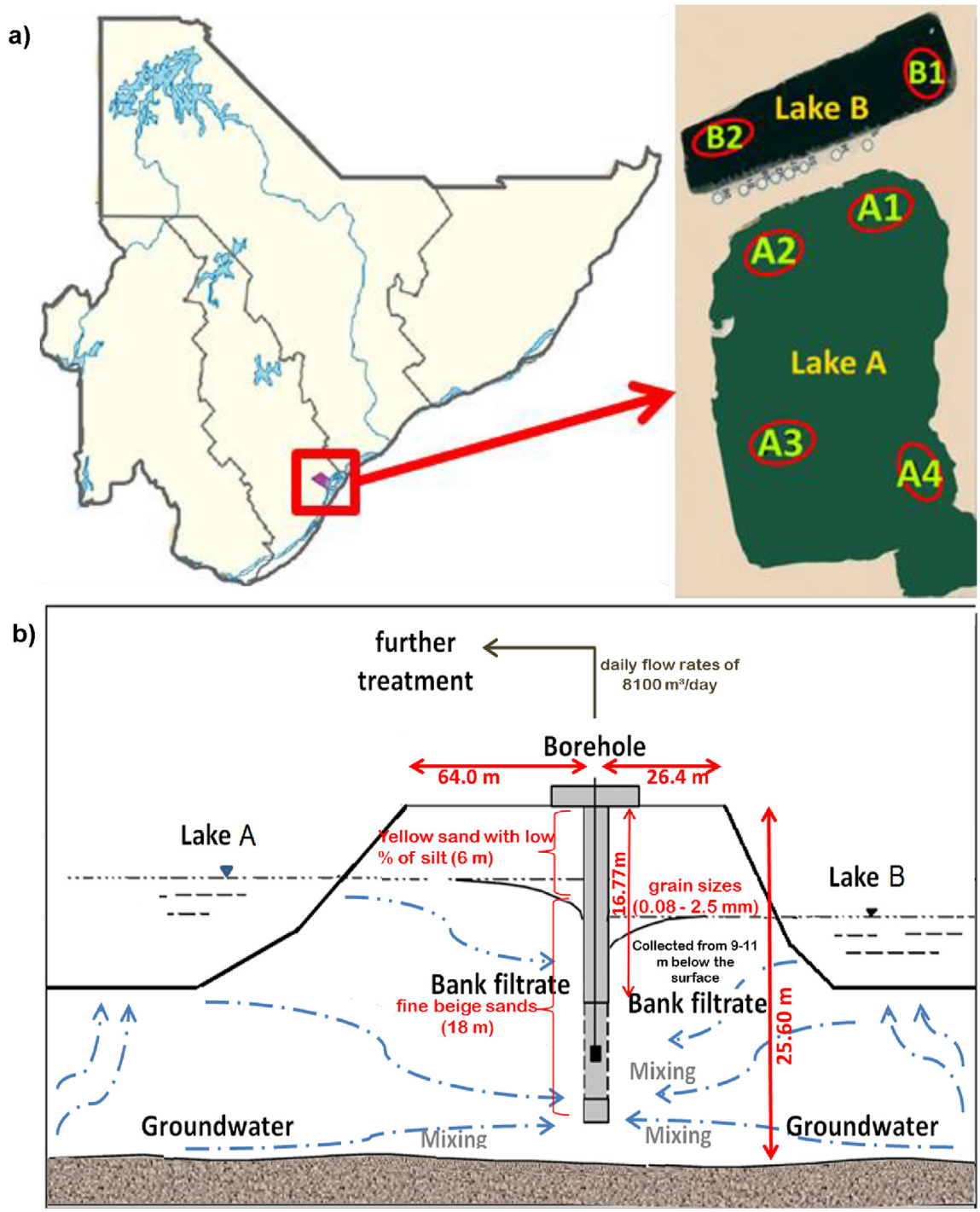

Fig. 1. a) Location of the study site, Lake A and Lake B situated in southern Quebec, Canada; b) Schematic of bank filtration system.

counts and preserved with Lugol's iodine solution directly after sampling and stored at 4 °C; and 2) toxin analyses and frozen prior to analysis for microcystin-LR within 7 days.

Following the late summer-early fall bloom period, sediment samples were collected twice in the winter to measure the accumulation of cyanobacteria in lake sediments. Sediment samples were collected from the surface to a depth of 30 cm using a Wildco® Hand Core Sediment Sampler with 30 cm Liner Core Tube. Lake sediment core samples were taken at 10 m below the surface of both lakes on the January 23 and March 13, 2014 in the presence of a stable ice cover. The samples were taken from the same point for both days on both lakes near the banks. Water samples were also collected from both lakes and the well water on the January 23 and March 13, 2014.

Microcystin-LR measurements were performed using ELISA kits (an enzyme-linked immunosorbent assay - Abraxis LLC, Pennsylvania, USA) with a detection limit of 0.15 μg L^{-1} MCLR eq. Cyanobacterial cells were lysed by three freeze-thaw cycles as described by (McQuaid et al., 2011) in order to measure total (intra and extracellular) toxins. Taxonomic analyses were conducted in

Limnology and Aquatic Environment Laboratory (GRIL) at the University of Quebec at Montreal (UQAM) using an inverted microscope as described in (McQuaid et al., 2011). Microbial indicators (total coliforms, enterococci, *Escherichia coli* and aerobic spores) were analyzed from samples collected over the course of 9 weeks from both lakes and in bank-filtered well water using Standard Methods (APHA et al., 2012). Water samples were collected for microbial indicators as per cyanobacterial samples.

2.3. Statistical analyses and log removal calculations

Probe and cyanobacteria biovolume data were lognormally distributed and were thus log transformed prior to statistical analyses. Statistical analyses (correlation analyses, linear regressions, Mann-Whitney U Tests) were conducted using the Statistica 12 software package (Statsoft, Tulsa, Oklahoma, USA). Origin6.0 (OriginLab, Northampton, England) and Microsoft Excel (Microsoft, Redmond, WA) were used as graphing and data analysis software packages. Depth profiles using interpolated phycocyanin RFU data (from the PC multi-probe) from both lakes and well water were used to estimate mean phycocyanin concentrations for each lake and were compared to RFU data from well water. A principal component analysis (PCA) was used to compare mean biovolumes of cyanobacteria species among sites using the R program, version 3.1.3 (Borcard et al., 2011). A Hellinger transformation was applied on data for PCA analyses to reduce the asymmetry of the species distributions since the distribution was composed of heavily skewed abundance data (Legendre and Legendre, 2012). The length of a PCA vector is proportional to the importance of the descriptor to the sites.

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

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

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

A second method for calculating cyanobacterial log removal used interpolated depth-averaged probe readings in the Lakes with probe readings from the well water. A third method considered probe readings at 10 m depth in Lake B with well water probe readings. Log removals were also compared with removals modified to include the effects of travel time (approximately a week for Lake A, no lag for Lake B since Lake B's travel time is less than 1 week which was generally the frequency of sampling).

3. Results and discussion

3.1. Lake water characterization and in situ monitoring

Table 1 summarizes the results of environmental monitoring at the surface of the two lakes and environmental variable profiles measured by the probe are presented in the Supplementary Information section (Fig. S1). Chlorophyll *a* and cyanobacterial biovolumes were within expected ranges for mesotrophic lakes in Quebec (MDDELCC, 2016). RFU values were lower than those measured in Missisquoi Bay, a eutrophic bay of Lake Champlain (Quebec, Canada) (McQuaid et al., 2011), also as expected. From all six samples for total phosphorus in both lakes, five were under limit detection (0.01 mg L^{-1}) and only the sample collected from A4 in Lake A was above the detection limit (0.03 mg L^{-1}). The Kjeldahl nitrogen concentration was above the detection limit at points A2 (0.70 mg L^{-1}) and A4 (0.73 mg L^{-1}) in Lake A and the remaining samples were below the detection limit (<0.4 mg L^{-1}). The higher pH in Lake A is consistent with a greater nutrient enrichment of Lake A (i.e. more photosynthesis and removal of CO_2 from the water column); however, nutrient samples were not collected throughout the period of study. *In situ* monitoring revealed similar temperature profiles in both lakes (Supplementary Information Fig. S1). Dissolved oxygen concentrations and pH followed similar trends, but with lower overall pH in Lake B. Dissolved oxygen was lowest in well water as was expected given lower concentrations at greater depths in the lakes (Fig. S1) and in groundwater as a result of biodegradation activity. Stratification is a key factor influencing the growth rate of cyanobacteria in many water bodies (Paerl and Otten, 2013).

Fig. 2 summarizes cyanobacteria densities estimated by the *in situ* probes (RFU) in the two lakes sampled weekly at different depths for the period of July 27th to October 15th. Sampling was carried out during the morning (between 9 and 11 a.m.) on Lake A and in the early afternoon (between 11 and 1 p.m.) in Lake B. *In situ* readings show a reverse gradient of phycocyanin RFU in the two lakes, with almost the same range of RFU value from 0.5 to 2.8 RFU in Lake A and 0.5 to 3 RFU in Lake B, with Lake B showing the highest RFU values at a depth of 10 m. The turbidity was also higher at 10 m depth in Lake B (Supplementary Information, Fig. S1). Cyanobacteria contribute to turbidity, but turbidity can also influence the probe's RFU readings (Zamyadi et al., 2012b). The higher turbidity and RFU values at 10 m depth cannot be explained by the probe disturbing the bottom sediments because Lake B's depth was greater than 10 m and the probe did not reach the lake's bottom sediments. The time of day of sampling could affect results (morning for Lake A versus afternoon for Lake B) as cyanobacteria can move through the water column according to light exposure. Cyanobacteria use light as a source of energy through photosynthesis. Conceptual models of cyanobacteria buoyancy suggest that cells increase their density following light exposure and move down towards the sediments where nutrients are available (Kromkamp and Walsby, 1990; Howard, 1997). However, these conceptual models are overly simplistic for full-scale systems and the relationship between light and cell density is affected by other factors that have not been fully elucidated (Ndong, 2014). Little variation in the water column was observed in Lake B over the

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

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

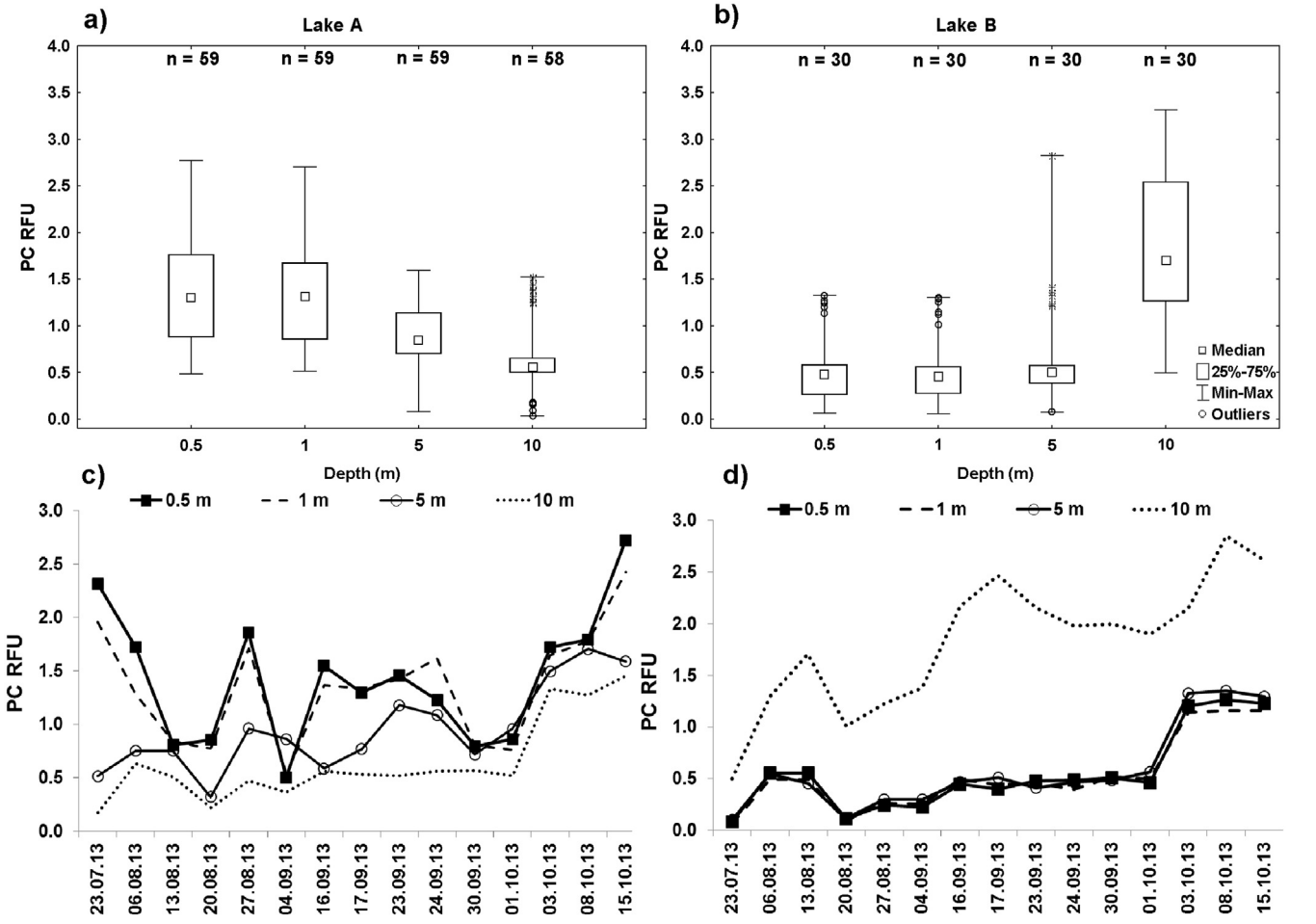

Fig. 2. PC fluorescence (RFU) at various depths in (a) Lake A and (b) Lake B for all samples combined; Time series of PC fluorescence as a mean of (c) points A1 and A2 in Lake A between July and October 2013, and (d) and of B1 and B2 in Lake B.

course of 1 day (data not shown).

Fig. 2c and d shows an increase in RFU values from the beginning to the end of the monitoring period, suggesting a progressive accumulation of cyanobacteria. Dense accumulations of cyanobacteria appeared as scums and were present towards the end of the study. *In situ* PC fluorescence demonstrated statistically significant correlations between concentrations in Lakes A or B and in well water (Fig. 3, $R > 0.86$ and $p < 0.0003$) as well as between Lake A and Lake B ($R = 0.66$, $p < 0.008$). Thus, as expected, the appearance of cyanobacterial cells in well water is associated with the source water quality of both lakes and environmental factors influencing cyanobacterial growth and accumulation. The correlation was stronger between well water and Lake B, suggesting a larger influence of Lake B water quality on well water potentially related to the shorter travel time. Cell concentrations and spatial heterogeneity can influence correlations and removal estimates and are further discussed in Section 3.4. Even without including the scum samples, probe readings from both lakes were significantly correlated with measured cyanobacterial biovolumes ($r = 0.59$, $p < 0.00001$) (Supplementary Information, Fig. S2).

The uncertainty of probe readings as percentage errors are higher when the cyanobacterial biomass is less 1 mm^3 L^{-1} (McQuaid et al., 2011), which was the case for about 60% of the water samples analyzed during the campaign sampling. Below 1 mm^3 L^{-1}, the PC probe may underestimate the cyanobacterial biovolume by about 20%. Probe readings for log PC were correlated to log chl *a* in RFU ($r^2 = 0.71$, $p < 0.001$) and could indicate a partial interference from green algae (Zamyadi et al., 2012b). However, the

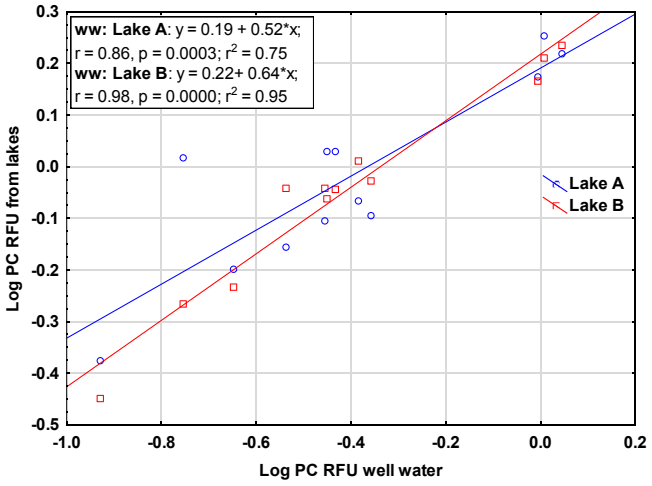

Fig. 3. Correlations between PC RFU estimated by probes in Lakes A and B with well water. The RFU in Lake B is the log transformed mean value of interpolated probe readings from all measured depths (0.5 m, 1 m, 5 m, and 10 m) from stations B1 and B2. RFUs in Lake A are the log transformed mean of interpolated probe readings from all measured depths (0.5 m, 1 m, 5 m, 10 m) from the two points closest to wells (A1 and A2).

probe was able to detect the breakthrough of cyanobacteria into the wells. A useful threshold of the probe for decision-making purposes with regards to cyanobacteria breakthrough in the well was 0.6 RFU that was related to a total cyanobacteria biovolume of 0.2 mm^3/L, corresponding to Alert Level 1 as proposed by Bartram et al. (1999). Thus, a value of 0.2 mm^3/L could be considered as the lower cyanobacteria biovolume monitoring threshold for this system.

Lake A had a trend of slightly higher PC RFU values in surface layers especially at 0.5 and 1 m (Fig. 2a). Cyanobacteria blooms in Lake A were more dispersed throughout the lake, potentially the result of quarrying activities in Lake A that would increase its mixing. Cyanobacteria blooms in Lake B were more concentrated at specific locations. For each of the bloom days sampled, data from the nearest meteorological station located at Latitude 45.47° N, Longitude 73.74° W (Government of Canada, 2016) showed that winds from the south-southwest were observed most frequently with speeds always lower 12 km/h. On two of the sampling days, the winds came from the north-northwest and north-northeast with speeds of less than 9 km/h. Winds from the southwest would tend to push blooms from Lake A towards the filter bank. Wind is an important factor influencing cyanobacteria accumulation in both lakes. Accumulation at the water's surface near the filter bank in Lake A is of concern because Lake A is the main source of pumped water consisting of 80% of the flow (Richard et al., 2010). Although Lake A contributes a greater proportion of flow towards the wells, travel time is also an important factor as are the specific phytoplankton characteristics that could influence cell passage through bank filtration that are discussed in Section 3.2.

3.2. Phytoplankton species in lakes, wells and sediments

Excluding samples from visible bloom days, the highest cyanobacteria biovolume (7.3 $mm^3\ L^{-1}$) was measured at the beginning of sampling campaign on August 20th in Lake A (Fig. 4). The observed cyanobacteria biovolumes varied from 0.01 to 8.5 $mm^3\ L^{-1}$in Lake A and from 0.003 to 1.7 $mm^3\ L^{-1}$ in Lake B (Fig. 4). As expected, the lowest biovolume values in the lakes were observed during the winter sampling on March 13, 2014. A Mann-Whitney *U* Test comparing biovolumes with Lake A and Lake B showed that measured biovolumes from samples collected at a depth of 0.5 m

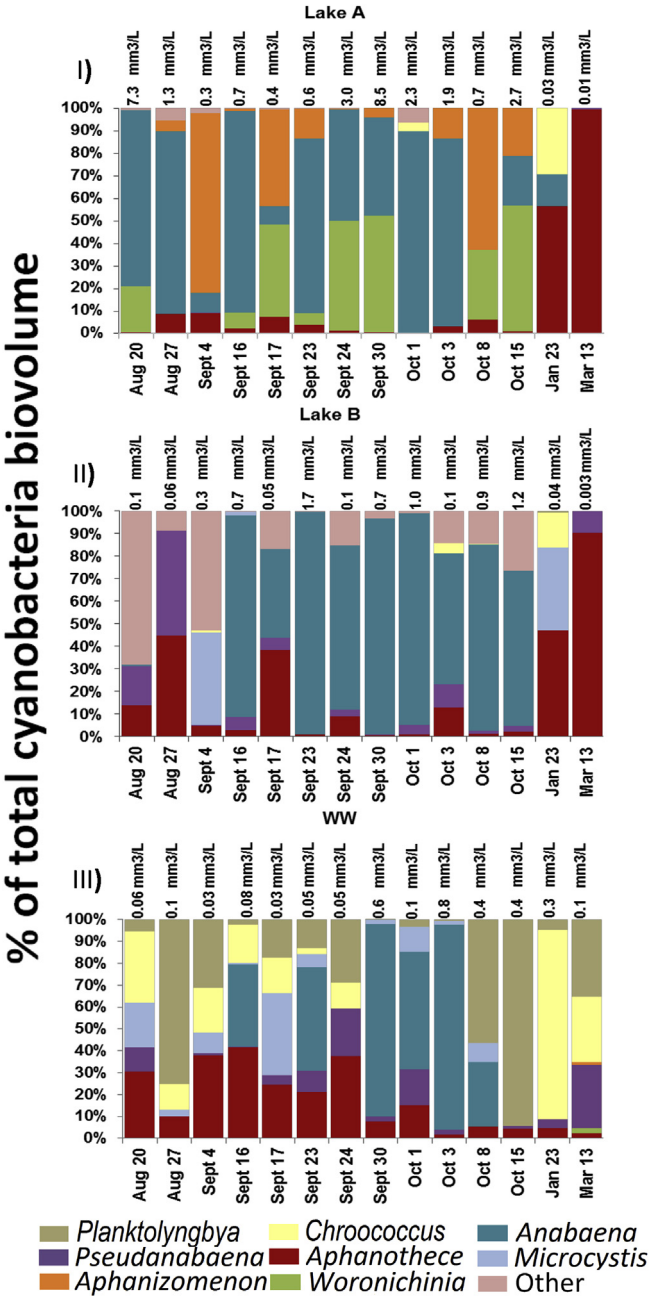

Fig. 4. Distribution of the biovolume of cyanobacterial genera in I) Lake A, II) Lake B, III) well water samples from August 2013 to March 2014. The total cyanobacterial biovolume in mm^3/L is shown above each bar in the graph. Other genera include: Merismopedia sp., Coelosphaerium sp., Cyanodictyon sp., Cryptomonas sp.

were slightly higher in Lake A ($p < 0.022$), which is consistent with the higher pH values (Zamyadi et al., 2012a) from the water observed in Lake A (Table 1). The mean cyanobacteria biovolume during non-bloom periods of study (8 days out of 12 days of sampling) varied from 0.1 (Lake B) to 0.4 $mm^3\ L^{-1}$ (Lake A). All well water samples were positive for total cyanobacteria, but had the lowest biovolume concentrations (0.02–0.4 $mm^3\ L^{-1}$). The cyanobacteria scum samples collected from surface during visible blooms are shown in Fig. 5.

The succession of cyanobacteria species over the course of the study is also shown in Figs. 4 and 5 with overall phytoplankton succession provided in the Supplementary Information section Figs. S3 and S4. Table 2 provides a list of the cyanobacterial species

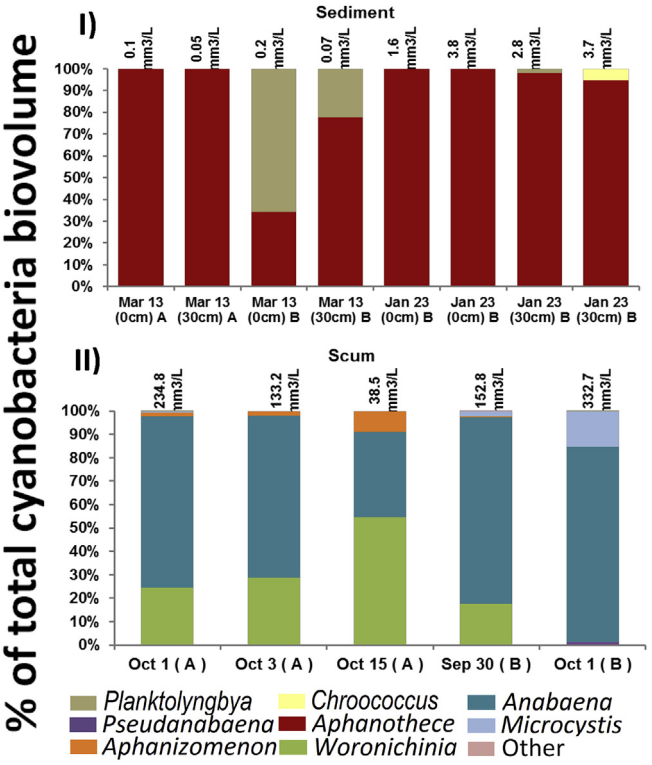

Fig. 5. Distribution of the biovolume of cyanobacterial genera in I) sediments, II) scum. The total cyanobacterial biovolume in mm^3/L is shown above each bar in the graph. Other genera include: Merismopedia sp., Coelosphaerium sp., Cyanodictyon sp., Cryptomonas sp.

Table 2
Mean cyanobacteria cell volumes from Lakes A and B and well water during the period of study.

Cyanobacterial species	Mean cell volume (μm^3)
Anabaena flos-aquae[a]	182
Anabaena solitaria planctonica	268
Anabaena solitaria solitaria	524
Anabaena flos-aquae treleasii	28
Aphanizomenon flos-aquae	88
Aphanizomenon flexuosum	42
Aphanothece clathrata brevis[a]	0.50
Aphanothece clathrata[a]	5.3
Chroococcus dispersus[a]	14
Chroococcus minimus	4.2
Chroococcus prescottii	118
Cryptomonas borealis	1570
Coelosphaerium kuetzingianum	9.4
Cyanodictyon imperfectum	0.50
Merismopedia minima	0.27
Microcystis aeruginosa[a]	38
Planktolyngbya limnetica[a]	13
Pseudanabaena biceps	32
Pseudanabaena limnetica[a]	9.2
Woronichinia naegeliana	57

[a] Indicates the cyanobacterial species was observed in well water.

including their mean cell volumes from all samples from Lakes A and B, well water, sediments and scums. The PCA analysis comparing cyanobacteria from all sites revealed that mean biovolumes of species found in well water were most similar to species found in Lake B as compared to Lake A or to winter sediments (Fig. 6). The passage of cyanobacteria to well water occurred year round demonstrating that cyanobacteria were always present in the bank filter. Fig. 4 shows cyanobacterial species observed in Lakes A and B and in well water in winter. It is possible that cells accumulate within the bank filter during blooms and continue to be released throughout the year. No relationship was observed between well water concentrations and sediment concentrations from the lakes, but no samples were collected from deeper bank filter materials. It has been demonstrated that drinking water treatment plants with only low densities in the raw water may be vulnerable to an accumulation of cells within various treatment processes (Zamyadi et al., 2013). Thus, it is conceivable that bank filter materials may also serve as media for cell accumulation. Although cells were intact, their ability to produce metabolites under such conditions is, to the best of our knowledge, unknown.

Lake A is considered to contribute a greater proportion of the flow, but is located at a greater distance from the wells. These results demonstrate the importance that distance and therefore travel time plays in the removal of cyanobacterial cells. Although it is known that travel time and morphotype of cyanobacteria influence bank filtration removal, few studies have been able to suggest a distance below which cell breakthrough will occur as they have not documented the breakthrough of cells (e.g. Romero et al., 2014). Our results show that travel times less than a week can lead to the breakthrough of cyanobacterial cells. Cyanobacterial cells can survive and produce metabolites in sludge systems (systems that are also devoid of the sunlight needed for photosynthesis) for up to 7–10 days (Ho et al., 2012; Pestana et al., 2016). Therefore, cells that pass through bank filters could potentially continue to produce metabolites, such as toxins, although this has not been confirmed. An important aspect of this research is the paired lake system that allows a direct comparison of cyanobacterial species from both lakes. Travel time is shorter for Lake B and the distance from the lake to the well is less than half that of Lake A. Given the greater similarity of species from Lake B and well water, the travel distance or time is more influential than relative proportion of flows or even the Lake cyanobacterial cell concentrations (as Lake A had higher concentrations and growth of cyanobacteria).

Sediment samples collected in winter demonstrated that diatoms were the most common type of phytoplankton with a mean biovolume value of 0.9 $mm^3\ L^{-1}$ in Lake A and 5.7 $mm^3\ L^{-1}$ in Lake B (Supplementary Information Fig. S4). The presence of cyanobacteria in sediment samples (mean of 0.07 $mm^3\ L^{-1}$ from Lake A and 2.02 $mm^3\ L^{-1}$ from Lake B) shows a greater accumulation of cyanobacteria at a greater depth and in sediments in Lake B (Fig. S4). In winter well water samples, 91% of observed phytoplankton consisted of cyanobacteria (0.1 $mm^3\ L^{-1}$) and diatoms were not detected (Fig. S4), which is similar to summer and early fall samples that show the selective passage of some cyanobacterial species and not other types of phytoplankton (Fig. 5). Variations in cell morphology in relation to their growth cycle could potentially influence cell passage. Although variations were observed in cell morphology, specifically for *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Pseudanabaena limnetica* and *Planktolyngbya limnetica* that were found in well water, no trends were observed.

3.3. Toxins in lakes and well water

Several of the cyanobacterial species that were present throughout the sampling period were potential toxin producers. A total of 58 water samples taken from both lakes (n = 44) and well water (n = 14) were analyzed for total (intra + extracellular) microcystin LR-eq (MCLR-eq). The total also includes all samples collected during visible blooms. The limit of detection was 0.15 $\mu g\ L^{-1}$ and only three samples out of 58 had a MCLR-eq concentration above the detection limit. The 3 samples with MCLR-eq above the detection limit were from scum samples collected from visible bloom areas on Lake B on the 1st (1.6 $\mu g\ L^{-1}$ of MC LR-eq),

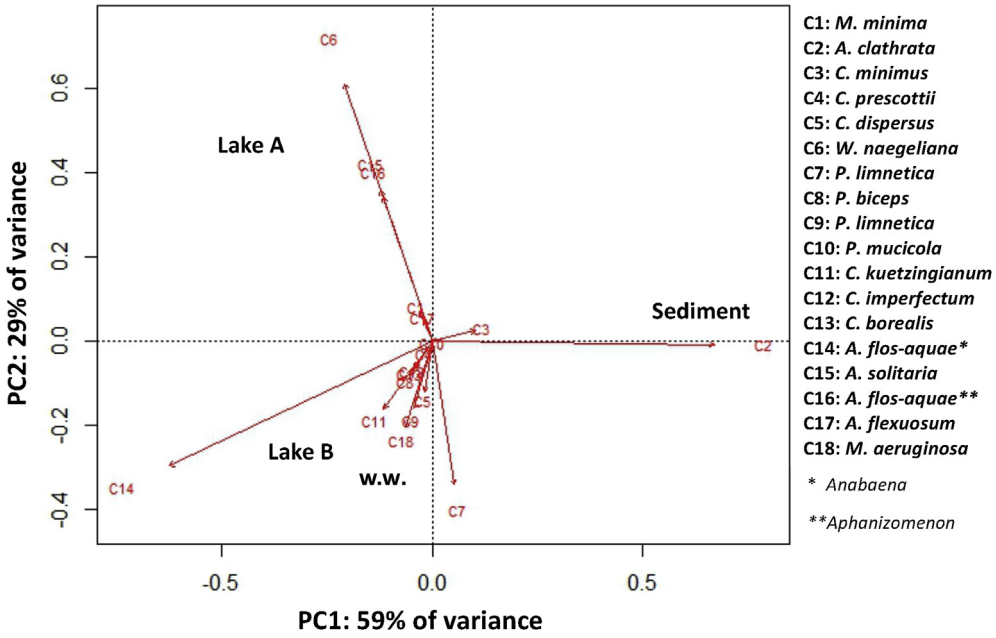

Fig. 6. Principle Component Analysis of mean biovolumes of cyanobacteria species (mm^3/L) at each site.

3rd (7.02 μg L^{-1}) and 15th (1.2 μg L^{-1} MCLR eq) of October. *Microcystis* spp. and *Anabena* spp. were the dominant species in samples collected during the blooms on those dates. Several factors could have influenced the production of toxins by cyanobacteria, including: 1) a succession of non-toxic genotypes to toxic genotypes (Kardinaal et al., 2007), and 2) toxin production only began in toxic genotypes once sufficient cell density was reached (Wood et al., 2011). Although no well water samples were above detection limits for microcystins, the greatest concern would be the passage of cells containing intracellular toxins, as most toxins are intracellular (e.g. Chorus et al., 2006; Zamyadi et al., 2013). Chorus et al. (2006) observed only intracellular toxins in groundwater observation wells. Many cyanotoxins have been shown to sorb readily to lake sediments (Maghsoudi et al., 2015). However, Klitzke et al. (2010) found that for cylindrospermopsin, a toxin frequently found in extracellular form, biodegradation was a more important process than retention in filter materials.

Although no toxins were measured in well water samples, it is possible to estimate the maximum potential microcystin concentration as a worst-case scenario of toxin production using measured cell biovolumes in the wells and values of microcystin per cell from the literature. Two values for the maximum production of microcystins per cell of *Microcystis* sp. were used: 0.2 pg from the World Health Organization (WHO) (WHO, 1999) and 0.63 pg from New Zealand's Ministry for the Environment and Ministry of Health (Ministry for the Environment and Ministry of Health, 2009). The highest calculated maximum potential microcystin concentration in well water samples occurred on October 8 when the highest density of *Microcystis* sp. was found in well water samples (0.59 μg/L, Table S3) and remains below drinking water standards in Québec. The main treatment post bank filtration is chlorination. Given the presence of cyanobacteria species that potentially produce anatoxin-a, a concern is that chlorination does not effectively oxidize anatoxin-a (Zamyadi et al., 2013). However, no samples were collected for anatoxin-a analysis.

3.4. Log removal during bank filtration

Log removals based on observed phytoplankton biovolumes in the Lakes and in well water are presented in Fig. 7 with and without considering the travel time. Chlorophyceae, Diatomophyceae and Dinophyceae had ≥3 log removal (i.e. none were detected in well water). Other types of phytoplankton were also largely removed with Cryptophyceae and Chrysophyceae having mean log removals of 2.9 ± 0.8 and 3.53 ± 0.9, respectively (considering travel time, Fig. 7). Cyanobacteria showed the lowest removal (0.89 ± 0.5) among the various types of phytoplankton (considering travel time, Fig. 7). Cyanobacteria and phytoplankton biovolumes in the lakes and well water used in the calculations are provided in Figs. 4 and S3(Supplementary Information), respectively. The variable results of cyanobacteria log removal could be explained by the heterogeneity of cyanobacteria species' morphology ranging in size from 0.27 μm^3 to 1570 μm^3 (Table 2). Cyanobacteria species occupy a

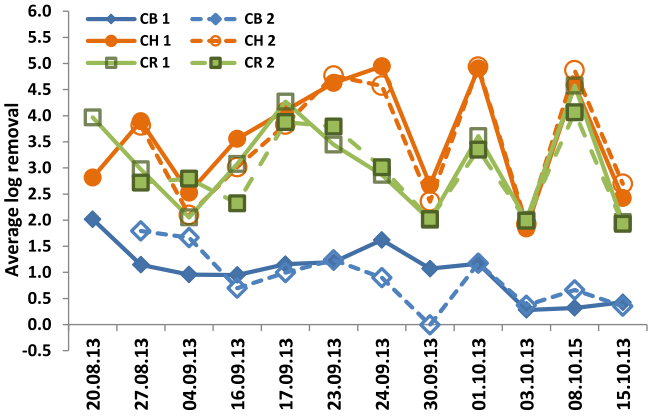

Fig. 7. Average log removal for Cyanobacteria (CB), Chrysophyceae (CH), and Cryptophyceae (CR) from taxonomic counts in Lakes A and B and in well water. 1) CB 1, CH 1, CR 1 are log removals estimated using flow weighted mean concentrations. 2) CB 2, CH 2, CR 2 are log removals estimated using flow weighted mean concentrations and travel time. For Chrysophyceae and Cryptophyceae values below detection limits in well water, the smallest measured biovolumes were used in log removal estimates (3.1E−5 mm^3/L and 3.9E−4 mm^3/L, respectively). Concentrations of Chlorophyceae, Diatomophyceae and Dinophyceae were below detection limits in well water, thus removal was considered >3 log.

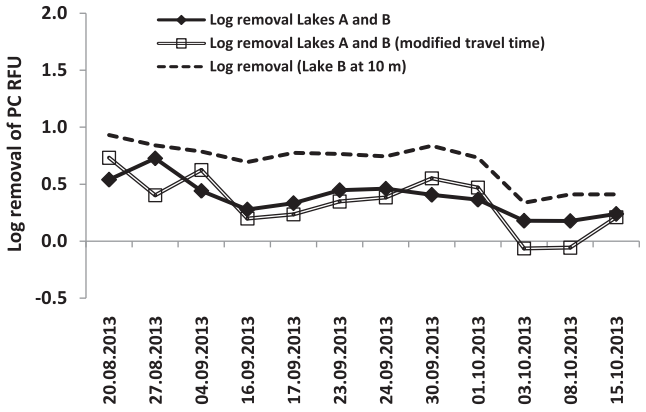

Fig. 8. Log removal based on phycocyanin RFU passing through bank filtration from Lakes A and B with and without considering travel time using flow weighted mean interpolated probe readings from B1 and B2 for Lake B and A1 and A2 for Lake A. The dotted black line is the Log removal of mean RFU from probe readings at 10 m depth in Lake B (B1 and B2) without considering flow weighted means.

variety of ecological niches and some are capable of regulating buoyancy, whereas others are predominantly benthic. However, even cyanobacteria that are typically found in surface scums (such as *Microcystis* sp.) were found in well water. Cell morphotype has been suggested to be an important factor affecting cell passage through filtration (Romero et al., 2014). We observed a significant negative relationship between cyanobacterial species' cell size and their frequency of observation in well water (Spearman rank correlation coefficient = −0.81, $p < 0.05$, Supplementary Information Table S2). That is, of well water samples that were positive for given cyanobacterial species, the smallest cells were most frequently observed. For example, the filamentous cyanobacterium, *Anabaena flos-aquae*, was observed half as frequently in well water samples as compared to the much smaller *Aphanothece clathrata brevis* that was observed in all well water samples.

The majority of previous studies have not observed the full-scale passage of phytoplankton through bank filtration, with the exception of Rachman et al. (2014), where passage was observed for 1 of 4 well systems tested in a system with the potential for direct hydraulic connections to the seawater used for a desalination plant. Although few studies have examined the passage of cyanobacterial cells, the passage of low concentrations of microcystins has been documented (Lahti et al., 2001; Chorus et al., 2006), but was not the case in our study despite cell breakthrough.

Fig. 8 presents the evolution of log removal throughout the study using the probe measurements in RFU. The use of the probe enables a more representative characterization of mean concentrations of cyanobacteria in the lakes. In addition, it was possible to determine the log removal for the system considering only the RFU values at a depth of 10 m from Lake B as being more representative of concentrations entering the bank. RFU-based log removal calculations were comparable to calculations using taxonomic counts. However, when considering only RFU concentrations at a depth of 10 m in Lake B, the log removal was slightly higher, but generally remained below 1.0.

Table 3 summarizes the performance of bank removal of all microbiological indicators. Relatively low concentrations of microbial indicators were present in the raw water and consequently their concentrations in well water were below the limit of detection with the exception of the aerobic spore-formed bacteria. Log removals of microbial indicators were higher than log removals of cyanobacteria. However, the higher log removals could be an artefact of lower concentrations of microbial indicators in the lakes as compared to cyanobacteria that led to non-detects in well water. Thus, the higher log removals cannot be considered as an indication of better performance of bank filtration for indicator bacteria, especially considering their relatively small cell size. Given that *E. coli* were below detection limits in well water, it was useful to monitor a series of bacterial indicators since aerobic bacterial spores and total coliforms have also been shown to be appropriate indicators in filtration studies (Bauer et al., 2011; Betancourt et al., 2014; Jenkins et al., 2011). Cyanobacteria log removals may provide a more robust estimate of the potential log removals of other types of bacteria such as indicators given that all well water samples were above detection limits for cyanobacteria.

4. Conclusions

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

- Distance and travel time are important factors for the removal of cyanobacteria through bank filtration. Travel times of less than 1 week can lead to cyanobacterial breakthrough in bank filtration with the potential for transport of intracellular cyanotoxins to wells.
- Removal of cyanobacterial cells in bank filters with travel times between a few days to a week were frequently below 1 log.
- Cyanobacteria can accumulate within bank filters, leading to breakthrough of cells in winter following the active cyanobacterial bloom season.
- Although no toxins were measured in filtered well water, the potential for cyanobacteria cells to contain intracellular toxins is of concern. Cyanobacteria generally represented less than 50% of the total phytoplankton biovolumes in Lakes A and B, yet made up more than 90% of the total phytoplankton biovolumes in well water
- Smaller cyanobacterial species such as *Aphanothece clathrata brevis* (mean cell size = 0.5 μm^3) were more frequently observed in wells as compared to larger cyanobacterial cells such as *Anabaena flos-aquae* (mean cell size = 185 μm^3).
- The application of an online PC fluorescence probe is a useful tool for rapid monitoring of the spatial and temporal distribution of cyanobacteria.
- A threshold of 0.6 RFU can be used for identifying cyanobacteria breakthrough into well water and was associated with a total

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

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

[a] Travel time of approximately 7 days from Lake A and same day from Lake B.

cyanobacteria biovolume of 0.2 mm^3/L, corresponding to Alert Level 1 as proposed by Bartram et al. (1999).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.06.037.

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