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Article

The Impact of Lake Water Quality on the Performance of Mature Artificial Recharge Ponds

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Abstract: Artificial groundwater recharge is commonly used for drinking water supply. The resulting water quality is highly dependent on the raw water quality. In many cases, pretreatment is required. Pretreatment improves the drinking water quality, although how and to what extent it affects the subsequent pond water quality and infiltration process, is still unknown. We evaluated two treatment systems by applying different pretreatment methods for raw water from a eutrophic and temperate lake. An artificial recharge pond was divided into two parts, where one received raw water, only filtered through a microscreen with 500 µm pores (control treatment), while the other part received pretreated lake water using chemical flocculation with polyaluminum chloride (PACl) combined with sand filtration, i.e., continuous contact filtration (contact filter treatment). Water quality factors such as cyanobacterial biomass, microcystin, as well as organic matter and nutrients were measured in both treatment processes. Microcystin condition was screened by an immunoassay and a few selected samples were examined by ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) which is a chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. Results showed that cyanobacterial biomass and microcystin after the contact filter treatment were significantly different from the control treatment and also significantly different in the pond water. In addition, with contact filter treatment, total phosphorus (TP) and organic matter removal were significantly improved in the end water, TP was reduced by 96% (<20 µg/L) and the total organic carbon (TOC) was reduced by 66% instead of 55% (TOC content around 2.1 mg/L instead of 3.0 mg/L). This full-scale onsite experiment demonstrated effective pretreatment would benefit a more stable water quality system, with less variance and lower microcystin risk. From a broader drinking water management perspective, the presented method is promising for reducing cyanotoxin risk, as well as TP and TOC, which are all predicted to increase with global warming and extreme weather.

Keywords: groundwater; pretreatment; contact filtration; infiltration ponds; nutrients removal; TP; cyanobacteria; cyanotoxin; microcystin-LR equivalent; eutrophic lakes; TOC; UPLC–MS/MS

1. Introduction

Artificial groundwater recharge plays an essential role in the sustainable management of groundwater resources as the demand for high quality drinking water continuously increases with growing populations in combination with the overexploitation of groundwater resources [1]. Artificial recharge of surface water for drinking water supply is commonly used in Sweden and elsewhere, such as in the Netherlands, Australia, China, and United States [2–6]. About 100 treatment plants in Sweden—representing about 25% of the Swedish municipal drinking water supply in

volume—apply artificial recharge [7]. The water quality after the artificial infiltration is important for drinking water safety and is highly dependent on the raw water quality. Hägg et al. showed that the final drinking water quality in terms of organic content might follow the same pattern as the raw water quality in conditions without pretreatment, according to his study on 16 artificial groundwater treatment plants in Sweden [8]. Moreover, experience from Finnish waterworks examining the occurrence of microcystin in raw water sources and treated drinking water showed that efforts should be made to stabilize the operational conditions of the artificial recharge to meet the high and rapid variations in raw water quality [9]. Therefore, pretreatment is often required before the water enters the artificial recharge ponds. It is a way to optimize treatment operations [10], prevent cyanobacteria regrowth, and reduce cyanotoxin risk. Meanwhile, the extent to which cyanotoxin risk in drinking water can be reduced by introducing pretreatment is rarely studied.

Therefore, a full-scale onsite project at one of the largest drinking water treatment plants, i.e., Vomb Waterworks in south Sweden, was performed to demonstrate the impact of pretreatment on water quality. Mature artificial recharge ponds have been applied at Vomb Waterworks for more than 60 years. Past observation of cyanotoxin with quantifiable anatoxin-a, microcystin-LR, and microcystin-RR as well as identifiable homoanatoxin, cylindrospermopsin, microcystin-YR, and many more microcystin variants [11] had prompted operators to improve the treatment process by introducing pretreatment to complement the pond infiltration performance. This is important for identifying proper pretreatment methods/barriers for a safe drinking water supply to meet future challenges as higher concentrations of cyanotoxin in the recharged water may occur more often due to hypereutrophic condition of raw water and the likely warmer water temperature due to climate change [12].

Pretreatment is highly valuable for drinking water from a hypereutrophic lake, such as Lake Vomb (south, Sweden), particularly during algal bloom season. On one hand, it reduces algae cells and nutrients level in the pond water, prohibiting cyanobacteria regrowth as small water body provides favorable conditions with easier light access and warmer water. On the other hand, pretreating lake water also reduces cyanotoxin risk before water enters infiltration ponds, as pond infiltration might not be enough to remove cyanotoxin, such as in situations of high infiltration rate and low temperature. Those situations would lower the microbial biodegradation activities [13]. Therefore, a complementary approach, such as pretreatment, is needed especially in the autumn, when cyanobacteria cells might decay and release toxins [13]. Groundwater contamination by microcystin from cyanobacterial blooms pose a significant health risk for residents in Lake Chaohu, China, when it is used for drinking water [14]. Moreover, rivers and lakes in the boreal regions or those surrounded by large agricultural areas tend to trigger algal blooms [15,16]. Cyanobacterial blooms in freshwaters have become a worldwide problem for both ecosystem function and human health [13].

The best opportunity for cyanotoxin removal is to remove intact cyanobacterial cells and other harmful compounds physically [17]. This can be effectively achieved by standard drinking water treatment processes [18,19], such as chemical flocculation and filtration treatments, while the removal of extracellular toxins is more complex. Activated carbon, membrane filtration, and chemical inactivation (ultraviolet (UV), disinfectants, and oxidants) are common treatment techniques for the removal of extracellular toxins [20]. Furthermore, the efficiency of different treatment processes for the removal of cyanotoxins can be referred to in many previous studies and authorities [17,20,21]. Bank filtration (sand filtration), like artificial recharge infiltration, is commonly used for removing cyanobacteria and cyanotoxins. Romero and his colleagues summarized a few studies and did a pilot study at the Lake Lagoa do Peri in Brazil and showed that bank filtration is efficient in removing phytoplankton and *Cylindrospermopsis raciborskii* (10^5 – 10^6 cells/mL) and removing up to 7.26 µg STX Eq./L, i.e., saxitoxin equivalent, each toxin analogue is converted to STX equivalents using Toxicity Equivalency Factors [22], in raw water before reaching a sampling well at 12 m deep and located 20 m from the lakeshore. The biomass was mainly removed in the upper 2 cm of the sand and no cyanotoxins were found in the monitored well water [23].

In addition, as it is likely that microcystin coexists with other type of toxins in this water source, a method for screening microcystin condition that covers many microcystin variants was encouraged to be used to screen the cyanotoxin risk, and its operability was discussed. The best approach for monitoring microcystin is to use a combination of screening methods to indicate positive samples and then use a more sophisticated quantification method such as HPLC or LC–MS/MS for confirmation [24]. Commercially available enzyme-linked immunosorbent assay (ELISA) test kits are one of the more commonly utilized cyanotoxin testing methods due to low cost and that less training is required for its use. Immunoassay based microcystin test is not particular for any specific variant. It measures all variants with results expressed as microcystin-LR equivalent (MC-LR Eq.). It is also recommended by the Swedish National Food Agency as a screening tool for microcystin monitoring in drinking water resources [21]. In this study, ELISA was used to screen the microcystin condition and a few samples were sent to the Swedish National Food Agency for confirmation by ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS).

Furthermore, eutrophic conditions tend to enhance the growth and bloom formation of algae, specifically cyanobacteria [25], and particularly when total phosphorus is above 20 µg/L [26]. The nutrient conditions in the pond water were examined. The removal of natural organic matter (quantified as TOC) from lake water is also of considerable importance as it may affect the drinking water quality in various ways, such as causing formation of disinfection byproducts and supporting biofilm in the distribution systems [27]. Moreover, synergies between climate warming and increasing levels of natural organic matter were observed to trigger an increase in cyanobacteria biomass [28] as well as a reduction in the biodiversity of phytoplankton [29,30].

In conclusion, the purpose of this project was to test how much nutrients, cyanobacteria biomass, and cyanotoxins were reduced through a pretreatment process, and how it would affect the pond water quality and final infiltrated water. The novelty of this project is that it is a full-scale onsite experiment examining how and to what extent pretreatment combined with artificial recharge can cope with one of the most important issues threatening drinking water safety, i.e., cyanobacteria and its toxins, in addition to discussion of the practical operability of using an immunoassay as a screening method for cyanotoxin risk monitoring. This supports local water operators to identify proper pretreatment methods/barriers for the safe drinking water supply to meet future challenges accompanying, e.g., climate warming.

2. Material and Methods

2.1. Case Study Description

The study was performed at the Vomb Waterworks, an artificial groundwater treatment plant in south Sweden which has been applying artificial recharge for more than 60 years. Vomb Waterworks is run by the largest drinking water supply company in south Sweden (Scania), Sydvatten, representing about 900,000 inhabitants from 17 municipalities. Raw water with a flow rate around 1000 L/s is taken from the Lake Vomb which is situated about 3 km northeast of the treatment plant and water is pumped to a sieve station with a microscreen of 500 µm pore size, removing macroparticles and reeds. The water is then distributed into infiltration ponds around the waterworks. There are 54 ponds in total (covering a surface of 430,000 m²) but only half of them are in use at the same time. The water seeps slowly, with an average velocity of 0.4 m/day through the fine sand (grain size is about 0.5–0.8 mm), and it takes about 2–3 months for the water to reach the groundwater wells. The water is then pumped up through 120 wells and led to the treatment plant. In the drinking water treatment plant, the main treatment process is to remove iron, reduce the hardness, adjust pH, and disinfect the water before distribution to consumers [2].

The catchment area of the lake is 450 km². The treatment plant has been used since 1948 for drinking water production due to the unique natural features. The area is rich in natural groundwater and suitable for artificial groundwater recharge as the area south of the lake is mostly composed of

sand, pebbles, and gravel [31]. With an average precipitation of 750 mm/year, it is estimated that about 5 m³/s water, on average, infiltrated into the groundwater aquifer [31].

In this study, one of the fifty artificial recharge ponds at Vomb Waterworks was used. The pond was selected for this experimental test based on both practical and technical considerations such the access to the pretreatment facility, heterogeneity of the natural layers of the whole area, and the location of the area where residence time was longer compared to the average residence time of all ponds. That was to avoid negative effect on groundwater quality if anything went wrong. The pond was divided into two parts, where one received untreated lake water while the other received pretreated water through chemical flocculation combined with continuous contact filtration.

2.2. Continuous Contact Filtration

The continuous contact filtration technique in this study used an upflow solids contact filter, which eliminates the need for settling tanks since liquid–solid separation, filtration, and sludge removal are done in a single unit process. After addition of coagulant, flocs which are formed are removed continuously in a moving sand bed. This purifies water and, at the same time, washes the sand without interruption and additional maintenance (for a detailed description of the technology, see [32]). The filters are commercially available. In our study, DynaSand filters (Nordic Water, Gothenburg, Sweden) were applied. The process involves chemical precipitants (polyaluminum chloride) which are added to a mixer in the pipe system before it reaches the filters. Hydroxide flocks are formed and then filtered and separated directly inside the filter bed. The sand grain size is around 1.2–2.0 mm, and the filter covers an area of 3 m² with a filter bed height about 2 m. This process is suitable for the treatment of surface water [33] and is commonly used in small-scale water treatment plants (<20,000 m³/day) in Sweden, and examples can be seen in Kalmar, Karlskoga, and Karlskrona [8].

The coagulant dosage was tested at the beginning of the project as it is an important factor for the removal of fine suspended solids, dissolved organic matter, and phosphate. In the end, 195 µL/L PAX 15 (polyaluminum chloride (PACl), KEMIRA, Sweden) was used for this pilot test. To guarantee good efficiency, the following conditions were chosen: maximum flow 10.5 L/s; sand movement average speed 9.79 mm/min; pH around 6.4. The consumption of PAX 15 was 177 L/day. The weekly consumption of PAX 15 was around 1240 L.

2.3. Study Design

The project design is illustrated in Figure 1. The artificial recharge pond was divided into two separate parts of similar size and shape: one receiving lake water only treated through a microscreen (500 µm pores) (control treatment) and one after coagulation, flocculation, and continuous contact filtration (contact filter treatment). Both sides of the pond were maintained in the same way and received the same water flow. To be able to follow the treatment process during the study, four observation wells were used for groundwater sampling. They were located 2 m from the sides of each pond (Figure 1). The ground level was used as reference. The pond was 2 m deep. The four observation wells were, on average, 13 m deep and the groundwater table was around 5–7 m under the ground. The pond was divided by a bank of fine material in the middle approximately 0.5 m under the surface of the sand. Water flows entered the pond from the furthest sides, the groundwater collected in the closest wells from both sides and was assumed to be unaffected by water from the opposite pond. The initial condition of the pond was with the top ground surface scraped after not being used for half of year. The experiment setup started in the second week of April 2014. It took 2 months (in June) to have a stable system before taking samples from both inlets of the pond and it took 4 months (in August) to have enough standing water in the both half ponds to take pond water samples.

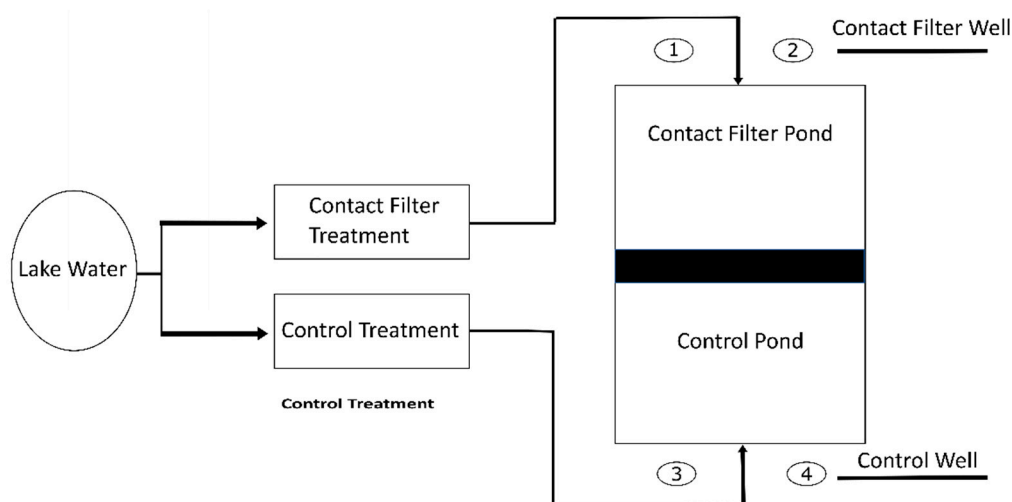


Figure 1. Study site overview. The lake is Lake Vomb from which water is led either through contact filter treatment or control treatment to their corresponding pond, i.e., contact filter pond and control pond.

2.4. Samplings

Samples were taken from 10 June to 20 October 2014 (every other week) at several sites along the treatment process, including from the incoming lake water, pretreated water after contact filter treatment and control treatment, water from the contact filter pond and control pond (from the end of August), and infiltrated water from all four wells (Figure 1). The number of samples for each parameter are summarized in Supplement Table S1.

The water quality parameters that were assayed were turbidity (FAU), UVA_{254nm} , color (436 nm), chemical oxygen demand (COD; mg/L), total phosphorus (mg/L), pH, total organic carbon (TOC; mg/L), nitrate (mg/L), orthophosphate (mg/L), cyanobacteria, and microcystin-LR.

A Hach Ratio XR Turbidimeter (Hach Lange GmbH, Germany) was used for turbidity measurements and a WTW 197-S pH meter (Xylem Analytics, Weilheim, Germany) for pH measurements. An DR6000™ UV VIS Spectrophotometer (Hach Lange GmbH, Germany) was used to measure $UV(SAC)_{254nm}$ and VIS_{436nm} absorbance (water color). Colorimetric titration with potassium permanganate was used for COD measurement. TOC samples were sent to specialized laboratory (Eurofins, Sweden). Orthophosphate and total phosphorus were measured by HACH LANGE test kits at the above spectrophotometer. Algae samples were fixed by Lugol's solution and kept overnight to allow sinking of particles onto the bottom of an Utermöhl chamber before analysis. A reverse microscope with maximal enlargement of 400× was then used to count cells. The cell density was transformed to a biomass scale so that the number represents the percentage of algae area covered on the chamber area (1 = 10%, 2 = 20%, and so on). This was used to give a simple estimation which gives an overview of the abundance of cyanobacteria. The following taxonomic groups were distinguished by their morphological characteristics:

1. *Woronichinia/Snowella/Microcystis/Radiocystis*
2. *Chroococcus*
3. *Beggiatoa*
4. *Achroonema/Limnothrix/Planktolyngbya*
5. *Anabaena/Nostoc*

The immunoassay for microcystin test was Beacon Microcystin Tube Kit (Beacon Analytical Systems Inc., Saco, MA, USA). It is a polyclonal antibody based, semi-quantitative method. The test process was as in [34]. Several microcystin variants can be detected due to cross-reactivity. According to the instructional brochure, the percentage cross-reactivity (% CR) of other microcystin variants

relative to microcystin-LR are microcystin-LR (100%), microcystin-RR (73%), microcystin-YR (58%), microcystin-LA (2%), microcystin-LF (3%), microcystin-LW (4%), and nodularin (126%). This suggests that it is suitable as a screening method. The official detection level for the microcystin test is 0.3 µg/L [35]. According to the test kit supplier, through spike and recovery experiment with standard solution, values below 0.3 µg/L might also be valid. In this study, no validation was done. Therefore, all measured data were considered valid for analysis.

(UP)LC-MS/MS is a chemistry technique, combining the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. It has very high sensitivity and selectivity. In general, its application aims to not only for the general detection but also for potential identification of chemicals in the presence of other chemicals such as in a complex mixture). UPLC-MS/MS was developed for simultaneously analyzing 22 intra- and extracellular cyanotoxins in raw water and drinking water, including anatoxins, cylindrospermopsins, nodularin, and microcystin [11]. UPLC-MS/MS was used to confirm the toxin profile.

The guideline value of microcystin-LR monitoring in drinking water suggested by the World Health Organization (WHO) is 1 µg/L [35]. This value was newly listed in the revised Drinking Water Directive proposal (2018) which is the main piece of EU legislation regarding regulations on water quality intended for human consumption [36]. A group of experts from the EU project CYANOCOST proposed that the parametric value of 1.0 µg/L should include all microcystin variants, not only microcystin-LR [37]. As most tested microcystin variants exhibit strong toxicity, the inadequacy of current microcystin guidelines based only on microcystin-LR might pose health risks for drinking water supply [37]. More cautious microcystin-LR values are used in some regions, such as 0.16 µg/L in Vermont [38] and 0.1 µg/L in Minnesota in the United States [39].

2.5. Bootstrapping Resampling

Data resampling refers to methods for economically using a collected dataset to improve the estimate of the population parameter and to help quantify the uncertainty of the estimate. Bootstrapping is one of the resampling methods which relies on random sampling with replacement. It allows assigning measures of accuracy—defined in terms of confidence intervals in this study, for example—to the estimated resamples. This technique allows for estimation of the sampling distribution of almost any statistic using random sampling methods [40].

Bootstrapping was used to test the significance of the difference of MC-LR Eq. after two treatment processes and in the pond water during the bloom season (June–October 2014). The advantage of using this method is that it does not require any tests for its distribution type, and it is therefore possible to do resampling and generate a close to population distribution based on limited sampling points, such as is the case in this study. Comparison of the mean value, variance, and standard deviation were done, and 1000 resampling iterations were generated. In every iteration (random sampling from the original set of data), for example, the mean difference was calculated. After 1000 iterations, the distribution of the mean difference thereafter was plotted, and the significance can be decided based on the location of the sample difference.

3. Results

Site observation showed continuous contact filter treated water developed slower resistance in the pond and higher infiltration rates. Until the end of August, i.e., after running the project for 4 months, the whole bottom in the control pond was covered by water, while only two-thirds of the contact filter pond was covered by water. Water quality with mean value and Min.–Max. values at different stages along the treatment process are presented in Supplementary Table S1. The sections below focus on the removal of organic matter, nutrients, cyanobacteria, and MC-LR Eq.

3.1. Organic Matter and Particle Removal

One major task for drinking water producers is to remove particulate materials, i.e., inorganic and organic particles. Particulate materials from the raw water and flocs formed during chemical flocculation are physically removed by filtration processes. This can be seen in Figure 2, where contact filter treatment removes about 50% of particles from the raw water, measured as turbidity (FAU), and it remains low level in the following pond water. On the control treatment side, turbidity has a slight decrease due to the microscreen, which was designed to remove particles larger than 500 μm . The increase of turbidity with big variance in the pond water might partly be due to algae regrowth in the control pond (Figure 3).

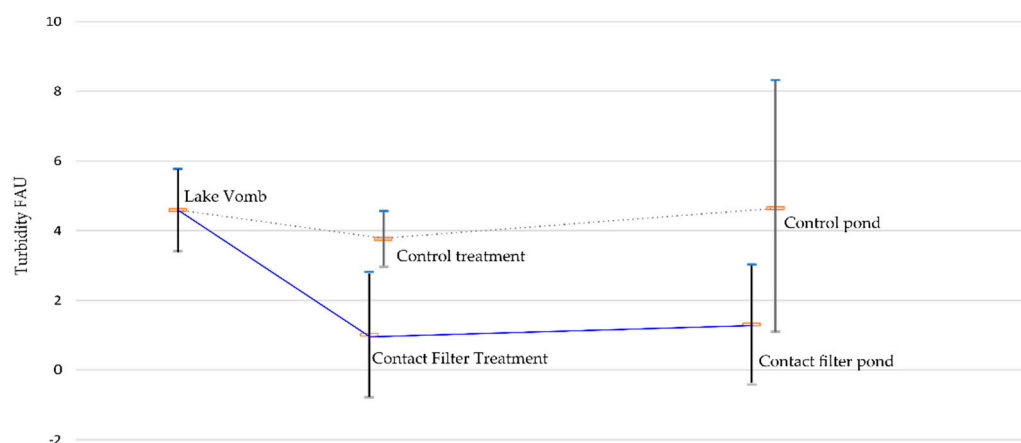


Figure 2. Turbidity measurements at different stages along the two treatment processes (FAU). The middle bar is the mean value and the top and lower bars show the standard deviation. The dotted line is the contact filter treatment process and the solid line is the control treatment process.



Figure 3. Onsite observation of two sides of pond in August 2014: (a) control pond and (b) contact filter pond (photo taken by Marie Baehr and Petra Larsson).

The organic content removal in the form of TOC content shown in Figure 4 is much more efficient by contact filter treatment process than the control treatment. TOC content was already reduced by 50%, after contact filter. This is a similar result to the previous studies on flocculation for TOC content removal by using the same source water [41] and within the observed range of other drinking water treatment plants (DWTPs) in Sweden using contact filtration [8,33,41]. The final TOC content in the well water on the contact filter side was 2.1 mg/L on average, while in the control wells, the TOC content was 3.0 mg/L on average.

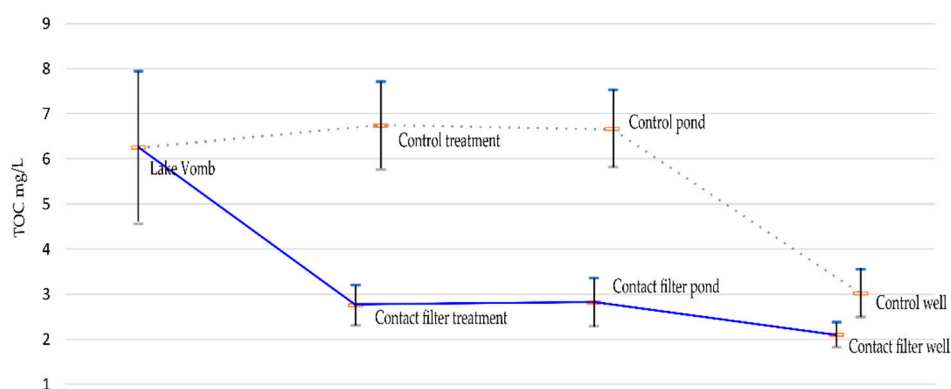


Figure 4. Total organic carbon (TOC) measurements at different stages along the two treatment processes (mg/L). The middle bar is the mean value and the top and lower bars show the standard deviation. The dotted line is the contact filter treatment process and the solid line is the control treatment process.

Similar trends were observed for other indicators of natural organic matter content (NOM), i.e., chemical oxygen demand (COD) and UVA_{254nm} , where these parameters were significantly reduced after contact filter process (Supplementary Materials Figures S1 and S2).

3.2. Nutrient Removal

Phosphorus removal using chemical coagulation and rapid sand filtration has been proven to be very effective [42]. This is also verified in this study, where up to 95% of the total phosphorus (TP) was removed after contact filter treatment (Figure 5) and the TP concentration was reduced to below 20 $\mu\text{g/L}$. Keeping a low concentration of phosphorus (<20 $\mu\text{g/L}$) is crucial for cyanobacterial bloom control [17]. This can be seen in Figure 3 in which intensive cyanobacterial blooms were present in the control pond where the phosphorus remained the same as in the raw water, while very clear water was present in the contact filter pond where TP content was low.

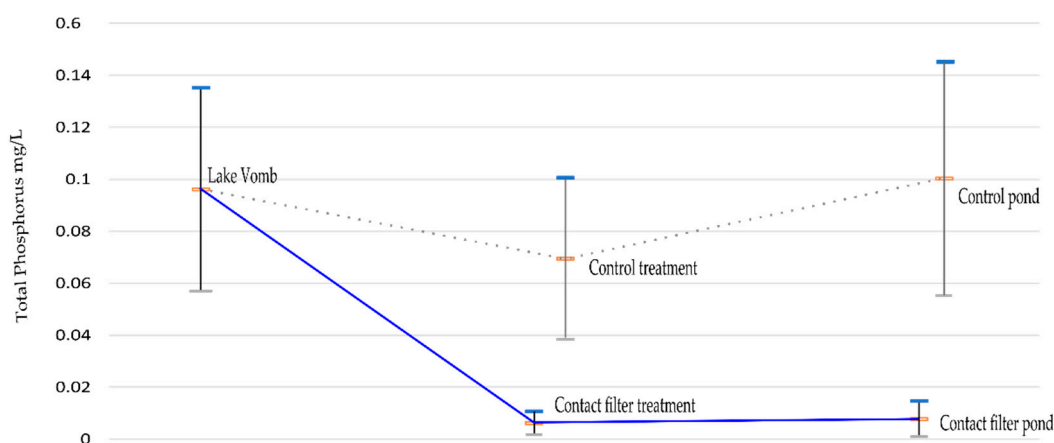


Figure 5. Total phosphorus removal at different stages along the two treatment processes. The middle bar is the mean value and the top and lower bars show the standard deviation. The dotted line is the contact filter treatment process and the solid line is the control treatment process.

Monitoring nitrogen level is important for final drinking water quality and controlling cyanobacteria-favorable growth conditions. The nitrate concentration in these two different processes was significantly different both in the pond water and in the well water (Figure 6). The nitrate level in the control pond was around 25% lower than the contact filter pond and the value in the control well was almost double that of the value in the contact filter well. The nitrogen concentration in the form of ammonium is almost the same through these two treatment processes (Figure 7).

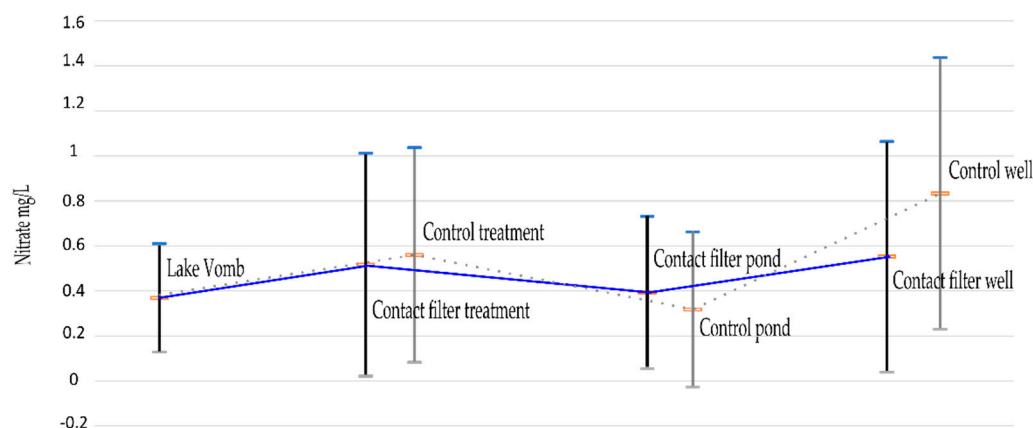


Figure 6. Nitrate content at different stages along the two treatment processes (mg/L). The middle bar is the mean value and the top and lower bars show the standard deviation. The dotted line is the contact filter treatment process and the solid line is the control treatment process.

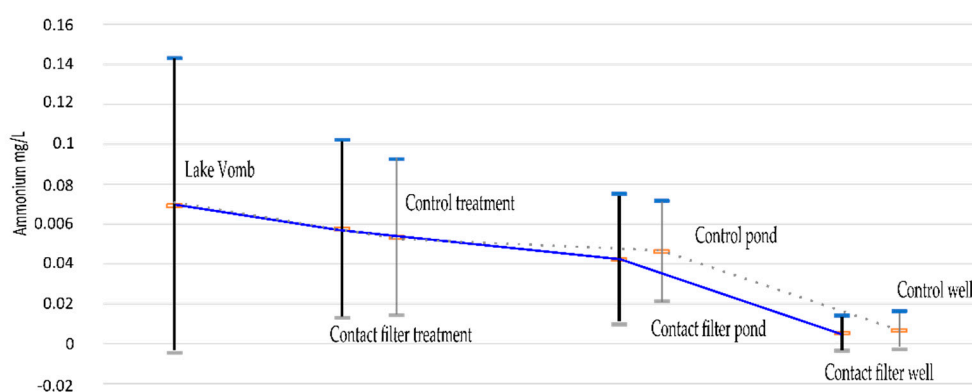


Figure 7. Ammonium content at different stages along the two treatment processes (mg/L). The middle bar is the mean value and the top and lower bars show the standard deviation. The dotted line is the contact filter treatment process and the solid line is the control treatment process.

3.3. Cyanobacteria Removal

Onsite observation (Figure 3) has already showed clear evidence of water quality improvement by introducing contact filter treatment. The result of onsite visual inspection is shown in Supplementary Table S2, and a subjective evaluation result about the comparative amount of cyanobacteria in different groups is shown in Supplementary Material Figure S3. There was a significant difference in cyanobacteria biomass between the two sides of the pond.

The dominating species of cyanobacteria in the control pond are group 1 (*Woronichinia/Snowella/Microcystis/Radiocystis*) followed by group 5 (*Anabaena/Nostoc*) and group 2 (*Chroococcus*) (Supplementary Material Figure S3). Only group 1 (*Woronichinia/Snowella/Microcystis/Radiocystis*) and 4 (*Achroonema/Limnothrix/Planktolyngbya*) are present in the contact filter pond (Supplementary Material Figure S3). The differences in the groups are mainly morphology and size. Group 1 and 4 are the main dominating species. Group 1 species are the main ones which regrew in the control pond. Some cyanobacteria species with smaller cell size passed the contact filter, such as group 1 (2–3 μm) and group 4 (1–3 μm), while they are limited in the contact pond compared to the control pond (Supplementary Material Figure S3). No cyanobacteria cells were found in the well samples.

3.4. Cyanotoxins Analysis Result

Microcystins are hepatotoxic products of freshwater cyanobacterial blooms of *Microcystis*, *Anabaena*, and *Nostoc* with *Microcystis aeruginosa* being the most common [35]. *Anabaena* might also produce

anatoxin and saxitoxins [35]. Different variants of microcystin have been detected from blooms where *Woronichinia naegeliana* was the dominant species among secondary metabolites [43,44]. The biological effects on planktonic crustaceans were investigated with relation to a fraction containing microginin-FR3 [44].

Microcystins measured as MC-LR Eq. appeared higher during late August and late September 2014. Boxplot results of MC-LR Eq. at different stages (Supplementary Material Figure S4) clearly show that the contact filter treatment system has much less variance than the control system, although all median values are similar (thick line in the middle).

During the experiment period, the microcystin content both after pretreatment and in the pond water follow the same trend in raw water (blue triangle) with much lower values and less variance in the contact filtration process (left in Figure 8). A steady development of microcystin level in the control pond (green diamond in the figure on the right, Figure 8) is observed from early September to the middle of October 2014. It was likely partly produced by the cyanobacteria regrowth in the pond, specifically on 13 October 2014. This situation was under control in the left figure, and the microcystin risk increases along with the increase in the raw water at beginning, while it does not follow at certain level. The contents corresponding to the higher values on 29 September and 13 October in the raw water were prevented from entering the artificial recharge system.

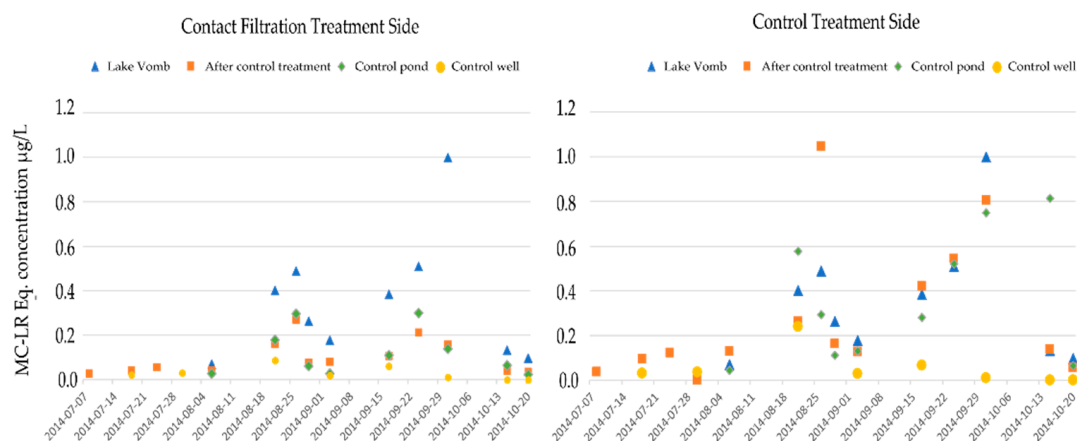


Figure 8. Microcystin development along the sampling period (left is the microcystin condition in the contact filtration treatment process and right is the control situation).

A bootstrapping method was used to test the significance of the difference of MC-LR Eq. after two treatment processes and in the pond water during the bloom season (June–October 2014). After running 1000 iterations, the distribution of the mean difference between microcystin condition after pretreatment was plotted in Figure 9. This demonstrates all possible difference between these two sets of data. The sample difference 0.2 (the blue line) which is close to the median of the distribution, suggesting a high possibility of occurrence. The mean difference in MC-LR Eq. after two pretreatment steps is significant based on 95% confidence (between the red lines). This applies to the mean difference of 0.24 in the pond water. Their variance and standard deviation difference were also significant. There is no significant difference of the MC-LR Eq. concentration in the well water.

Few samples with higher MC-LR Eq. were sent to the Swedish National Food Agency to study the toxin profile by using UPLC–MS/MS. Results confirmed the presence of MC-LR, MC-RR, MC-YR, MC-D-Asp3-LR, and anatoxin-A.

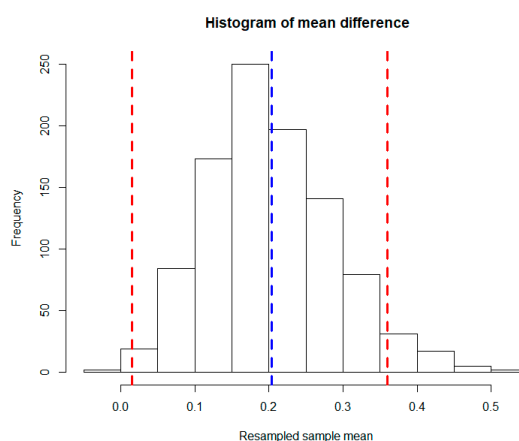


Figure 9. The distribution of mean difference of toxin level after reference treatment and new treatment during the bloom months (June–October 2014).

4. Discussion

This in situ, full-scale experiment demonstrated the efficiency of pretreatment on the removal of nutrients, organic contaminants, and its impact on pond water quality, reducing the cyanotoxin risk in drinking water. Such is shown in Supplementary Table S1, that the pretreatment resulted in more stable water quality with less variance, reduced total organic matter, prevention of cyanobacteria regrowth, and elimination of cyanotoxin in the pond water.

4.1. Regarding to Organic Matter Removal

Pretreating raw water reduced the high variability of organic matter entering the pond, as well as in the well water. The variance in turbidity, TOC, and COD measurements in the control pond was higher than the contact filter pond. The same applies to the well water.

Efficient reduction of particles and natural organic matter, on the one hand, will result in less clogging and maintenance of artificial recharge ponds and a reduction in organic pollutants in drinking water. On the other hand, a certain amount of organic matter is also expected, contributing to a certain resistance in the infiltration process for longer absorption and degradation times, and a certain fraction is also used as food for microbial organisms. Unfortunately, we were not able to observe the starting phrase of organic content changes since it took 2 months for the system to stabilize before taking samples. For a seeping rate of 0.4 m/day and average depth of the groundwater table to the bottom of the pond of around 3–5 m, it was estimated that it might just take around 10 days to reach the closest observation wells.

The pretreatment complemented the TOC content removal during the pond infiltration process where microbial degradation occurred. This resulted an overall TOC reduction of 52–66%. This was achieved partly by the two different techniques' abilities to remove different fractions of organic matter. Organic matter in the form of particles and cells are removed by physical separation. The different fractions in the dissolved form (DOM: dissolved organic matter) can be removed selectively by different treatment methods. An examination of DOM composition in drinking water production showed that coagulation was highly selective towards to remove fraction resembled terrestrial endmembers and DOM with microbial endmember characteristics was especially reactive during slow sand filtration [45].

4.2. Regarding Nutrient Removal

Contact filter filtration has been known for its high removal of phosphorus and this was also observed in our case study. This brought about a positive impact on preventing the cyanobacteria regrowth in the pond water. The difference between the nitrate concentration level in the pond water and the well water for these two treatment processes might be due to the difference of the

cyanobacteria biomass and different species. The difference might influence the nitrogen cycle in the pond. More cyanobacterial biomass in the control pond and regrowth of cyanobacteria might consume more nitrate than the contact filter pond. The decay of cyanobacteria might contribute to the higher nitrate level in the control well. As the ammonia condition did not vary, this might also indicate that some extra nitrogen from the air might also be fixed by cyanobacteria into the pond and have ended up in the infiltration system.

4.3. Regarding the Removal of Cyanobacteria and Cyanotoxin

Our experiment allowed us to see the fate of different groups of cyanobacteria influenced by pretreatment. It was highly dependent on their morphology and size as well as the changed growing conditions. The microscreen had a large pore size and could only remove small number of cells, and neither intracellular nor extracellular toxins were removed. In addition to the cyanobacteria regrowth in the control pond water, this resulted in a significant difference in cyanobacteria cells between the two sides of the pond both after pretreatment and in the pond water. The apparent domination of group 1 in the control pond needs more attention. *Microcystis* and *Anabaena* (*Dolichospermum*) are listed as the most toxic cyanobacterial genera, and often succeed each other during harmful algal blooms [46]. *Microcystis naegeliana* appears frequently in freshwater and one study showed that blooms of *Woronichinia naegeliana* were toxic towards invertebrate zooplankton [47].

The group with larger cell size and filaments might be filtered out or have limited growth due to a low nutrient supply. Smaller-sized harmful species, such as in group 1 and 4, might pass through the contact filter, though they did not show signs of regrowth in the contact filter pond probably as they met with reduced nutrients and unfavorable N:P conditions. Nitrogen-limiting conditions favor nitrogen-fixing cyanobacterial blooms. In this case, *Woronichinia naegeliana* [48] was present in the control pond where N:P was below 10, while in the contact filter pond, where N:P was above 40, the nitrogen-fixing *Anabaena* species were not found in the pond water although they were found after the contact filtration process (Supplementary Figure S3). This is an important confirmation on how to influence the nutrient conditions in water to prevent cyanobacteria growth, reducing the risk of cyanotoxin in drinking water. In a previous study, it was also discussed how low N:P may trigger higher cyanobacterial blooms [49], while no trace of cyanobacteria in the well water suggests the infiltration process in the pond was able to remove cyanobacteria cells even when there were intensive blooms in the control pond.

Artificial recharge infiltration ponds were recorded to effectively remove cyanotoxins with the help of microbial degradation [50], which was also observed during this study. With the help of pretreatment, there is a significant difference in microcystin conditions between the pond waters while no significant difference was observed in the well water, though there was less variance in the control well water. Many physical and microbial processes have been involved in the process. Coagulation might also remove certain extracellular cyanotoxins that are hydrophobic, such as MC variants RR, YR, LR, and LA [18]. The majority that are extracellular are removed in the infiltration pond, likely by microbial degradation [50].

In the current system, infiltration ponds are the only barriers for cyanobacteria and cyanotoxin removal. Biodegradation of cyanotoxins was dependent on the pH and temperature [51], which might require other barriers to complement microbial activities especially when it comes to the autumn, when it is likely that cyanobacteria start to decay and release toxins into water [46]. Meanwhile, in our observations, we only found slightly lower microcystin conditions with less variance in the contact filter well water than the control well water and this might not be the situation for all the other ponds and at other times.

Furthermore, the mechanism of cyanotoxin removal by microorganisms needs to be further studied as microorganisms mainly consist of organic carbon, nitrogen, and phosphorus. Therefore, the C:P:N ratio in the surrounding environment might also affect their full potential for water treatment [52].

4.4. Discussion around Microcystin Detection and Monitoring

Immunoassay based microcystin test kit which can detect other microcystin variants due to its cross-reactivity might be a good start as a screening method. With the help of advanced analytical methods, toxin profiles can be further confirmed and even quantified. UPLC–MS/MS confirmed the presence of MC-LR, MC-RR, MC-YR, MC-D-Asp3-LR, and anatoxin-A in the lake water and pond water samples.

The current guideline value applied in Sweden is MC-LR < 1 µg/L, which might be legislated as EU law as suggested in the revised draft for EU Drinking Water Directive. As we can see in our case, we have much more MC variance beyond MC-LR and other types of cyanotoxin such as anatoxin. In only focusing on MC-LR monitoring, other potential risks might be missed. As discussed before, experts in the cyanobacteria research field also suggest this parametric value to involve all type of MC variants. As examples, Minnesota and Vermont have even stricter levels due to health concerns or public initiatives. This might push water suppliers to initiate targeted monitoring [38]. Drinking water operators need more practical support for cyanotoxin detection and monitoring.

Pretreatment would help to reduce the hazard risk in general, even though no significant difference of cyanotoxin was represented by MC-LR Eq. in the well water. Accumulation of cyanobacteria and toxin in drinking water process [53] might occur even though the toxin level is not high in the raw water, such as due to high toxin content in the sludge. This presents more challenges for drinking water operators for both monitoring and treating cyanotoxins.

4.5. Regarding the Impact of Final Water Quality

This project demonstrated the positive aspects of introducing effective pretreatment to reduce the risk of cyanotoxin presence in the drinking water. The effect was limited, however, since the toxin was analyzed in well water taken after a pond which was selected to have better performance than the average level of all the ponds due to its original safety concern. On the one hand, we fulfilled the project purpose of examining the effect of continuous contact filtration on cyanobacteria cells, nutrients, and cyanotoxin removal, and the impact on the pond water quality. On the other hand, we could also get an indication that during the experiment period, under the current toxin level represented by MC-LR Eq., the selected pond could deal with cyanotoxin. However, this does not mean that it would be the same situation for other ponds with less retention time and, in the future, when global warming continues, cyanotoxin levels are likely to increase.

As this is a small-scale study with a 10 L/s flow rate and with the consideration of mixing with natural ground water and a final production of 1000 L/s from the waterworks, the effect of the experiment on the total drinking water production is almost neglected. Furthermore, the setup took space to build, and it might not be suitable for a large water treatment plant like Vomb Waterworks, though it is highly likely to be applicable for a small-scale water treatment plant processing ca. 20,000 m³/day. Future pretreatment investigations might be focused on a filter system with smaller pore size, like 10 or 30 µm, for removal of organic matter such as algae [54].

5. Conclusions

Cyanobacteria in drinking water is a complex issue, and multibarrier approach should be applied. On a small-scale, in situ experiment, we have demonstrated how and to what extent pretreatment of raw water can reduce the cyanobacteria and cyanotoxin risk in artificial recharge ponds before infiltration, and how the treatment process can benefit from introducing effective pretreatment. It might be suitable for a small-scale water treatment plant processing ca. 20,000 m³/day.

In general, pretreating raw water contributes to more stable water quality in through reduced organic load, reduced phosphorus in the infiltration ponds, and higher water quality in the infiltrated water. Water quality supplied to the infiltration pond can be improved considerably, especially regarding TOC reduction (by 66% on average), COD reduction (by 75% on average), UVA reduction (by 74%

on average), and TP reduction (by 95% on average). Compared to the control treatment, this led to a reduction of the total TOC concentration in the final product from around 3.0 to 2.1 mg/L, a total phosphorus content of below 20 µg/L, and a significance decrease in cyanobacteria and microcystin content. This results in preventing intensive cyanobacteria blooms in the artificial recharge ponds and reducing the risk of additional formation of cyanotoxin.

In practice, the detection of cyanotoxin in drinking water is challenging. Only focusing on MC-LR might not be ideal. Detection methods that can cover most of the toxic variants are needed. In future, there might be continuous discussion around the parametric value of microcystin for drinking water, and more types of cyanotoxin should also be included in the legislation. How microbial activities are functional for microcystin removal in the artificial recharge pond and how they are influenced by the surrounding physical and chemical condition need to be further studied.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4441/11/10/1991/s1>, Figure S1: COD measurements at different stages along the two treatment processes (mg/L). The middle bar is the mean value and the top and lower bars show the standard deviation. The dotted line is the contact filter treatment process and the solid line is the control treatment process.; Figure S2: UVA 254 nm at different stages along the two treatment processes. The middle bar is the mean value and the top and lower bars show the standard deviation. The dotted line is the contact filter treatment process and the solid line is the control treatment process.; Figure S3: Changes in algae types in the two treatment processes, the biomass scale is the area covered by algae, i.e., 1 = 10%, 2 = 20%, and so on.; Figure S4: Microcystin concentrations at different treatment processes; Table S1: Water quality at different stages along the treatment processes and changes in the toxin data; Table S2: Global amount of cyanobacteria in different steps of the pretreatment process.

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