Managing Eutrophic Waters in Artificial Recharge Plants

Cyanotoxin risk in Swedish freshwaters

Li, Jing

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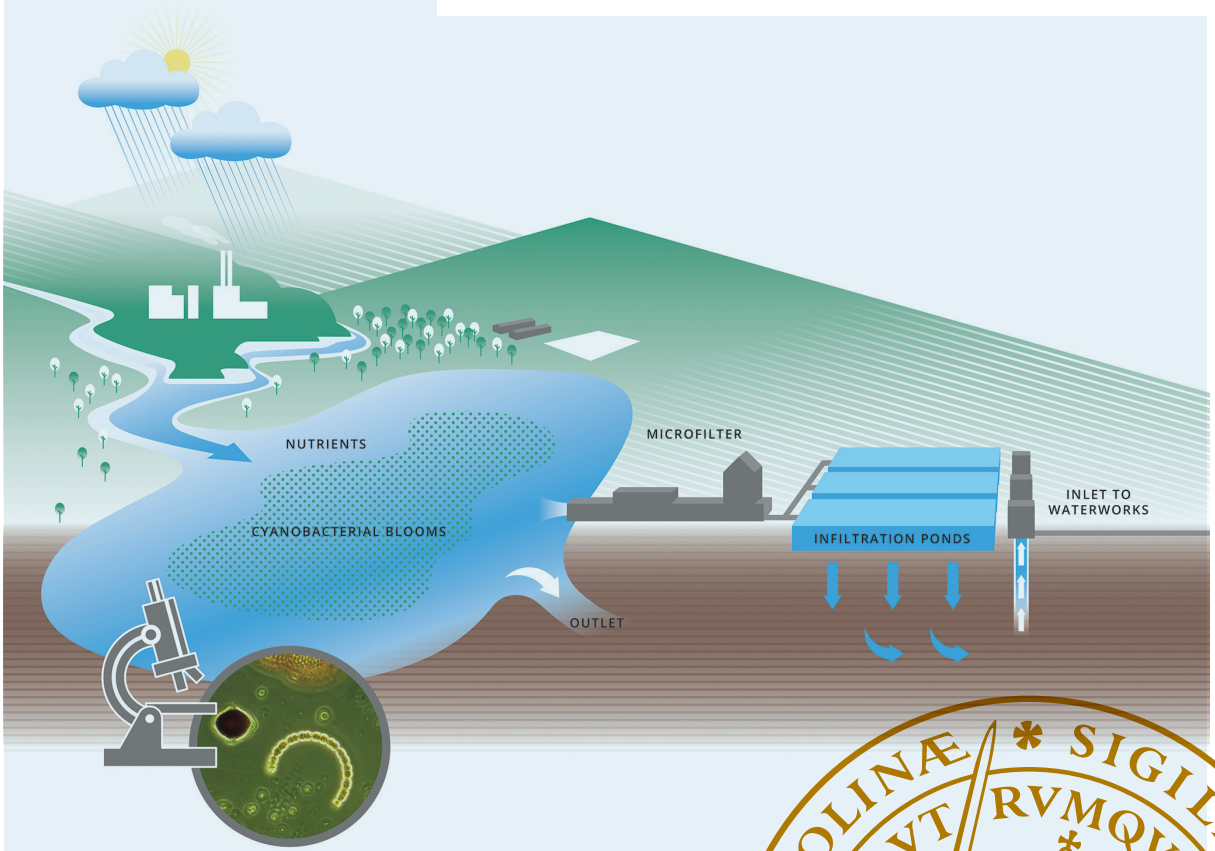
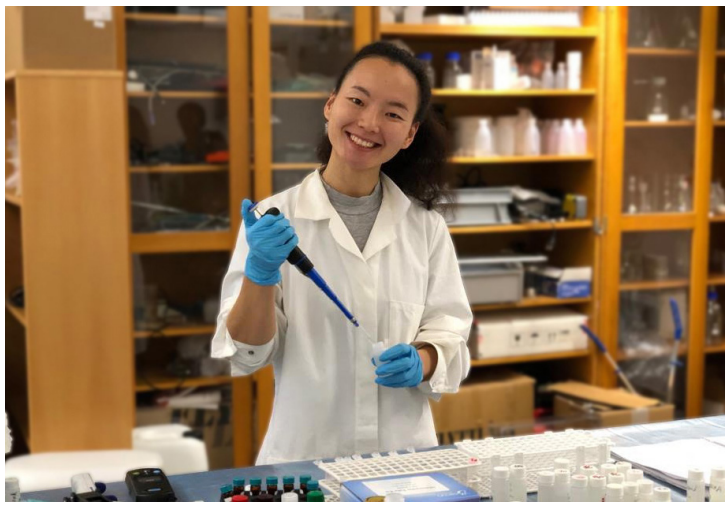
JING LI | FACULTY OF ENGINEERING | LUND UNIVERSITY





I started my career as a water researcher at a start-up company in Stockholm after graduation as an environmental engineer at Lund University in 2011. After two years' applied research work on investigating methods for monitoring drinking water microbial contamination, my ambition of pursuing higher research level brought me back to Lund University. In April 2017, I finally started my doctoral research on investigating methods and strategies for safe drinking water. Since 2014, I have been working as a project coordinator for a multidisciplinary water network, so called LU Water: www.water.lu.se. I also own a private consultant company regarding environment and water, driving Swedish and Chinese collaboration in water innovations.

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DOCTORAL DISSERTATION

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Abstract

In the last decades, the frequency and intensity of cyanobacterial blooms have been of increasing concern. They have become a direct threat to the drinking water supply by clogging filters, bringing odour and unpleasant taste to the treated water and worst of all, causing elevated cyanotoxins, which can be difficult to remove, yet lead to severe health issues. This thesis aims to present a comprehensive knowledge base and tools for water managers and operators to understand cyanobacterial risk in their water so that bloom problems can be prevented or mitigated. Firstly, an adaptive approach for cyanobacteria management in drinking water supply is proposed, starting with an overview of this problem and resulting in a conceptual management tool design, a Cyanobacteria Management Tool (CMT) by which multi-indicators for actions are provided. Secondly, the magnitude of this problem in Swedish freshwaters was studied both on a national and local scale, including their geographical distribution, species dynamics, bloom seasonal pattern and their connection with eutrophication status, land use, and other factors. Thirdly, the study highlights impact of nutrients on cyanobacteria formation, including testing two hypothesizes, 1) if Total Phosphorus (TP) can be used to predict cyanobacteria risk and 2) if the Dissolved Inorganic Nitrogen and Phosphorus ratio (DIN/TP) is a better indicator for cyanobacteria risk than TN/TP. The results were also verified by a full-scale on-site experiment study of pre-treating eutrophic water at a local water treatment plant. Lastly, cyanotoxin detection challenges and strategies are presented. The key findings are:

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- Most problematic lakes that experience intensive cyanobacterial blooms are located in southern Sweden; and the lakes are eutrophic or hypereutrophic due to intensive land use;
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Research findings presented in this thesis can be used to update a locally based CMT and applied as a workflow for water operators to improve their monitoring routines and develop their strategies. Measures to control nutrients in freswaters are necessary to protect our drinking waters from intensive cyanobacterial blooms.

Key words Drinking water, cyanobacterial blooms, cyanotoxin, nutrients, total phosphorus, DIN/TP, quick test kit

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To my family and dear friends for giving me, love, laughter and courage. Life becomes prettier with your smile :)

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感谢父母给予我生命，尊重我的人生选择。你们的善良和笑容是我一生最宝贵的一课。

Big love to my dear Peter for accepting me who I am, for your support to finish this project with good food, warm hugs and comfortable massages.

Abstract

In the last decades, the frequency and intensity of cyanobacterial blooms have been of increasing concern. They have become a direct threat to the drinking water supply by clogging filters, bringing odour and unpleasant taste to the treated water and worst of all, causing elevated cyanotoxins, which can be difficult to remove, yet lead to severe health issues. This thesis aims to present a comprehensive knowledge base and tools for water managers and operators to understand cyanobacterial risk in their water so that bloom problems can be prevented or mitigated. Firstly, an adaptive approach for cyanobacteria management in drinking water supply is proposed, starting with an overview of this problem and resulting in a conceptual management tool design, a Cyanobacteria Management Tool (CMT) by which multi-indicators for actions are provided. Secondly, the magnitude of this problem in Swedish freshwaters was studied both on a national and local scale, including their geographical distribution, species dynamics, bloom seasonal pattern and their connection with eutrophication status, land use, and other factors. Thirdly, the study highlights impact of nutrients on cyanobacteria formation, including testing two hypothesizes, 1) if Total Phosphorus (TP) can be used to predict cyanobacteria risk and 2) if the Dissolved Inorganic Nitrogen and Phosphorus ratio (DIN/TP) is a better indicator for cyanobacteria risk than TN/TP. The results were also verified by a full-scale on-site experiment study of pre-treating eutrophic water at a local water treatment plant. Lastly, cyanotoxin detection challenges and strategies are presented. The key findings are:

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Research findings presented in this thesis can be used to update a locally based CMT and applied as a workflow for water operators to improve their monitoring routines and develop their strategies. Measures to control nutrients in freswaters are necessary to protect our drinking waters from intensive cyanobacterial blooms.

Populär Sammanfattning

Det som vanligtvis kallas blågröna alger, men som inte är alger utan cyanobakterier, kan växa till snabbt i sötvatten. Under vissa fall kan de växa så snabbt att vattnet blir alldeles grönt, man säger att vattnet blommar. Vissa blomningar kan åstadkomma bildning av gifter, toxiner, som kan bli direkta hot mot badare och dem som hämtar dricksvatten från ytvattnen.

Denna avhandling handlar om hur cyanobakterier förekommer i ytvatten i Sverige i allmänhet och i Vombsjön i Skåne i synnerhet. Avhandlingen handlar också om hur väl konstgjord grundvattenbildning fungerar för att avskilja cyanobakterier och toxiner vid beredning av råvatten till dricksvatten. Slutligen redovisas några analysmetoder som kan användas för att mäta förekomst av cyanobakterier och toxiner i råvatten och dricksvatten.

Fokus har varit på att ge dem som använder och förvaltar ytvatten kunskap och hanteringsmetoder för att hjälpa dem att förstå hur cyanobakterier förekommer i råvatten så att de kan förhindra eller mildra problem med blomningar och toxinförekomst i vatten. I arbetet har ett antal indikatorer utvecklats som kan visa risken för förekomst av cyanobakterier i vatten, så kallade cyanobacteria management tool (CMT). För att förstå hur spridda cyanobakterieblomningar är i Sverige gjordes en undersökning på både nationell och lokal nivå utifrån Sveriges 108 trendsjöar som ingår i Havs- och vattenmyndighetens programområde för nationell miljöövervakning. Särskild undersöktes hur sjöarnas näringsinnehåll korrelerade mot cyanobakteriell tillväxt. Två hypoteser testades: 1) om total fosfor (TP) kan användas för att förutsäga risken för cyanobakterier i de svenska trendsjöar; 2) om förhållandet mellan löslig oorganiskt kväve och total fosfor (DIN/TP) är bättre än totalt kväve till fosforförhållande (TN/TP) för att visa på hög nivå av cyanobakterier. Mera mätdata från Vombverket användes också för en fallstudie. Ett antal resultat kom fram av arbetet:

• Med hjälp av kvantilregressionsmetoden går det att visa vid vilken nivå av TP man når den högsta möjliga cyanobakteriella nivår.

• Kvoten DIN/TP är mycket bättre på att förutsäga hög nivå av cyanobakterier än TN/TP; hög nivå av cyanobakterier sammanfaller med DIN/TP <10.

• Det är huvudsakligen sjöar i södra Sverige som har frekventa blomningar, på grund av intensiv markanvändning och påverkan av lantbruk och avlopp. De är eutrofa eller extremt eutrofa med hög halt fosfor i vattnet. Eutrofa skogssjöar är har de högsta nivåerna av cyanobakterier.

• Det finns ett tydligt säsongsmönster för cyanobakteriell biomassa i Vombsjön. Särskild uppmärksamhet bör ägnas från maj till nov, då finns höga cyanobakterieriell risk.

• Kemiska snabbmetoder såsom enzymbunden immunosorbentanalys (ELISA) eller lateral flödesimmunanalys (LFA) kan användas för regelbunden och snabb kontroll av förekomst av toxiner från cyanobakterier i vatten. För exaktare mätningar krävs betydligt mera avancerade analysinstrument där LC-MS / MS visade sig vara mycket användbart för toxinmätningar.

Resultaten av denna avhandling kan användas för att regelbundet uppdatera CMT och användas som ett arbetsflöde för vattenoperatörer för att förbättra sina övervakningsförfaranden och utveckla strategier för att förstå råvattnet och i tid veta när oönskade biologiska förändringar sker i vattnet. Näringskontroll är nyckeln till att förhindra återkommande cyanobakterieblomingar.

摘要

在过去的几十年中，蓝藻水华（又称，蓝绿藻，蓝细菌）的频繁发生和高强度越来越受到社会的关注。有毒蓝藻已经对饮用水的安全造成直接威胁，他们不只是会堵塞过滤器，带来异味，严重的是，他们会产生大量的很难处理的蓝藻毒素，从而导致严重的健康问题。

本文旨在为净水管理者和经营者提供全面的知识和管理方法，以帮助其了解水原地的蓝细菌风险，从而可以预防或减轻蓝绿藻水华问题。首先，该文从这个问题的综述出发，提出了一套在净水供应过程中应对蓝藻的管理方法并提供多个行动指标，即蓝藻管理工具（CMT）。然后为了了解瑞典淡水中蓝绿藻的整体情况，我们在瑞典国家和地方范围内进行了研究，包括其地理分布，物种组成，季节规律，及其与水中富营养化状况，土地利用等因素的联系；继而，我们重点研究了营养物的含量和比例对蓝细菌成长的影响。此研究验证两个假设：1）是否可以使用总磷（TP）来预测蓝细菌在瑞典淡水湖泊的风险；2）预测蓝细菌峰值，是否溶解性无机氮和总磷比（DIN/TP）优于总氮磷比（TN/TP）。瑞典南省的一个净水处理厂也作为案例验证研究结果。最后， 该文总结检测蓝藻毒素的挑战和策略。主要发现总结如下：

• 通过分位数回归方法该文研究总磷对蓝细菌生长能力的影响，提出该方法可用于设立总磷在饮用水水源地的治理指标。

• DIN/TP 是优于 TN/TP 的指标用来预测蓝细菌峰值；蓝细菌达到峰值和DIN/TP < 10 吻合。

• 密集蓝藻繁殖的湖泊集中在瑞典南部；这些湖泊大多数优于土地集约利用，呈富营养化或极度富营养化；富营养化严重的森林型湖泊蓝藻情况更为严重。

• 蓝藻生物量的季节性规律清晰，主要条件表明应在 5 月至 11 月注意蓝藻风险，此时蓝藻在浮游植物群落中占主导地位。

• 诸如酶联免疫吸附实验 ELISA 或侧向流免疫测定（LFA）之类的蓝藻毒素筛选工具可用于大规模筛选检测水中藻类毒素；而若对藻类毒素种类和成分进行分离、鉴定和定量分析，则需依赖（例如 LC-MS / MS）等先进的大型分析仪器。

本文的研究结果可用于持续更新 CMT，并用作水务运营商的工作流程，以改善他们的监测程序并制定策略。最后，我们的工作再次强调营养元素控制是预防密集蓝藻水华的关键。

Papers

Appended papers

This thesis is based on the following papers which will be referred to by their roman numerals in the body of the text. The papers are appended at the end of the thesis.

I. Li, J., Parkefelt, L., Persson, K., & Pekar, H. 2017. Improving Cyanobacteria and cyanotoxin monitoring in Surface Waters for Drinking Water. *Journal of Water Security, 3*. Retrieved from http://jws.asu.lt/jws/article/view/30

II. Li, J., Persson, K.M, Pekar H. and Jansson D. 2020. Evaluation of Indicators for Cyanobacterial Risk in 108 Swedish Trend Lakes Using 23 Years of Environmental Monitoring Data--Application to drinking water and recreational bathe hazard assessment, Manuscript.

III. Li, J., Hansson, L.-A.; Persson, K.M. 2018. Nutrient Control to Prevent the Occurrence of Cyanobacterial Blooms in a Eutrophic Lake in Southern Sweden, Used for Drinking Water Supply. *Water* **2018**, *10*, 919.

IV. Li, J., Hägg, K.; Persson, K.M. 2019. The Impact of Lake Water Quality on the Performance of Mature Artificial Recharge Ponds. *Water* **2019**, *11*, 1991.

V. Li, J., Persson, K.M, Paralytic Shellfish Poisoning toxins (PSPs) monitoring in freshwater for drinking water supply-A review. Manuscript.

VI. Li, J., Persson, K.M, Preliminary validation of a quick detection method for Paralytic Shellfish Poisoning toxins (PSTs) monitoring in freshwater for drinking water production. Manuscript.

Author's contribution to appended papers

I. The author planned the study together with the co-authors, analysed the results and was the main contributor to the writing of all sections and of the discussion and the final review of the paper, with the assistance of the co-author.

II. The author planned the study together with co-authors, performed the statistical analysis, analysed the results and was the main contributor to the writing of all sections and of the discussion and the final review of the paper, with the assistance of the co-authors.

III. The author planned the study together with the co-author, performed the experiments and analysed the results and was the main contributor to the writing of all sections and discussion, with the assistance of the co-author.

IV. The third author was the main contributor to the project plan and Sydvatten's engineering performed the pilot study. The author performed statistical analysis and was the main contributor to the writing of all sections and of the discussion with the assistance of the co-authors.

V. The author planned the study, analysed the results and was the main contributor to the writing of all sections and of discussion and the final review of the paper, with the assistance of the co-authors.

VI. The author planned the study together with the co-author, did experiments and analysed the results and was the main contributor to the writing of all sections and of discussion and the final review of the paper, with the assistance of the co-authors.

Other related publications

Conference Abstracts

Li, J. October 2016, 10th International Conference on Toxic Cyanobacteria, Wuhai, China: Dynasand filtration's impact on artificial pond's filtration and water quality--A case study in Sydvatten, Skåne Sweden

Li, J. November 2017, Nationell Dricksvattenkonferens (National drinking water conference), Stockholm, Sweden: Stopp för Cyanotoxiner i Dricksvatten

Li J.; Hägg K.; and Persson K.M. October 2019, 7th IWA Specialist Conference on Natural Organic Matter Research, Tokyo, Japan: Removal of Cyanobacteria and Cyanotoxin by Managed Aquifer Recharge (MAR) for Drinking Water Supply

Journals and Magazines

Li, J., Persson, K. M. 2017 Kan det finnas cyanotoxiner i dricksvatten? (Are there cyanotoxins in the drinking water?) ***VATTEN - Journal of Water Management and Research***, 73: 145-149, 2017 (in Swedish with English abstract).

Abbreviations

DWTP:	Drinking water treatment plant
MAR:	Managed artificial recharge
TP:	Total phosphorus
TN:	Total nitrogen
Chl-a:	Chlorophyll-a
ATX-a	Anatoxin-a
ATX-a(S)	Anatoxin-a(S)
CYN:	Cylindrospermopsin
MCYST:	Microcystin
MC-LR:	Microcystin-LR
NOD:	Nodularin
NSTX:	Neosaxitoxin
STX:	Saxitoxin
LC–MS/MS:	Liquid chromatography with tandem mass spectrometric detection methods
WHO:	World Health Organization
ELISA:	Enzyme-linked immunosorbent assay
LFA：	Lateral Flow Immunoassay
PBS：	Phosphate-buffered saline
CHABs:	Cyanobacterial Harmful algal blooms
DSP:	Diarrheic shellfish poisoning
ASP:	Amnesic shellfish poisoning
PSP:	Paralytic shellfish poisoning
PSTs:	Paralytic shellfish toxins

1. Introduction

"*Cyanobacteria are arguably the most successful group of microorganisms on earth. They are the most genetically diverse; they occupy a broad range of habitats across all latitudes, widespread in freshwater, marine, and terrestrial ecosystems, and they are found in the most extreme niches such as hot springs, salt works, and hypersaline bays. Photoautotrophic, oxygen-producing cyanobacteria created the conditions in the planet's early atmosphere that directed the evolution of aerobic metabolism and eukaryotic photosynthesis. Cyanobacteria fulfil vital ecological functions in the world's oceans, being important contributors to global carbon and nitrogen budgets.* " ~ (Stewart and Falconer, 2008)

In the last decades, cyanobacteria are mostly known as nauseous harmful algal blooms and have now become a global issue. Cyanobacterial blooms are not only intensified by nutrient pollution from human activities but also climatic induced changes, for example, temperature increase and extreme weather. Consequently, these have a huge impact on the ecosystem and human activities. For example, fishing activities are negatively affected by clogging fishing-nets, while operational problems are caused in drinking water by clogged filters in raw water and unpleasant odour and taste in the treated water. However, the worst of all, public health concern regarding cyanobacteria is intensified since some cyanotoxins produced by cyanobacteria cause severe and irreversible health effects. The major exposure routes of cyanotoxins to humans are food, recreational swimming and poorly treated drinking water.

In Sweden, 75% of the drinking water supply is based on surface water sources, which can be a problem since harmful cyanobacterial blooms have been frequently been found in many lakes in Sweden. Specifically, there is a risk that existing water purifiers fail to remove the toxic cyanobacteria, particularly at small water treatment plants.

This thesis aims to present comprehensive knowledge and tools for water managers and operators to understand their raw waters and its cyanobacterial risk, and hopefully inspire them to develop monitoring tools to evaluate treatment processes and establish toxin monitoring routines, so that problems can be avoided or at least mitigated. To achieve this, the objectives, including papers in this thesis contributing to each objective, are listed below.

1.1 Objectives

- Develop a multi-tiered cyanobacteria/cyanotoxin management tool (Paper I)
- Support nutrient control as the key for cyanobacterial risk control (Paper II, III and IV)
- Investigate hypothesis of using TP as indicator for cyanobacterial risk assessment (Paper II)
- Investigate hypothesis of low DIN/TP as an indicator for cyanobacteria risk in a local lake (Paper III)
- Investigate other cyanobacterial influencing factors (Paper II)
- Discuss impact of lake water quality on the performance of mature artificial recharge ponds (Paper IV)
- Validate a quick test kit for Paralytic shellfish toxins (PSTs) test (Paper VI)
- Develop a protocol for microcystin screening in raw water (Paper I&IV)

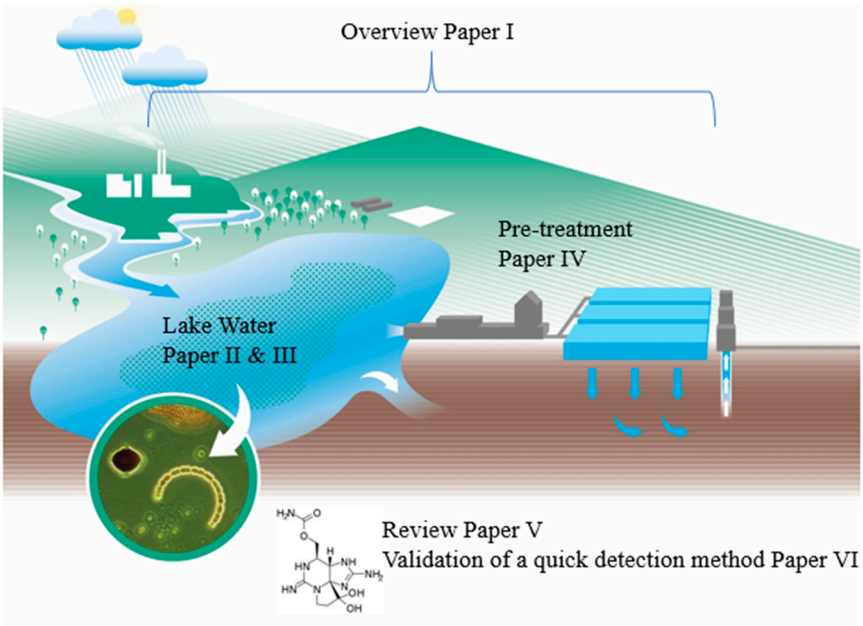

Figure 1. The overview of thesis projects

Figure 1 demonstrates an overview of the thesis content. Firstly, **Paper I** gives an overview of a systematic procedure to manage cyanobacteria in drinking water, including identifying alert levels for the condition of nutrients, cyanobacterial

biomass and cyanotoxin level. **Paper II&III** continue to explore connections between the dynamics of nutrients and cyanobacterial risk in lake water from both national and local perspectives. Furthermore, a local water treatment plant using eutrophic lake water as the drinking water source was utilized as a case study in **Paper IV** to demonstrate the impact of lake water quality on the performance of mature artificial recharge ponds by introducing pre-treatment. In **Paper V**, an emergent type of toxin in freshwater is reviewed, and challenges and opportunities are presented. **Paper VI** presents validation result of a quick test kit for Paralytic shellfish toxins (PSTs) in freshwaters. All the research findings from Paper II, III, IV, V and VI are utilized to update the systematic management concept of cyanobacterial issue in drinking water in **Paper I.**

2. Theoretical background

"*In theory there is no difference between theory and practice.*
In practice there is." ~Albert Einstein

Managing cyanobacterial blooms for drinking water supply requires a systematic approach; multi-tiered monitoring and management tools are needed, including understanding the interaction between blooms and their biological, physical and chemical condition to better predict bloom risk: understanding the species diversity, abundance and toxin profile, creating practical and proper monitoring routines and applying effective treatment methods.

2.1 Cyanobacteria

Algal blooms have grown to be a global issue during the last decades. This phenomenon, which is also called water bloom, is traditionally caused by planktonic cyanobacteria, also known as blue-green algae. They look and behave like algae when blooming occurs but are not algae. In fact, cyanobacteria are categorized together with bacteria in prokaryotic group. A prokaryote is a microscopic single-celled organism that has neither a distinct nucleus with a membrane nor other specialized organelles. Since the photosynthetic cyanobacteria contain chlorophyll like green plants, they use sunlight for fuelling chemical processes in their cells.

Cyanobacteria are found to be the first to produce oxygen on Earth, back to over 4 billion years ago and play an important role in oxygen, nitrogen and carbon cycles at the early life stage of Earth (Allen, Thake and Martin, 2019). Cyanobacteria account for approximately 20 to 30 percent of the photosynthesis on the planet today and continue to play an important role in the composition of the atmosphere. Even though over 2600 cyanobacterial species have been reported, it is believed that there are still many unknown species (Tokodi *et al.*, 2018). The use of discovery curves (i.e., time–species accumulation curve) estimates that this group must contain more than 6000 species (Nabout *et al.*, 2013).

It has been known for a long time that cyanobacterial toxins in lakes, ponds and rivers in various parts of the world cause poisoning of animals and humans. One of

the earliest reports of the toxic effects can be traced back to China 1000 years ago (Chorus and Bartram, 1999). Some cyanobacteria also produce Taste and Odour (T&O) compounds such as 2-methylisoborneol and geosmin, which has caused an earthy-musty taste and odour problems (Izaguirre *et al.*, 1982), for example, 2-methylisoborneol produced in Taihu Lake, China during ''Wuxi Drinking Water Crisis'' in 2007 (Qi *et al.*, 2012). Several cyanobacteria species produce toxins, which cause various adverse effects according to toxin type and route of exposure. Symptoms commonly include skin irritation, stomach cramps, vomiting, nausea, diarrhoea, fever, sore throat, headache, muscle and joint pain, blisters of the mouth and liver damage (Chorus and Bartram, 1999).

Algal blooms usually occur during July and August; however, they may also occur during the spring, late autumn and even winter under ice on the surface. Toxins produced by cyanobacteria may disappear in a few days, but can also remain in water for several weeks, depending on the circumstances. Freely dissolved toxins in the water can be degraded in a few hours, days or weeks, depending on the toxin involved, water and climate conditions, and so on. However, according to a Finnish study, microcystin remained for three months during a winter in the Vantaanjoki River, due to a complete lack of degradation (Kiviranta *et al.*, 1991).

2.2 Nutrient and cyanobacterial blooms formation

It is crucial to reduce nutrient input to aquatic ecosystems to reduce CHABs (Cyanobacterial Harmful Algal blooms) (Paerl, 2008; Xu *et al.*, 2015). Phosphorus is commonly considered as the limiting nutrient in freshwater ecosystem (O'Neil *et al.*, 2012) and examined in the occurrence of cyanobacterial blooms in many regions of the world, such as in the Great Lake and Taihu Lake (Steffen *et al.*, 2014; Huang *et al.*, 2016). To ensure a low probability of CHABs, it recommends that the TP values are less than 20 μg/L which indicates a low probability for cyanobacterial bloom (Carvalho *et al.*, 2013;Paper I). An enclosure experiment in the shallow, subtropical Lake Donghu, China showed that *Microcystis* blooms never occurred in the treatments with low P concentration despite the presence of enough N (Xie *et al.*, 2003).

Further, cyanobacteria should not be regarded as a single group when it comes to potential effects of changes in nutrient load on the structure of phytoplankton community (Dolman *et al.*, 2012; Dolman and Wiedner, 2015). Unlike planktonic algae, some cyanobacteria can fix atmospheric nitrogen in a nitrogen-limiting situation. The availability of nitrate or ammonia is an important factor in determining which cyanobacterial species become dominant. The lack of nitrate or ammonia favours the dominance of nitrogen fixing species such as *Anabaena* and

Aphanizomenon (Issa, Abd-Alla and Ohyama, 2014). This is also observed by our case study in a eutrophic lake in southern Sweden where cyanobacteria tend to dominate the phytoplankton community towards the autumn, when it is likely occur nitrogen limiting conditions, i.e. low TN/TP ratio.

Some reports also showed that a low TN/TP ratio appears to trigger cyanobacterial algal blooms and cyanotoxins (Smith, 1983; Orihel *et al.*, 2012). In a study of 17 lakes by Smith, blue-green algae tended to appear at TN:TP < 29 (Smith, 1983). In a Canadian study, high microcystin concentrations occurred only at low TN/TP ratios in nutrient rich lakes and rapidly decreased at higher TN/TP ratios (Orihel *et al.*, 2012). Certain species such as *Microcystis* has optimized TN/TP shown in microcosm and mesocosm experiments (Amano *et al.*, 2008). Therefore, in some projects such as Lake Taihu, local TN:TP ratio was examined to understand N or P limitation, for preventing blooms in the short term. However, for the long term, a strict dual N and P management strategy is necessary (Ma *et al.*, 2015).

This thesis contributes to the understanding of nutrients condition's impact on cyanobacteria formation and composition changes from both a national scale and local scale and focused on two hypotheses, 1) if TP concentration is suitable for evaluating cyanobacterial risk and 2) if low DIN/TP is a better indicator for cyanobacterial peak than TN/TP.

2.3 Cyanotoxin

As elevated cyanotoxin poses a major threat to ecological environment and human health, identifying specific cyanotoxins is an obvious and essential aspect of cyanobacterial research and management.

Cyanotoxins are a diverse group. According to chemical structure, cyanotoxins are mainly divided into cyclic peptides, alkaloids, lipopeptides non-protein amino acids and lipoglycans. Different chemical structures of cyanotoxins contribute to the diversity of their functional properties. Based on functional properties, cyanotoxins can be mainly classified into neurotoxins (affecting the nervous system), hepatotoxins (affecting the liver), and dermatoxins (affecting the skin), and cytotoxins (affecting cellular function) (Buratti *et al.*, 2017). Several cyanobacteria species may form various toxins. Multiple publications are provided on the principle of cyanotoxin classification, health effects and toxicities to mice and corresponding producer cyanobacteria genera (Chorus and Bartram, 1999; Bhattacharyya *et al.*, 2015; Du *et al.*, 2019; Svirčev *et al.*, 2019)

There is a gap between what can possibly exist in a natural water source and what can be measured. What is measured might just show an iceberg of what is potentially

there. The type of cyanotoxin suggested by the Swedish Food Agency for monitoring in drinking water (Chapter 3.4) has hundreds of different variants, for example, microcystins > 250 analogues(Martens, 2017); cylindrospermopsin > 3 analogues; anatoxins > 2-6 analogues and saxitoxins > 50 analogues (US EPA, 2019).

Mysterious toxin producing mechanism Cyanotxins are toxic secondary metabolites produced by cyanobacteria, and they are not produced during the cyanobacteria cells' entire life cycle, but rather in largely unknown circumstances that allow immediate toxin production (Sabart *et al.*, 2010; Westrick *et al.*, 2010). Cyanotoxins are found both inside the cells and in surrounding waters. Some toxin variants are mostly retained within the cells (i.e. intracellular toxins) during the growth stage of the bloom such as anatoxin-a and the microcystin variants (95 %). While some of toxins such as cylindrospermopsin, produced by *Cylindrospermopsis, Aphanizomenon and Umezakia* may be naturally released into water by living cells; 50 % intracellular and 50 % extracellular (United States Environmental Protection Agency, 2019). Currently, many cyanotoxins are known, but there is a possibility of other/new types of toxins still unknown to us.

Global distribution Global distribution of the common cyanotoxins and related poisoning cases are reviewed by Svirčev and his colleagues, and they found that microcystins were the most often recorded cyanotoxins worldwide, followed by cylindrospermopsin, anatoxins and saxitoxins (Svirčev *et al.*, 2019). This is also because microcystins are the most commonly analysed toxins. It is highlighted in a recent review that there is a lack of studies on cyanotoxins beyond microcystins, especially in environmental sciences (Janssen, 2019).

The above four types of cyanotoxins are listed on the drinking water hazard list (Livsmedelsverket, 2018) and the Contaminant Candidate List (United States Environmental Protection Agency, 2019). Many different toxins have been observed in lakes and rivers in Sweden. For example, microcystins, have been detected in Swedish lakes (Eva Willén, 2007; Larsson *et al.*, 2014) as well as homo-anatoxin, cylindrospermopsin, saxitoxin (Sundh and Lindberg, 2014) and anatoxin (Pekar *et al.*, 2016).

Regulations In 1998 the World Health Organization (WHO) first published a provisional Guideline Value of 1 μg L^{-1} for microcystin-LR, which is the most toxic, widespread and common toxin in water supplies (Sanseverino *et al.*, 2017). The value is likely to be legislated by European Commission in the revised Drinking Water Directive proposal 2020 (European Commission, 2019). The regulation value, however, also needs to address the possible presence of a wide range of other cyanotoxins, for which no guideline values can be derived due to insufficient toxicological data. The presence of microcystins (commonly expressed as microcystin-LR equivalents) may be used as a proxy for overall cyanobacterial risk, but this may miss certain risks, such as the presence of dissolved fractions of

cylindrospermopsin and cyanobacterial neurotoxins. Due to incomplete toxicological data for certain toxins, expert judgement becomes more relevant for the target value for cyanobacterial risk assessment. This leads to variations between countries' thresholds and trigger certain actions (Chorus, 2012; Ibelings *et al.*, 2014; Livsmedelsverket, 2018; United States Environmental Protection Agency, 2019).

As most tested microcystin variants exhibit a strong toxicity, the inadequacy of the current microcystin guidelines based only on microcystin-LR might pose health risks for drinking water supply (Meriluoto *et al.*, 2018). 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 (Meriluoto *et al.*, 2018).

2.4 Managing cyanobacteria and cyanotoxins in drinking water supply

Many countries have implemented a two or three tier alert level system with incremental severity (Ibelings *et al.*, 2014). From a management point of view, drinking water operators must be aware of the species of cyanobacteria that dominates the bloom and their growth patterns, cyanotoxins properties (i.e., intracellular or extracellular) while identify the most effective treatment processes of these toxins.

Managing cyanobacterial blooms is challenging since some species such as *Aphanizomenon* cells are poorly coagulated, pass through filters while not being trapped efficiently in sludge (Zamyadi *et al.*, 2013), and have blooms that can produce various cyanotoxins which may respond differently to treatment (Livsmedelsverket, 2018; United States Environmental Protection Agency, 2019). Cyanotoxins in extracellular form are extremely difficult to remove; they cannot even be degraded by boiling.

Standard treatment processes such as coagulation, flocculation, sedimentation and filtration are effective in removing some intact cyanobacterial cells (Chow *et al.*, 1999; Westrick *et al.*, 2010). Activated carbon, membrane filtration, and chemical inactivation (ultraviolet (UV), disinfectants, and oxidants) are common treatment techniques for the removal of extracellular toxins (United States Environmental Protection Agency, 2019). Furthermore, different types of treatment processes have different impacts on the removal of various toxins (Svrcek and Smith, 2004; Livsmedelsverket, 2018; United States Environmental Protection Agency, 2019).

This dissertation presents the gap between research and practice and tries to bridge it by providing management strategies, monitoring routines and developing protocols as examples for water operators.

3. Methods and materials

"*Be stubborn about your goals but flexible in your methods.*" ~Anonymous

The thesis framework is presented in Fig. 2. To improve cyanobacteria management in drinking water supply, firstly, an overview was performed in **Paper I** and three indicators for actions were identified, including nutrients condition, cyanobacteria biomass alert level and toxin regulations. Then four projects were executed to target these three aspects (Fig. 2). Figure 2 also illustrates the contributions from all five papers to their corresponding perspectives. **Paper II** used an interesting database of a list of so-called trend lakes in the Swedish Environmental Monitoring Program to illustrate the cyanobacterial problems in Swedish freshwaters. This dataset also contributed to test a hypothesis that TP can be used as an indicator for cyanobacterial risk. **Paper III** investigated a local drinking water resource, Lake Vombsjön, to understand how nutrient control can be used for preventing cyanobacteria formation. This dataset also contributed to test the second hypothesis that DIN/TP is a better indicator for cyanobacteria peaks than DN/TP. A case study at a local waterworks, i.e. Vomb Waterworks, in **Paper IV**, demonstrates an example of how improved raw water quality would affect nutrients condition, followed by cyanobacterial biomass and composition and cyanotoxin level. The case study also contributes to the understanding of the importance of introducing pre-treatment of eutrophic water as to improve artificial groundwater recharge performance. At the end, the complexity of cyanotoxin monitoring was presented together with a review of screening saxitoxin in freshwaters in **Paper V** and quick test validation experiment in **Paper VI**. To close the loop of managing cyanobacterial risk in drinking water, an updated CMT is presented as management strategies examples. Last, to secure a toxin free drinking water, more efforts need to be done upstream such as applying advanced treatment methods, or most preferably reducing nutrients in the raw water.

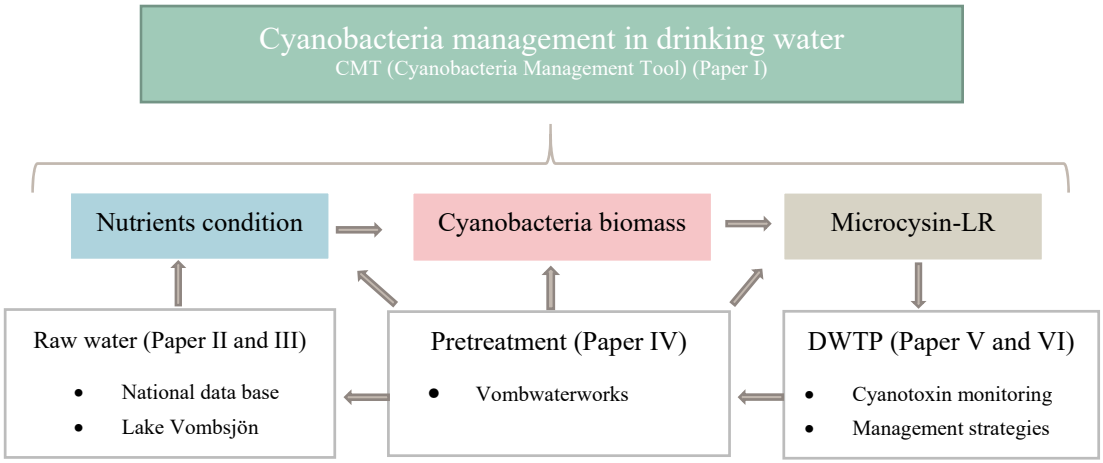

Figure 2. Thesis framework

A *literature study* was done to derive three critical indicators for actions in CMT including TP level, cyanobacteria biovolume and toxin level.

In order to test whether TP can be used as an indicator for cyanobacterial risk prediction, *quantile regression* was applied to explain the complexity of interactions between TP and cyanobacteria growth by using 108 Swedish trend lakes' 23 years of monitoring data in Swedish national freshwater program. TP targets were also defined, by applying WHO drinking water alert levels (**Paper II**).

In order to get a picture of the problem, various statistical analysis methods are used: *PCA analysis,* which can detect strong patterns in a dataset, was applied to visualize the most relevant water parameters in connection with cyanobacteria condition and their connection to different types of land use. The a *Mann Kendall Trend Test (M-K test),* which was useful for analysing data collected over time for consistently increasing or decreasing trends (monotonic) in response values, was applied to test the trend test of cyanobacteria peaks. *M-K test* is a non-parametric test, which means that it works for all distributions. A *change point analyser* was used to check significant break points of a time series data (Taylor Enterprises, 2019). The *Non-parametric Spearman's rank correlation coefficient* was used to assess the relationship between nutrients and phytoplankton biomass. The Spearman's correlation between two variables will be high when the observations have a similar rank (or identical for a correlation of 1) or consequently low when the observations have a dissimilar (or completely opposite for a correlation of −1) rank. p-Value is the significant level which shows the likelihood that the two variables are uncorrelated (Lehman *et al.*, 2013). *Boxplots* were utilized to visualize the seasonal pattern of cyanobacteria and the variation of its percentage in the phytoplankton

community. Boxplots graphically display a variable's location and its spread and provide some indication of the data's symmetry and skewness as well as display outliers.

A case study about pre-treating eutrophic water for groundwater artificial recharge (also called Managed Artificial Recharge, MAR) at Vomb Waterworks was designed to study cyanobacteria formation in two different raw quality conditions as to evaluate the effect of pre-treatment for reducing cyanobacterial risk.

The enzyme-linked immunosorbent assay (ELISA) was applied to screen the microcystin condition, and high-performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) was used for toxin profile confirmation done at Swedish National Food Agency. Beacon Microcystin Tube Kit (Beacon Analytical Systems Inc., Saco, MA, USA) is commercially available. Details refer to their website. Lateral flow immunoassay (LFA) was validated for saxitoxin screening test. The Scotia Rapid Test for Paralytic Shellfish Poisoning (PSP) is also commercially available. Details refer to their website and **Paper V** and **VI**.

3.1 SwAM Trend lakes

There are around 110 lakes (Fig.3) listed as reference lakes as part of a national freshwater program, in terms of point sources and intensive different local land use and may be affected by large-scale airborne pollution and extensive land use (Folster *et al.*, 2014). The actual number varies according to SLU between 106 and 112 depending on the studied year. In the thesis, 108 lakes are evaluated. Sampling routines and database are managed by the Swedish Agency for Marine and Water Management (SwAM) and operated by the Swedish University of Agricultural Sciences (Department of Aquatic Sciences and Assessment, 2019).

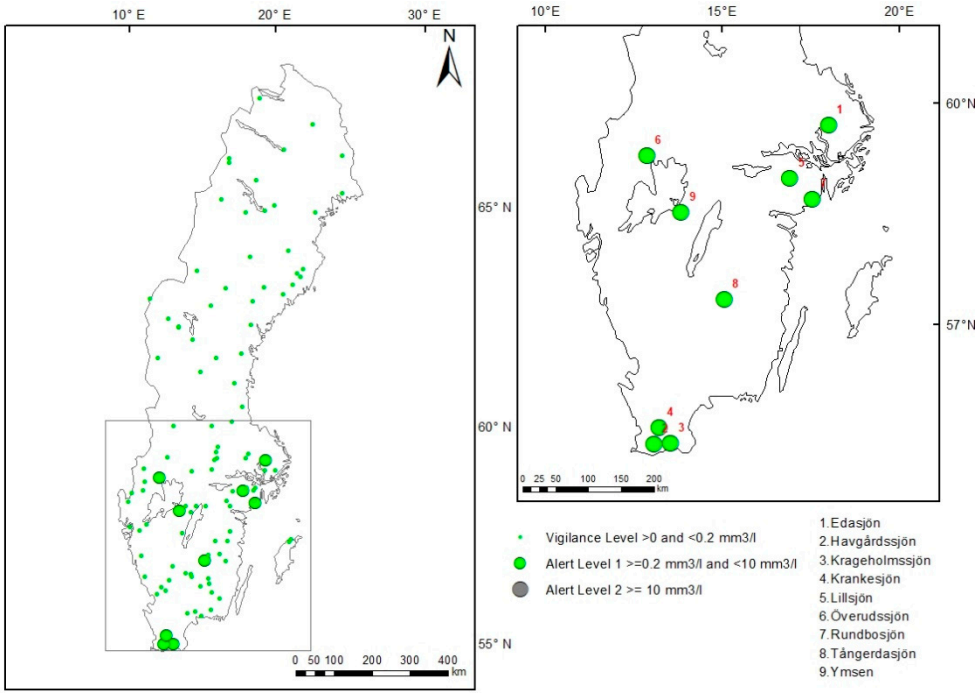

Figure 3. 108 Swedish Trend lakes under environmental monitoring program; 9 lakes are selected for further study regarding cyanobacteria occurrence.

Sampling and biological and chemical analysis

Phytoplankton was sampled yearly in August between 1995-2018 in 108 lakes across Sweden of varying sizes, including 49 lakes < 0.5 km^2, 21 lakes > 0.5 to 1 km^2 and 38 lakes > 1 km^2. The qualitative sampling was made to determine the composition of phytoplankton species and community. All qualitative samples were taken at the lake surface, using a landing net, in the centre of the lake. Total biomass and biomass of the different species were analysed in the quantitative sample collected using a 2 m tube sampler. Samples were collected in the photolytic zone between 0 - 5 meters. In larger lakes (> 1 km^2), the sample was taken at the centre of the lake while in smaller lakes (< 1 km^2) samples at five different locations were taken and then mixed to a composite sample. Surface water samples for water chemical analysis were collected using a Ruttner water sampler.

Phytoplankton analysis comprises 14 classes, with quantitative measures of bio-volume per water volume by using the Utermöhl technique (Utermöhl, 1958). The methods for species and subspecies identification are described elsewhere (Guiry, 1995). The data is freely downloaded from open access (http://miljodata.slu.se/). Relevant parameters for this work are: Cyanobacteria biovolume, Chlorophyll-a, Transparency, Total Phosphorus (TP), Total Nitrogen (TN), Total Organic content

(TOC), pH, Fe, Si, Alkalinity/Acidity, and Conductivity. Analysis methods for chemical parameters and measurement uncertainty are presented in detail elsewhere (Swedish University of Agricultural Sciences, 2019).

3.2 Case studies at Vomb waterworks

Lake Vombsjön is situated 20 km east of the city of Lund and is part of Kävlingeån River's catchment area (Fig. 4). The main types of land use within the catchment area are agriculture (72%) and forestry (23%). The lake has a surface area of 12 km^2; average depth 9.4 m and max. depth 16.0 m (SMHI, 2019a). The lake retention time, which is the average time that water spends in a lake, is 1.04 years. The main inflow to the lake (76%) is from the Björkaån River. The inflow taken from Lake Vombsjön for drinking water supply is about 31 million m^3 of water /year, i.e. 20% of Lake Vombsjön is used for drinking water. Lake Vombsjön is one of the two ordinary raw water sources for Sydvatten, which is one of the largest water suppliers in southern Sweden, representing about 900,000 inhabitants from 17 municipalities.

Lake water quality suffers considerably from the leaching of nutrients and pesticides from the wastewater and agriculture. More than 85% of the external phosphorus and nitrogen load is from agricultural activities (SMHI, 2019b). The accumulation of large amounts of nutrients in the lake sediments has also become a challenge for nutrient management of the lake.

Data source

Nutrients data at the outlet of Lake Vombsjön was collected from 1990 to 2016 within the framework of the regional and national environmental water recipient monitoring program "Vattenanknuten recipientkontrollprogram" (Swedish Agency for Marine and Water Management 2014), led by the Kävlingeåns Water Protection Agency (Kävlingeåns vattenvårdsförbund, 2018). Data was downloaded from Kävlingeåns vattenvårdsförbund: http://kavlingeans-vvf.com/undersokningar/karta/. It is connected to water monitoring system managed by Swedish University of Agricultural Sciences. Since 2019, a new environmental data platform has been utilized, i.e. Miljödata MVM (En webbtjänst med mark-, vatten- och miljödata). Water quality analysis methods are the same as above national reference lakes. Parameters are: TP µg/L, TN µg/L, NOx-N µg/L, NH4-N µg/L, PO4-P µg/L. Phytoplankton data from 1989 to 2010 was provided by the county board of Scania and was analysed using the method of Utermöhl for quantitative assessment of phytoplankton. Data for 2016 was provided by Sydvatten AB, and samples were taken of the incoming water to the drinking water treatment plant (Jacquemot *et al.*, 2016).

Figure 4. The location of Lake Vombsjön

3.3 Managed artificial recharge (MAR)

MAR 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 (Megdal and Dillon, 2015). A standard way of MAR is that surface water is diverted to pass through sand in infiltration ponds into a groundwater aquifer; thereafter, groundwater is extracted and taken to DWTP for, at the Vomb waterworks, mainly reducing hardness and disinfection before distributing to consumers. MAR is commonly used in Sweden and elsewhere, such as in the Netherlands, Australia, China, and the United States (Tielemans, 2007; Dillon *et al.*, 2009; Lu *et al.*, 2011; US EPA, 2012). About 100 treatment plants in Sweden—representing about 25% of the Swedish municipal drinking water supply in Water volume—apply artificial recharge (Svenskt Vatten, 2019). The water quality after the artificial infiltration is important for drinking water safety and is highly dependent on the raw water quality.

Managed artificial recharge (MAR) at Vomb Waterworks

Vomb Waterworks has been using MAR since 1948 for drinking water production due to the unique natural features. Details refer to Paper IV. Vomb Waterworks is run by the largest drinking water supply company in south Sweden (Scania), Sydvatten. Raw water with a flow rate around 1000 l/s is taken from the Lake Vombsjön which is situated about 3 km northeast of the treatment plant; and 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 and filtered through a fine sand layer into a groundwater aquifer. It takes 2 to 3 months

for infiltrated water to reach wells and extraction to Vomb waterworks. After 2017, the sieve station started to apply microscreen of 40 µm pore size.

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 (Pekar *et al.*, 2016) had prompted operators to improve the treatment process by introducing pre-treatment to complement the pond infiltration performance.

Project design

One of the artificial recharge ponds was divided into two separate parts of similar size and shape by a bank of fine material in the middle placed approximately 0.5 m under the surface of the sand; one side received lake water only treated through a microscreen (500 µm pores) as control and another side received water pre-treated by coagulation, flocculation, and continuous contact filtration as 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, located 2 m from the sides of each pond, were sampled (Fig. 5). 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 deep. Groundwater collected in the closest wells from both sides was assumed to be unaffected by each other. The initial condition of the pond was with the top ground surface scraped after not being used for half of a year.

Experiment phrase

Samples were taken every other week after the system became stable, from 10 June to 20 October 2014 at several sites along the treatment process, including incoming lake water, pre-treated water after contact filter treatment and control treatment, water from the contact filter pond and control pond (from the end of August, when there was enough standing water for sampling). The number of samples for each parameter are summarized in Supplement Table S1 in Paper IV. Parameters analysed were turbidity (FAU), UVA254nm, 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. Details of instruments, analysis methods and algae analysis refers to Paper IV.

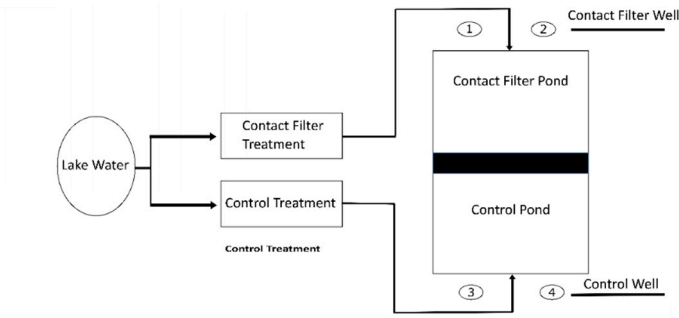

Figure 5. Pre-treatment project design. The lake is Lake Vombsjön from which water is led either through contact filter treatment or control treatment to their corresponding pond, i.e., contact filter pond and control pond.

3.3 Statistical analysis

Quantile regression

Quantile regression differs from the least square regression (linear regression) where the conditional mean of the response variable is estimated by giving values of the predictor variables. The advantage of quantile regression is that its estimates are robust against outliers in the response measurements, facilitating a more comprehensive analysis of the relationship between variables (Koenker and Hallock, 2001). Quantile regression is highlighted in a large European project to derive a quantitative understanding of total phosphorus on cyanobacterial abundance in over 800 European freshwater lakes and set nutrient targets to sustain recreational services and more (Carvalho *et al.*, 2013). In this project, quantile regression is used to study the situation in SwAM trend lakes and set nutrient target to sustain both recreational and drinking water services and provide different levels of precaution for decision making.

Software R offers several packages that implement quantile regression, most notably quantreg by Roger Koenker (Roger *et al.*, 2019). In this project nonlinear quantile regression is used by applying Self-Starting Nls Logistic Model (SSlogis). SSlogis as selfStart model evaluates the logistic function and its gradient. It has initial attributes as initial estimates of the parameters Asym, Xmid and scal. The equation used is

Log10 (cyanobacteria+1) = Asym/ (1+exp ((xmid-log10 (TP))/scal)) (Eq.1.)

Where, **Asym** a numeric parameter representing the asymptote.

Xmid a numeric parameter representing the TP value at the inflection point of the curve. The value of SSlogis will be Asym/2 at xmid.

Scal a numeric scale parameter on the input axis.

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 (Fox, 2008). 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 our study for example—to the estimated resamples. This technique allows for estimation of the sampling distribution of almost any statistic using random sampling methods (Fox, 2008). 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. This method can be used to test the significant mean value difference of two sets of samples such as in our case in Paper IV: if there is significant difference between toxin level with pre-treatment and without treatment.

3.4 Guideline values

WHO (**1999**, **2003**, 2015) recommends 'a series of guideline values associated with incremental severity and probability of health effects'(Chorus and Bartram, 1999; WHO, 2003; World Health Organization, 2015). WHO thresholds for cyanobacterial abundance in recreational waters and drinking waters were applied for cyanobacterial risk assessment.

Drinking Water Alert Level 1: cyanobacterial biomass as 2,000 cells per ml or 0.2 $mm^3 L^{-1}$ biovolume or 1 $\mu g L^{-1}$ chlorophyll-*a*. It requires an assessment of potential toxic cyanobacteria concentration and toxins and consultation with health authorities for ongoing assessment of the status of the bloom and of the suitability of treated water for human consumption. Weekly monitoring is suggested throughout the source water body.

Drinking Water Alert Level 2: cyanobacterial biomass of 100,000 cells per ml or 10 $mm^3 L^{-1}$ bio-volume or 50 $\mu g L^{-1}$ chlorophyll a (with the presence of toxins confirmed by chemical or bioassay techniques). It describes an established and toxic bloom with high biomass and possibly also localised scums. Effective treatment is required, and an alternative drinking water source should be suggested together with media releases of the emergency information, and even direct contact with consumers.

Thresholds for recreational waters: three health alert categories: low (< 2 $mm^3 L^{-1}$), moderate (2 $mm^3 L^{-1}$ to 10 $mm^3 L^{-1}$) and high (> 10 $mm^3 L^{-1}$). A high alert (or high probability of adverse health effects) is assigned when surface scums are present, where cell densities and toxin concentrations can be very high and severe health

risks are possible. The ‘low’ and ‘moderate’ probabilities of adverse health effects are associated with less severe symptoms such as skin irritations and gastrointestinal illness.

In Sweden, the National Food Agency provides action limits applied to drinking water (outgoing and users) but can also be used as guide values for the cyanotoxin content in raw water (Livsmedelsverket, 2018). The action limits for each cyanotoxin are shown below. The motivation for the value selection and the health concerned refer to Cyanobacteria Handbook (Livsmedelsverket, 2018)

- Microcystins 1 µg / l
- Anatoxin-α and homoanatoxin-α 1 µg / l
- Cylindrospermopsins 1 µg / l
- Saxitoxins 3 µg / l
- Nodularins 1 µg / l

3.5 Field tests for cyanotoxin screening

Portable and cost-effective methods that are faster and simpler are acknowledged for practical screening of cyanotoxin at a drinking water source. In our study, we evaluated two types of immunoassay-based screening method: ELISA for microcystin (**Paper IV**) and LFA for saxitoxin test (**Paper V**).

ELISA (Enzyme-linked Immunosorbent Assay)

Instead of using pre-made test strips, ELISA requires samples, microcystin-enzyme conjugate and antibody solution, which are added into the plate wells step by step (Fig.7). Once added, the toxins in the samples compete with enzyme conjugate for a limited number of antibodies. The antibody to bind many microcystin-enzyme conjugate molecules is captured by anti/rabbit lgG coated on the tube wall. After washing, incubating and adding substrate, the solution is converted to a blue compound when there is the presence of bound microcystin-enzyme conjugate. The colorimetric reaction is catalysed by the enzyme that is conjugated to the antibody. Since there is the same number of antibody binding sites in every tube, and each tube receives the same number of microcystin-enzyme conjugate molecules, a sample containing a low concentration of microcystin allows the antibody to bind many microcystin-enzyme conjugate molecules, thereafter, showing darker signal (blue colour). The signal is therefore inversely proportional to the amount of toxins present in the sample (Beacon Analytical System Inc., 2019). With standard toxin concentrations, it can calculate the toxin concentration in the sample. The range is from 0.3 µg / l to 5 µg / l.

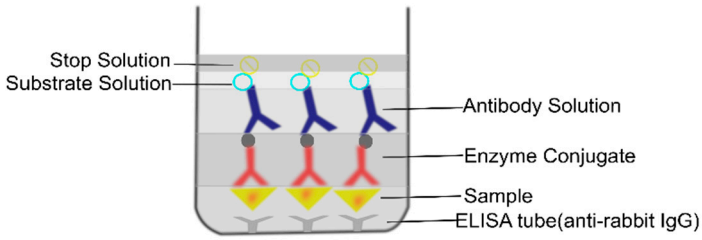

Figure 6. Example of ELISAS Principle (Picture, Jing Li)

LFA (Lateral Flow Immunoassay)

LFA gives qualitative results by indicating a positive/negative (Yes/No) signal. Several different formats of LFA have been described. The following format is generally used for toxin analysis (Mcleod, Burrell and Holland, 2015). Pre-made strips of carrier material that contain regions where antibodies and toxin have been bound are used (Fig. 6). After the extract mixed with a buffer solution, it is pipetted in the Sample Pad, where it flows downstream over an adjacent reagent pad containing labelled antibodies. Any toxin in the extract competes with toxin bound on the test line for the labelled antibody. The antibody bound with toxin on the test line will give distinct colour, such that the colour intensity of the test line is inversely proportional to the amount of toxin in the extract. There is a control line downstream, which has the bound antibodies that always bind the labelled antibody, which yields intense colour, confirming the assay's proper functionality. There are several commercially available LFIAs based products for some of the major algal toxin groups such as Diarrheic shellfish poisoning (DSP), Amnesic shellfish poisoning (ASP) and Paralytic shellfish poisoning (PSP). They are mainly provided by Scotia Rapid Testing Ltd (formal name: Jellet Rapid Testing Ltd.) or Neogen Europe Ltd.

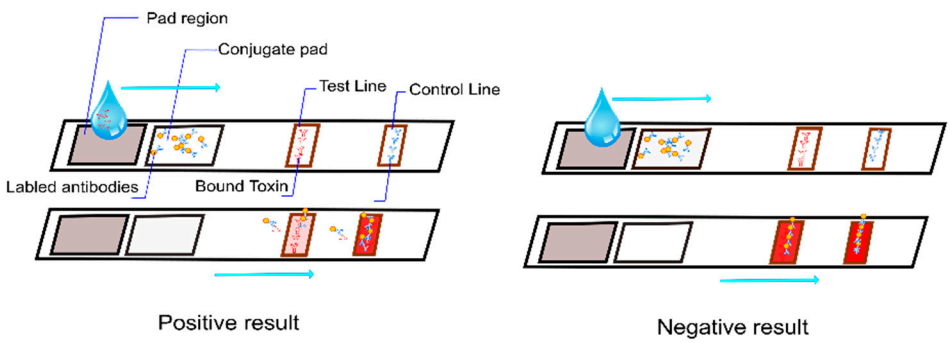

Figure 7. Example of LFA principle: the left shows that toxins in the sample compete with toxins bound on the test line with the labelled antibodies and show less color thanin the right figure that all labelled antibodies are bound to the toxins on the test line (Picture, Jing Li)

4. Cyanobacterial risk in a Swedish context

"It's the small pieces that make the big picture" ~Anonymous

This chapter aims to give a picture of the size of the problem regarding the presence of cyanobacterial blooms in Sweden and explores its connection with land use and eutrophication statues and seasonal patterns. 108 Swedish lakes' 23 years of environmental monitoring data and a case study at a local water resource, Lake Vombsjön are used.

4.1 General distribution of cyanobacterial biomass

Geographical distribution of all 108 SwAM Trend lakes is presented in **Paper II,** shown in Fig. 2. In the figure, it shows a high occurrence of cyanobacteria (i.e. median value > 0.2 mm^3 L^{-1}) present in 9 lakes (8 % of SwAM trend lakes) mainly located in the south of Sweden. Lillsjön has the highest median value and in more than 60 % of the cases, cyanobacteria are dominating (> 50%). Krageholmssjön has the largest variance. All 108 lakes' cyanobacterial biomass values with min, max and median refer to Supp. Tab.1 in **Paper II**.

Svirčev and his colleagues reviewed 1118 recorded identifications of major cyanotoxins in 869 freshwater ecosystems from 66 countries throughout the world and showed that microcystins were the most often recorded cyanotoxins worldwide (63%), followed by cylindrospermopsin (10%), anatoxins (9%) and saxitoxins (8%). The most commonly found toxic cyanobacterial genera were *Microcystis spp. Anabaena spp. Aphanizomenon spp. Planktothrix spp*. and *Oscillatoria spp.* (Svirčev *et al.*, 2019)

In Swedish trend lakes, the most frequent taxa and species are summarized in **Paper II** and Fig. 8**,** by analysing samples that are of a biovolume > 0.2 mm^3 L^{-1} (424/ 2410 samples from 1995 to 2018). The most frequent taxa are presented in Fig. 8 A and the most frequent species are presented in Fig. 8 B. In Fig. 8, it shows that *Dolichospermum spp. (*previously named *Anabaena)* and *Aphanizomenon spp.* were the most frequent species, followed by *Microcystis spp.* and *Woronichinia spp.* and *Planktothrix agardhii.*

To discover the potential toxins produced by the most frequent cyanobacterial species, a literature study was done and summarized in **Paper II** (Sup. Tab. 4). This information also refers to Svirčev' s recent review (Svirčev *et al.*, 2019). In summary, cyanobacteria in those 108 trend lakes have the potential to produce several variants of microcystins, anatoxins, cylindrospermopsins, and saxitoxins. As the most frequent cyanobacterial species comprise almost 90 % of the occurrence, it indicates that cyanobacteria biovolume > 0.2 mm^3 L^{-1} in those lakes were potentially toxic.

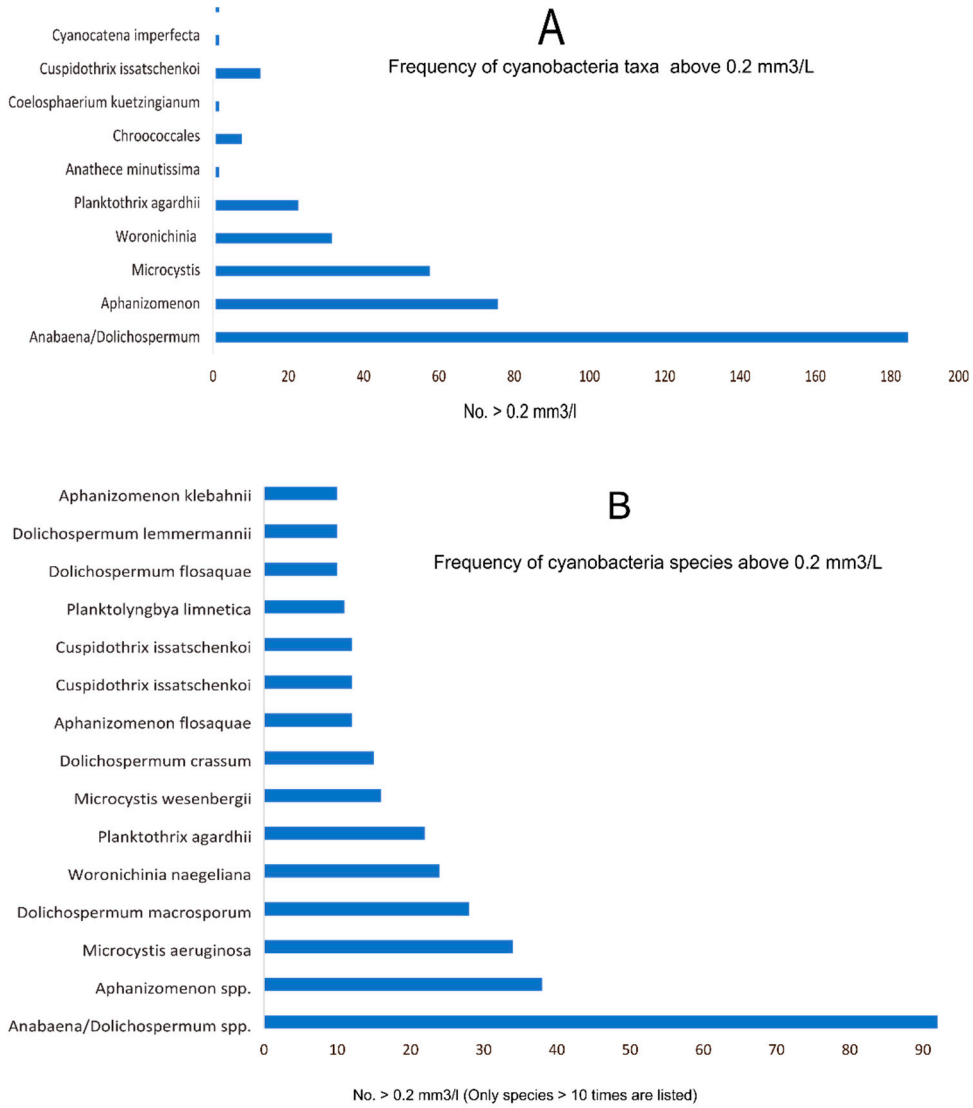

Figure 8. Cyanobacteria biovolume in the Swedish trend lakes, exceeding 0.2 mm3 L-1 (Drinking water alert level) divided into A) dominating taxa and B) dominating species. Spp. are sum of unidentified species.

4.2 Seasonal pattern of cyanobacteria

Lake Vombsjön has been experiencing heavy algal blooms almost every summer since the 1970s. **Paper III** used long-term phytoplankton monitoring data from 1989 to 2002 to demonstrate a clear seasonal pattern in the cyanobacterial development in Fig. 9. Figure shows that bloom might start in the spring, followed by an increase during July and August, which generally peaks in September and then eventually declines.

In the figure, compared to WHO alert levels, most samples from May to Nov (80 % of total samples), are above WHO Drinking Water Alert Level 1 (Blue line) and in September, more than 25 % of the cases are above WHO Health Threshold 2 / Drinking Water Alert Level 2 (Red line). This means that advanced treatment is required, and alternative water sources should be considered for drinking water supply while recreational activities should not be allowed due to high health risk. Since in general 50–75 % of algal blooms are toxic (WHO, 2003), this suggests that raw water taken from the lake for drinking water purposes might frequently be toxic during bloom seasons.

We also found that the later in the season a bloom occurs, the more likely it is dominated by cyanobacteria (Fig. 10). During the period of Sep to Nov the percentage of cyanobacteria present in the phytoplankton community was often above 80 %, suggesting that the monitoring of algal blooms in late autumn is crucial.

In this context, from June to Nov., weekly monitoring in raw water with toxic species concentration and toxin concentration are suggested; advanced treatment methods should be installed if MAR or other biological treatment methods are lacking, or alternative water sources should be provided.

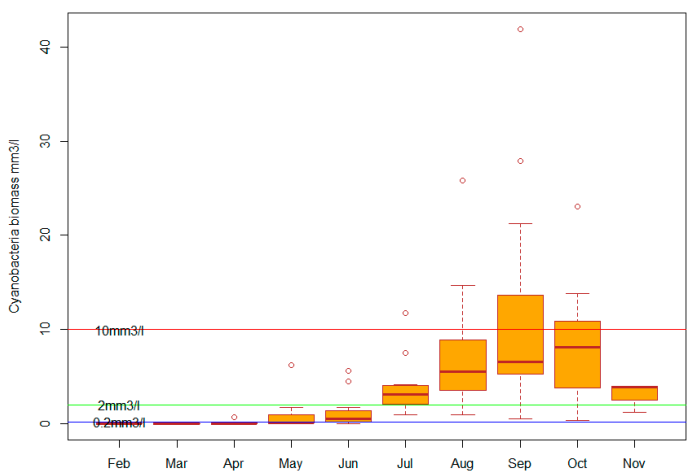

Figure 9. Seasonal pattern of cyanobacteria biomass in Lake Vombsjön from 1989 to 2002

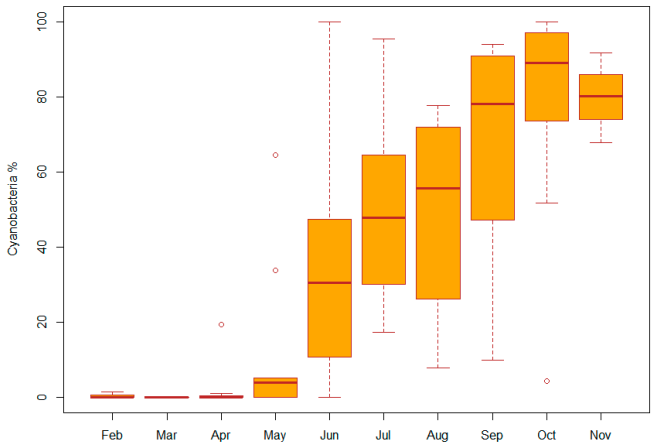

Figure 10. Cyanobacteria percentage in plankton group in Lake Vombsjön increases towards the end of bloom season

4.3 Species diversity

Eutrophication is the major process stimulating the growth of algal and cyanobacterial biomass and affect the diversity of species (**Paper II**). The more europhic the water is, the more multi toxin producing species are likely co-occuring (Rantala *et al.*, 2006; **Paper II**). In Finland, a study showed in oligotrophic lakes, the occurrence of only one MC producer was most common while the combination of *Microcystis* and *Planktothrix* was slightly more dominant than others in mesotrophic lakes, and the co-occurence of all three MC producers i.e. *Microcystis, Planktothrix* and *Anabaena spp* were most widespread in both eutrophic and hypertrophic lakes (Rantala *et al.*, 2006). The connection between eutrophication status and the diversity of cyanobacteria taxa is also observed in our SwAM lakes' study in Fig.11 and **Paper II.** Fig. 11 illustrates the diversity of cyanobacteria species in the 9 lakes plus Lake V. as a reference lake. Figure shows that the reference lake with the lowest level of phosphorus has only one specie while the lake with highest level of phosphorus, i.e. Krageholmssjön, has more than 7 taxa. The total phosphorus concentration has a significant correlation (0.54) with the number of taxa surpass 0.2 mm^3 L^{-1}.

By studying, the historical background of land use in **Paper II,** summarized in Supp. Tab. 2, that all those 9 lakes with high occurrence of cyanobacterial biomass can be categorized as having a bad or unsatisfied ecosystem status mainly due to high eutrophication, which is highly influenced by urbanization, agriculture and being historically exposed for not having well treated wastewater. How nutrients load and its changes affect cyanobacteria formation and phytoplankton community composition is presented in Chapter 5.

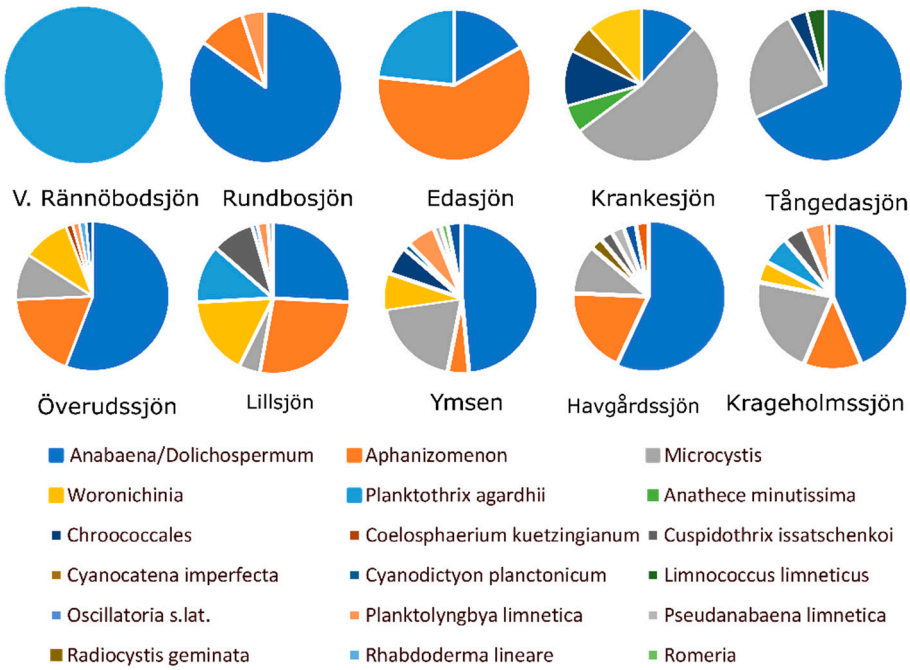

Figure 11. Diversity of cyanobacteria species increases with eutrophication status; eutrophication statues is increasing from left to the right, from the first line to the second line.

4.4 Factors beyond nutrients

Cyanobacterial blooms in freshwater bodies are intensified not only by eutrophication but also by climatic induced changes. Experimental evidence has shown that the synergistic interaction between climate warming, eutrophication and brownification (increase of organic matter in water) on cyanobacterial biomass (Rigosi *et al.*, 2014; Urrutia-Cordero, Ekvall and Hansson, 2016; **Paper II**).

Cyanobacterial blooms are predicted to become even more common due to climate warming (Paerl and Huisman, 2008; Jeppesen *et al.*, 2009). In Sweden, ¨mini-lakes¨ studies have demonstrated that synergies between climate warming and increasing levels of humic substances in runoff are able to trigger an increase in cyanobacteria biomass (Urrutia-Cordero, Ekvall and Hansson, 2016), as well as a reduction in the biodiversity of phytoplankton (Hansson *et al.*, 2007; Ger *et al.*, 2016). In **Paper II**, a correlation analysis between water quality parameters in above mentioned 9 lakes shows that Total organic matter is positively significantly correlated with cyanobacterial biomass except for nutrient conditions such as TP and TN, Chl-a content and alkalinity condition. It is also confirmed by PCA (Principle Component

Analysis) plot (Fig. 12), which illustrates their spatial connections, that in eutrophic water, TOC and cyanobacteria abundance are correlated.

The figure also clearly illustrates that the lakes surrounded by more forest form a distinct cluster on the top left, and lakes that are surrounded by more agricultural land, are clustered in the bottom right. The former ones are closer to high cyanobacterial biomass and cyanobacteria dominating situation, total organic matter and Fe and the latter is closer to nutrient condition. Additionally, we can also notice that there are two lakes very distinct from others: one is a lake with mixed land use (Ymsen), located in-between the two clusters and a reference lake (V. Rännöbodsjön) with comparatively clearer water is located far from others.

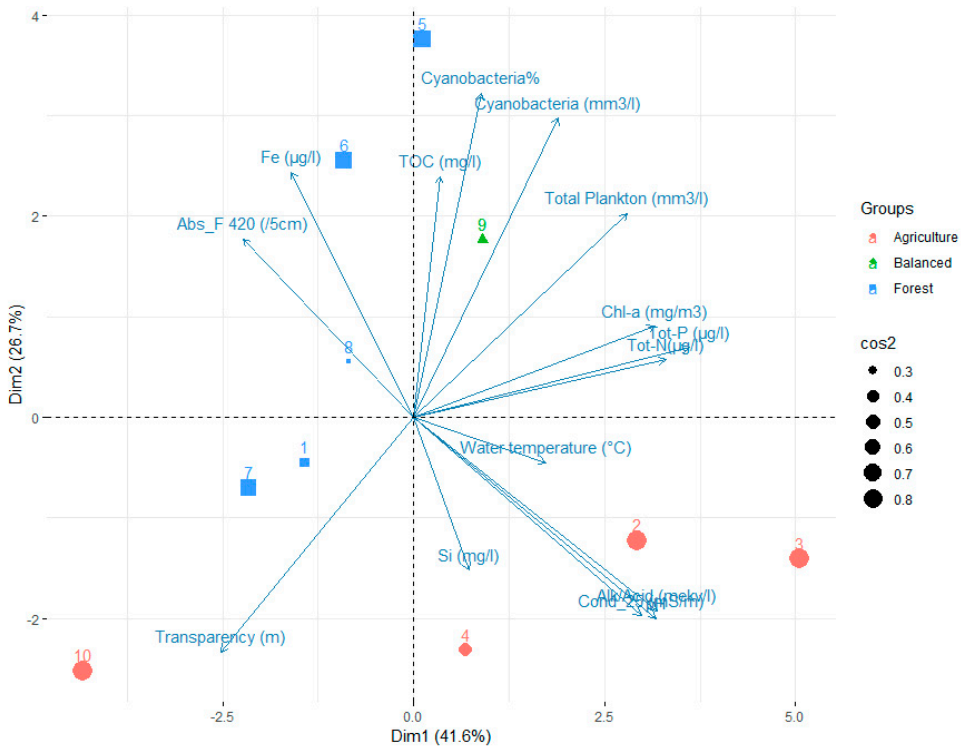

Figure 12. PCA plot of water quality analysis based on 23yrs' median data in 9 selected lakes, plus lake 10 as a reference.

4.5 Limitations and application

Among SwAM trend lakes, only 8% lakes displayed problematic cyanobacteria abundance between 1995 –2018. The percentage is probably valid for Swedish Lakes, but cannot be extrapolated and cannot be applied to source waters for drinking water supply. Source waters for drinking water production are often located close to cities and in general more impacted by anthropogenic activities than many of the Swedish trend lakes, for example, Lake Vomb. Being above WHO drinking water alert level 1 requires more frequent monitoring in those lakes. We should also be aware that the samples were taken in August, so that it is highly likely that we missed other peaks in different months and lakes. For example, *Aphanizomenon* was abundant also in November and *Planktothrix agardhii* has been identified in considerable volumes during winter in some lakes (Willén and Mattsson, 1997). We have also already shown that the highest peaks might appear in September as it is shown in Lake Vombsjön (Fig. 9).

This study can be used as a reference for managing cyanobacteria in eutrophic lakes. Our study confirms that eutrophication is the major process stimulating the growth of algal blooms. All selected 9 lakes are categorized to be eutrophic or hypereutrophic. It also confirms that the increase of eutrophication would increase the number of toxic species that are co-occuring. This might also indicate a diverse toxin profile.

Our study also shows that water managers can benefit from a long-term monitoring dataset from which cyanobacteria species' dynamics and seasonal pattern can be derived. One immediate application in Lake Vombsjön is to increase raw water monitoring during June to November. Sufficient treatment or alternative water resources are required.

In the next Chapter, we will continue to investigate how nutrients load and how the changes of load affect cyanobacteria formation and present the results of the two hypotheses. Details also refer to **Paper II and III.**

5. Nutrients and cyanobacterial blooms formation

"*We all live downstream.*" ~ Award-winning geneticist, David Suzuki

Despite the complexity of the various environmental factors influencing cyanobacteria abundance as well as their various survival strategies (Reynolds, 2006), it is still of great importance to understand more fully the response of cyanobacterial abundance in relation to nutrient pressures. In this chapter, we focus on the interaction between nutrients and cyanobacterial formation, discuss the probability to use TP to indicate cyanobacterial risk, and support nutrient control as a fundamental measure for cyanobacterial control.

5.1 How changes of nutrient load affect cyanobacterial formation?

Cyanobacteria should not be regarded as a single group when it comes to potential effects of changes in nutrient load on the structure of phytoplankton community (Dolman *et al.*, 2012; Dolman and Wiedner, 2015). Unlike planktonic algae, some cyanobacteria can fix atmospheric nitrogen in a nitrogen-limiting situation. The availability of nitrate or ammonia is an important factor in determining which cyanobacterial species becomes dominant. The lack of nitrate or ammonia favours the dominance of nitrogen fixing species such as Anabaena and Aphanizomen (Issa, Abd-Alla and Ohyama, 2014). Although some species are not nitrogen fixing, they are very competitive to get access to nitrate or ammonia at low concentrations. For example, *Woronichinia naegeliana* can thrive in low nitrogen to phosphorus ratios (N:P < 15) condition (iNaturalist Netværk, 2019; **Paper IV**). This is also observed by our case study in a eutrophic lake in southern Sweden where cyanobacteria tend to dominate when there is likely nitrogen limiting conditions, i.e. low TN/TP ratio, which happens at the end of the summer and early autumn.

Some reports also showed that a low TN / TP ratio appears to trigger cyanotoxins, except for cyanobacterial algal blooms (Smith, 1983; Orihel *et al.*, 2012). In a study of 17 lakes by Smith, blue-green algae tended to appear at TN: TP < 29 (Smith,

1983). In a Canadian study, high microcystin concentrations occurred only at low TN/TP ratios in nutrient rich lakes and rapidly decreased at higher TN/TP ratios (Orihel *et al.*, 2012). Certain species such as *Microcystis* has optimized TN/TP shown in microcosm and mesocosm experiments. They grow significantly with a decrease of the ratio and a decrease with an increase of the ratio. Significant suppression of *Microcystis* growth (70 %) could occur when the TN:TP ratios exceed 21 (Amano *et al.*, 2008). Therefore, in some projects such as Lake Taihu, local TN: TP ratio which indicating N or P limitation, was derived for preventing blooms effectively in the short term. However, for the long term, a strict dual N and P management strategy is necessary (Ma *et al.*, 2015). **Paper III** particularly investigated how TN / TP influence the formation of cyanobacteria and found that the changes of TN / TP might be the reason behind the seasonal pattern of cyanobacteria dominating condition, where the highest cyanobacteria biomass (Fig. 9 in Chapter 4) corresponds to the lowest TN / TP condition (< 15, below the blue line) Fig. 13 , i.e. in September.

A case study in **Paper IV** also demonstrates that in raw water with an average of TN/TP < 10, intensive cyanobacterial blooms (left in Pic. 1) could occur. Species are likely *Woronichinia/Snowella/Microcystis/Radiocystis* (shown in a global overview of species in **Paper IV**). While on the pre-treated raw water side, where TN/TP > 40 (right in Pic. 1), blooms are prevented; even though some species passed the filter, such as *Anabaena spp.*; they didn't develop in the pond water. This might also be due to low TP in the pond water thanks to pre-treatment (Chapter 2. 3.2).

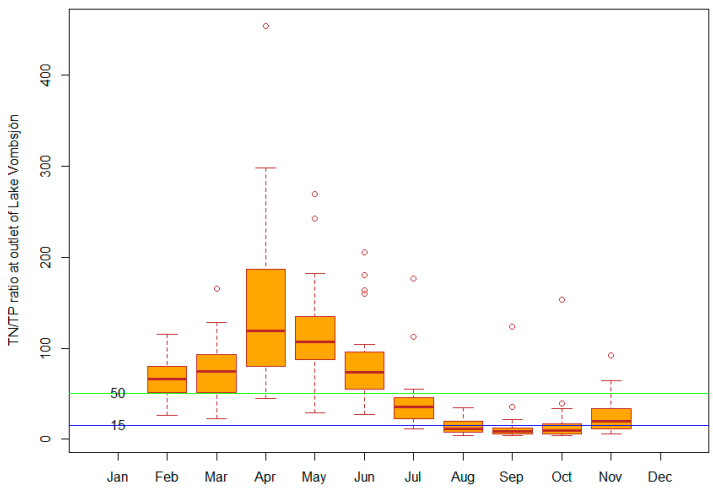

Figure 13 Seasonal pattern of N:P ratio from 1990 to 2016

Paper III further investigated the correlation between cyanobacteria biomass and TN, DIN (dissolved inorganic nitrogen), TP, DP (dissolved phosphorus), TN/TP and DIN:TP in Lake Vombsjön by using the data from 1990 to 2002, described in Chapter 2. Correlation in Table 1 shows that TP has stronger positive correlation than DP with cyanobacteria biomass and DIN/TP has stronger correlation than TN/TP with cyanobacterial biomass. These results suggest that both TP and DIN/TP might be useful for indicating the cyanobacterial risk.

Table 1.Correlations between concentration of cyanobacteria and TN, DIN (dissolved inorganic nitrogen), TP, DP (dissolved phosphorus), TN:TP and DIN:TP in Lake Vombsjön, and resulting P-values (Spearman's Rs)

Item	TN	DIN	TP	DP	TN:TP	DIN:TP
Correlation Coefficient	-0.56	-0,65	0.62	0,31	-0.63	-0.66
P-Values	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

5.2 TP as indicator for cyanobacterial risk prediction

As TP is highly correlated with cyanobacteria biomass, it is commonly considered as the limiting nutrient in a freshwater ecosystem (O'Neil *et al.*, 2012). Phosphorus has been examined in the occurrence of cyanobacterial blooms in many regions of the world, such as in the Great Lakes in the US and Taihu Lake in China (Steffen *et al.*, 2014; Huang *et al.*, 2016). An enclosure experiment in the shallow, subtropical Lake Donghu, China showed that *microcystis* blooms never occurred in the treatments with low P concentration despite the presence of enough N (Xie *et al.*, 2003). To ensure a low probability of CHABs, it recommends that TP < 20 μg L^{-1} which indicates a low probability for cyanobacterial bloom (Carvalho *et al.*, 2013) and **Paper I and IV.** When raw water was improved by pre-treatment to a level of TP < 20 μg L^{-1}, shown in **Paper IV**, that clear difference was observed in the water quality in the artificial recharge ponds (Pic.1). This is also contributed by TN/TP changes (Chapter 5.1)

Picture 1. Onsite observation of two sides of pond left: standard way, right after contact filtration(Paper IV, Photos: Marie Baehr and Petra Larsson)

As a key message in this thesis, TP concentration is critical for evaluating cyanobacterial risk and TP control is key for preventing cyanobacteria blooms. It was done by quantile regression as it has an advantage to give a comprehensive analysis of the relationship between variables.

Quantile regression with a fitted 3-parameter sigmoid nonlinear model are displayed in Fig. 14 by using all 108 lakes' data (Chapter 2). **Paper II** presented that all quantiles, including lower quantile (0.05), show a significant relationship between cyanobacteria biomass and TP. It might suggest that those SwAM trend lakes are sensitive in response to phosphorus concentration. This relationship differs from Carvalho and his colleagues' study, involving more than 800 European lakes (Carvalho *et al.*, 2013), in which a significant relationship was only present in higher quantiles (0.25 and above).

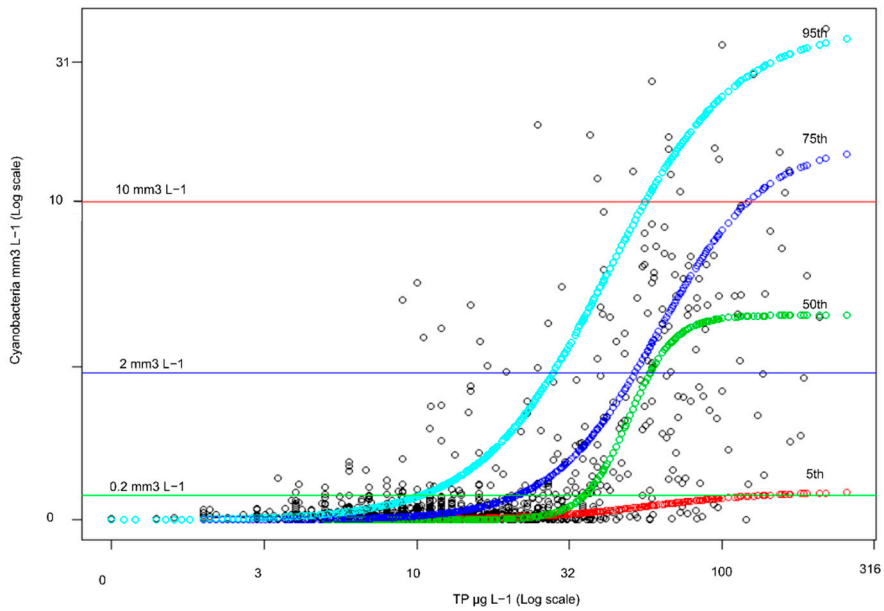

Figure 14 Scatter plot for log10(cyanobacteria+1) and log10(TP) (Total phosphorus) for Swedish trend lakes (n=108, 1995 to 2018). Quantile regression curves (0.5–0.95) using a fitted 3-parameter sigmoid nonlinear model are displayed. Thresholds relating to approximate WHO (1999) alert level 1 (0.2 mm3 L^{-1})and 2 (2 mm3 L^{-1})for drinking water are indicated together with thresholds relating to approximate low (2 mm3 L^{-1}) and medium health risk thresholds (10 mm3 L^{-1}) for recreational water.

Paper II also derived regression algorithms for predicting the bloom capacity at present temperature regimes. For example, the 95th quantile (i.e. 5 % exceeding rate) in Fig. 14, can be used to estimate the potential maximum capacity of cyanobacteria in response to the increase of TP. Both Fig. 14 and the calculation of 95% quantile fitted values (**Paper II**) demonstrate that the capacity for cyanobacteria increases with increasing TP, until TP concentrations > 150 µg L^{-1}; 5 % of samples would exceed drinking water alert level 1 at TP of 10 µg L^{-1}; and exceed drinking water alert level 2 at TP of 53 µg L^{-1}.

Nutrient targets in relation to alert levels for drinking water can be derived by applying quantiles algorithms with quantiles > 0.5 in Tab. 2; nutrient targets are calculated in relation to alert levels for drinking water. The results in Tab. 2 demonstrates that at a TP of about 13 µg L^{-1}, 10 % of samples exceeded the WHO drinking water alert level 1, if TP increases to 35 µg L^{-1}, the percentage of exceedance will be 50%. Similarly, it shows at a TP concentration level of 72 µg L^{-1}, 10 % of samples would surpass the drinking water alert level 2 and medium health risk. By using WHO health risk thresholds for recreational water, TP target can also be derived (**Paper II**).

Table 2.Total phosphorus (TP) concentrations for a given likelihood (quantile) of being below low and medium risk World Health Organisation (WHO 1999) threshold levels for cyanobacteria volume. TP concentrations are obtained from the fitted quantile regression models

Quantile		50th	60th	70th	80th	90th	95th
% exceeded		50%	40%	30%	20%	10%	5%
Drinking water alert 1 0.2 mm^3 L^{-1}	TP µgL^{-1}	35	30	24	19	13	10
Low health risk 2 mm^3 L^{-1}	TP µgL^{-1}	58	56	54	49	35	28
Drinking water alert 2/Medium health risk 10 mm^3 L^{-1}	TP µgL^{-1}				117	72	56

By applying results from Tab. 2 to our selected 9 lakes (Chapter 2), of which all have a median value of TP above 33 µg L^{-1}. Comparing with models' results in Table 2, it might indicate that at least 40 % of samples are above the drinking water alert level 1 and 10 % samples are above the low health threshold. Lake Krageholmssjön with the highest median value of TP, around 129 µg L^{-1}, is likely to be more than 20 % of samples above the medium health risk and drinking water alert level 2. TP target for low health risk of bathing in SwAM trend lakes allowable 10 % exceedance rate is 35 µg L^{-1}.

5.3 Challenges of cyanobacterial risk monitoring and predicting

Besides the influence of nutrients, there exist various factors in this business such as temperature, wind, land use, DO, salinity, pH, lake geometry, species competition, grazer as well as their various survival strategies (Reynolds, 2006). Fig. 14 also indicates that there are likely other factors involved, as the continuous increase of TP after 150 μg L^{-1} has little impact on influencing cyanobacteria biomass formation. Hydrodynamic factors such as turbulence/vertical mixing and water residence time/flushing are very important factors influencing cyanobacteria formation. Small movements in the water column and low turbulence conditions favor cyanobacteria, and long residence time increases cyanobacteria dominance (Paerl and Otten, 2013).

Transparency and Chl-*a* are listed as important factors for assessing the potential presence of cyanobacterial biomass in water (World Health Organization, 2015). If transparency is low (less than 1-2 m) and accompanied by a blue and green water colour, high cyanobacterial biomass is likely. Many research projects have used Chl-*a* as indicator or proxy for water quality in such algal blooms (Kreakie *et al.*, 2015; Lee and Lee, 2018). Taste and odour problems begin occurring once Chl-*a* values reach 10 μg L^{-1} (Kansas Department of Health and Environment Bureau of Water, 2011). A Chl-*a* concentration of 1 μg L^{-1} in raw water triggers Alert Level 1 in WHO management system for cyanobacterial blooms in the perspective of drinking water (Chorus and Bartram, 1999) and is used as a national guide implemented in the Czech Republic and in Turkey (Chorus, 2012).

However, our evaluation on transparency and Chl-*a* as an indicator for cyanobacteria peaks shows that this only fits for certain lakes. Using monitoring of Chl-*a* or Secchi depth may fail to indicate water quality degradation by extreme nutrient concentrations (Filstrup and Downing, 2017). A study of a lake from an agricultural region showed that extreme nutrient regimes in lakes can produce novel relationships between phytoplankton and nutrients. For example, Chl-*a* was weakly related to TN when TP was ≤ 100 μg L^{-1} but displayed a stronger response to TN at higher TP; When TP exceeded 100 μg L^{-1}, Chl-*a* increased along with increasing TN until reaching a threshold of about 3 mg L^{-1} and decreased thereafter, resulting in a high nutrient, low Chl-*a* region that did not coincide with shifts in nutrient limitation, light availability, cellular Chl-*a* content, phytoplankton composition, or zooplankton grazing pressure. The study also showed that beyond the threshold, nitrate comprised most of TN and occurred with reduced dissolved organic matter (DOM). These observations suggest that photolysis of nitrate may produce reactive oxygen species that damage DOM and phytoplankton. Reduction in N loading at

high P could therefore increase Chl-*a* and decrease water clarity, resulting in an apparent deteriorating water quality (Filstrup and Downing, 2017).

By using eutrophic water for drinking water supply with intensive algal bloom problem, advanced treatment processes are required to secure drinking water safety. The next chapter demonstrates management strategies.

6. Management strategies and future work

"*A good plan today is better than a perfect plan tomorrow*" ~ Proverb

This chapter aims to present ideas on how to update the Cyanobacteria Management Tool by integrating the above research findings and knowledge beyond. A short summary of future work with MAR as a barrier for microcystin removal and cyanobacteria monitoring are presented, followed by a protocol for ELISA microcystin test and future work considerations.

6.1. An updated CMT

The CMT proposed in **Paper I** can be updated and adapted to any local situation based on findings provided by this thesis and beyond. Fig. 15 illustrates the framework as an example, which can be a complement to the handbook of managing cyanotoxins in drinking water (Livsmedelsverket, 2018). The handbook provides recommendations on how producers and drinking water providers can prevent/avoid the presence of cyanotoxins in drinking water.

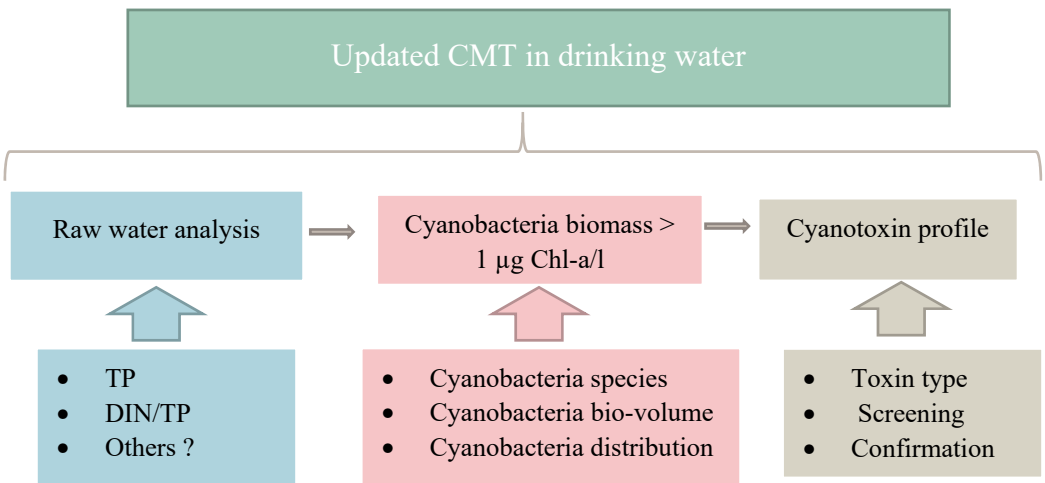

Figure 15. Framework of an updated CMT as an example

Raw water analysis

Alert level 1: TP > 20 µg L^{-1} proposed in Paper I, has been extended to TP > 13 µg L^{-1} and DIN/TP < 10 as indications for cyanobacterial risk in water. What to choose for alert levels to indicate cyanobacterial risk or cyanobacterial peaks depends on your local situation. Our study emphasizes the importance of understanding your raw water and how nutrients' condition and other water quality factors are influencing cyanobacteria formation. Therefore, monitoring routine in water is crucial for any analysis of significance.

Cyanobacteria profile

Alert level 2: *Chlorophyll-a* > 1 µg L^{-1}. A simple tool for a routine monitoring of cyanobacteria abundance was suggested and validated as a useful tool by Paper I, i.e. ALA (Algae Lab Analyzer) which measures concentration of cyanobacteria in the form of µg Chl-*a*/l. To get a picture of cyanobacteria risk in your water, alert values can be used such as cyanobacteria 1 µg Chl-*a* L^{-1}, corresponding to the WHO drinking water alert level 1 and the value of cyanobacteria 50 µg Chl-*a* L^{-1} corresponding to the WHO drinking water alert level 2. Other practical tools commercially are for example measuring phycocyanin concentration (any of a group of blue photosynthetic pigments present in cyanobacteria). Details refer to www.bbe-moldaenke.de.

For a qualitative analysis of identifying potential toxin producing species, it is necessary to collaborate with a research institute or a professional laboratory due to the qualification requirement which for this kind of analysis is high.

Cyanotoxin profile

Alert Level 3: microcystin-LR > 1 µg L^{-1} is not enough as an action limit if your water also contains other types of toxins. At least, you need to meet a requirement of the five most common toxin types in Swedish freshwater (see Chapter 3.4). Based on your cyanobacteria profile, potential toxins and corresponding screening tools can be selected. ELISA which are capable of screening all the listed common cyanotoxins in Swedish water can be integrated into a raw water cyanotoxin monitoring particularly from June to Nov. An example of protocol for this analysis is provided in 6.2. Advanced analytical methods are recommended for toxin confirmation occasionally. It is beneficial to build collaboration with professional laboratories as those analysis requires expensive equipment and highly qualified technicians.

Management strategies

A risk management plan is necessary for a cyanobacterial bloom occurrence, especially for those systems with source waters that are prone to CHABs. Elements of such a plan should include monitoring, treatment and communication components. The plan could include a monitoring program to determine sampling

locations and schedule; sample volume; whether to sample for cyanobacterial cells or specific cyanotoxins or both; which analytical screening test to use; and conditions when it is necessary to send sample(s) to an identified laboratory for confirmation. Regulations might vary (**Paper II**). You may find action limits that fit the local condition. For example, the Finnish National Supervisory Authority for Welfare and Health has chosen 0.1 mm^3 L^{-1} as national alert level 1 for operators of drinking water treatment plants to increase raw water monitoring and 1 mm^3 L^{-1} for actions to be initiated, such as risk assessment and information to authorities (Chorus, 2012). For more information, refer to American EPA (American EPA, 2019) and the Swedish Food Authority (Livsmedelsverket, 2018).

6.2 Pre-treatment for MAR

By using eutrophic water as source water for MAR, pre-treatment is often required to stabilize the operational conditions of the artificial recharge due to high and rapid variations in raw water quality (Lahti *et al.*, 2001;**Paper IV**). It reduces large amount of algae biomass, preventing clogging issue in the infiltration ponds as well as improves water quality in the infiltration ponds to a large extent (Pic.1).

If MAR is only applied as a main barrier for cyanobacterial risk, drinking water might be exposed to health problem, even though MAR or bank filtration can eliminate a wide range of substances utilizing adsorption and degradation processes. An example can be seen in Lake Chaohu, China, where groundwater contamination by microcystin from cyanobacterial blooms poses a significant health risk for residents when it is used for drinking water (Yang, Kong and Zhang, 2016).

Biodegradation which dominates cyanotoxin elimination such as MCYST in sandy aquifer, might be retarded due to changes of temperature condition, redox condition and sediment texture (Grützmacher *et al.*, 2002, 2005, 2010; Grützmacher, Bartel and Chorus, 2007). Retarded biodegradation can cause microcystin to break through infiltration materials. This might happen particularly in the autumn, as biodegradation is likely to be reduced by the decrease of temperature (Grützmacher, Bartel and Chorus, 2007); while at the same time cyanobacteria is likely to dominate (Chapter 4.2, Fig.10) and cyanobacteria cells might decay, releasing toxins (World Health Organization, 2015). Paper IV demonstrates the benefits of coagulation and filtration as pre-treatment for reducing cyanobacterial risk in the following infiltration ponds. To what extent that pre-treatment has improved the end water quality after MAR was difficult to observe in our case study except for more effective organic matter reduction and possible impact of intensive blooms on nitrogen content (**Paper IV**). The removal of cyanobacteria cells and cyanotoxin during the study period, were effective even without pre-treatment. The current

ongoing project is examining the extent to which cyanobacterial cells and cyanotoxin are removed by MAR in the different depths of sand layers and how these are connected to microbial activities.

Grützmacher and her colleagues in the German Federal Environmental Agency did a series of experiments on examining the condition for MCYST removal in slow sand infiltration and listed important factors of MAR performance regarding microcystin removal. A brief summary below can provide reference for future investigation of improving MAR for cyanotoxin removal.

- Retention of cells on the sediment surface is the most prominent process for eliminating these primarily cell-bound toxins. Middle to coarse grained sands (d10 = 0.41) eliminated more than 99.9 % of intracellular toxins within the first 10 cm of flow path (filter speed: 0.4 m/d). Elimination of extracellular microcystin during underground passage is mainly due to biodegradation. Laboratory experiments showed that the sediment structure, i.e. high clay/silt and organic content, is crucial for maximum adsorption. Reversible adsorption processes do not reduce the total load but lead to longer contact times for extended biodegradation (Grützmacher, Bartel and Chorus, 2007)
- The experiment with dissolved microcystins yielded very high elimination rates (> 95 %) inside the filter bed attributed to biodegradation, whereas retardation by adsorption was low. The second experiment, which was with mostly cell-bound microcystins, showed similar results during the first days after application of cyanobacteria (elimination > 85 %). As the population declined in late autumn, the proportion of extracellular to cell-bound microcystins increased. At the same time the elimination rates declined to values < 60 %. This decline is most likely attributable to retarded biodegradation at temperatures of < 4 ° C (Grützmacher *et al.*, 2002).
- Experiments conducted with virgin sand (no previous contact to MCYST) and high filtration rates compared with anaerobic conditions show that the greatest problem for MCYST elimination can be found under anaerobic conditions as degradation is not complete and may lead to harmful residual concentrations. Redox conditions play an important role for degradation rates: under aerobic conditions half-lives of less than one day occurred frequently, whereas anoxic conditions resulted in lag phases of one day and more, as well as in half lives of more than 25 days (Grützmacher *et al.*, 2005).
- 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 (Karlsson, 2015).

- An ongoing project at the same case study site at Vomb waterworks has shown that the top layer (50 cm) of MAR infiltration ponds can remove almost all intact cyanobacteria cells and microcystins during bloom season and the microbial community stabilizes at 1 m in the sand layer (Li *et al*, unpublished results).

6.3 Cyanotoxin monitoring

There is no single optimal method for the detection and identification of all types of cyanobacterial toxins, and each method has its applicability (Meriluoto, Metcalf and Codd, 2017; **Paper V**). Due to the diversity of cyanotoxin variants and chemical structure, the current routine methods cannot be used to detect all types and variants of cyanotoxins. Although there are methods that can provide an indication of concentrations such as enzyme-linked immunosorbent assay (ELISA) methods, routine monitoring for more than 250 MCYST variants in drinking-water is not the best way, which will only be present intermittently in source water during algal blooms.

Immunochemical kits such as ELISA and LFA are mostly applied as screening tools for cyanotoxin detection in water. Immunochemical kits can provide quick answers of whether certain cyanotoxins exist in the sample and give an approximate qualitative answer i.e. Yes/No (LFA, **Paper V**) or semi quantitative content (ELISA for microcystins, **Paper IV**), but they do not specify which variant of cyanotoxin. Immunochemical kits in general have challenges of cross reactivity and sensitivity (**Paper V** and **VI**). The increase of sensitivity to be able to detect low toxin concentration would sacrifice the cross reactivity that can cover various variants in the same group and vice versa.

Although LC-MS/MS gives a single measurement of each variant based on its standard concentration, it is expensive and only available at an advanced analytical laboratory. Immunochemical kits are, however, simpler and quicker and easier to adapt to a monitoring process at a water treatment plant.

Previous experience shows that combining these two methods can greatly improve the detection efficiency. For example, using a screening tool to screen many samples and select samples with high toxin concentration for further confirmation with advanced analytical technique. In our case study (**Paper IV**), ELISA was applied to measure approximate toxin concentrations to show the effect of pre-treatment on microcystin removal; the samples with a high toxin content were sent to an advanced laboratory for toxin profile confirmation by LC-MS/MS.

The detection methods to choose are affected by the variety and abundance of cyanotoxins. The choice of method is also inevitably influenced by the availability

of the analytical equipment and its applicability to the environment (Du *et al.*, 2019). For example, molecular methods fit for detecting potential toxic cyanobacteria, and immune sensors are suitable for monitoring the presence of toxins and bioassays are often used to evaluate the toxic effects. Consequently, research purpose, economic feasibility, speed of analysis, sensitivity and field applicability should be considered when selecting detection methods. There is still a need for new techniques that can detect and identify cyanotoxin more easily and sensitively.

Currently, there are commercial immunochemical kits for all four groups of cyanotoxins. The limit of detection of the immunochemical kits (Limit of Detection, LOD), i.e. the lowest content which can be measured should be below the Swedish Food Agency's recommended limits of action. Different types of immunochemical kits are needed to cover all groups of cyanotoxins. How to analyse with immunochemical kits is well described in the manuals.

Even though immunochemical kits are easier to use, they still require a certain knowledge and technique to perform. The Swedish National Food Agency recommends drinking water producers educate their employees, collaborate with other drinking water producers who have laboratories and competent personnel or send samples to an accredited laboratory. In Sweden, Swedac (Sveriges nationella ackrediteringsorgan) is recommended to contact for information on accredited laboratories for the analysis of cyanotoxins. The critical issue in cyanotoxin detection by using immunochemical kits is sample handling. Details are referred to Appendix 1. Appendix 1 presents a protocol for total MCYST test in lake water by using a commercial immunochemical kit.

LFA is simple to use with qualitative signals to indicate if there exist targeted toxin groups. It is highly recommended to apply as a screening tool. It also should be used with care as it has its limitations with cross reactivity and sensitivity. You must understand your water well and be aware of the type of toxins that would possibly exist so that your detection tool will not miss any of the harmful ones. Details can refer to **Paper V & VI**.

6.4 Future work consideration

From an operator's point of view, fast and easy measuring tools both for cyanobacteria cells and toxins are needed. Thus, applied research should be developed and commercialized, for example, a novel imaging-driven technique with an integrated fluorescence signature demonstrates great potential for fast and accurate quantification of cyanobacterial cells (Jin *et al.*, 2018). To measure different cyanotoxins simultaneously from a simple sample is an emerging issue. Anfossi reviewed LFA's potential for multiplexing point-of-need analysis due to its simplicity, rapidity, and cost-effectiveness and adaptability for multiplexing. (Anfossi *et al.*, 2018). Collaboration between research institutes and drinking water producers can be set up for hazard assessment and develop hands-on tools for practical application. For example, useful tools that can indicate cyanobacterial risk, such as florescence based cyanobacterial chlorophyll-a content or phycocyanin measurements should be further studied on its connection to toxicity or utilized for setting alert levels for decision making.

From a legislation point of view, the European Water Framework Directive (WFD) should be empowered to achieve its goals of protecting and establishing good water quality in European surface waters and securing drinking water supply by introducing a broader range of chemicals and their mixtures into ecosystem assessment proposed by SOLUTIONS (Brack *et al.*, 2019) and microcystin-LR, should be replaced by the sum of all microcystin group proposed by CYANOCOST (Meriluoto *et al.*, 2018). SOLUTIONS funded by the European Union, searches for new and improved tools, models, and methods to support decisions in environmental and water policies. CYANOCOST, which is an abbreviation of "Cyanobacterial blooms and toxins in water resources: Occurrence, impacts and management" is an EU H2020 project.

To reduce the threat of cyanotoxins to humans, it is essential to strengthen the monitoring of cyanobacterial toxins worldwide, especially in underdeveloped areas (Du *et al.*, 2019). The bright side of cyanobacteria has also attracted wide attention. For example, it was found that the lipopeptide cyanotoxins not only have neurotoxicity and cytotoxicity but also have anticancer, antifungal and molluscicidal activities (Ngo *et al.*, 2012). These specific types of natural toxins have unique pharmacological properties and demonstrate great potential of being developed and utilized in combating human disease. Particularly marine cyanobacteria are still a rich source of untapped natural products.

7. Conclusion

"*Jumping to conclusion can be bad exercise.*" ~ Lawrence Rasberry

Cyanobacterial blooms have become a direct threat to the drinking water supply among other issues such as ecosystem, seafood safety and recreational activities. Cyanobacterial toxins are widely distributed and varied, and their harmfulness cannot be ignored. This dissertation aimed to present a comprehensive knowledge and tools for water managers and operators to understand cyanobacterial risk in their water so that bloom problems can be prevented or mitigated.

Firstly, an adaptive approach for cyanobacteria management in drinking water supply was proposed, starting with an overview of this problem, resulting in a conceptual management tool design, i.e. Cyanobacteria Management Tool (CMT) by which multi-indicators for actions were provided. Secondly, a picture of this problem in Swedish freshwater was studied both on a national and local scale, including their geographical distribution, species dynamics, seasonal pattern and their connection with eutrophication status, land use, and other factors. Thirdly, the study highlights nutrient's impact on cyanobacteria formation, including testing two hypotheses, 1) Total Phosphorus (TP) can be used to predict cyanobacteria risk and 2) the Dissolved Inorganic Nitrogen and Phosphorus ratio (DIN / TP) is a better indicator for cyanobacteria risk than TN / TP. The results were also verified by a full-scale on-site experiment study of pre-treating eutrophic water at a local water treatment plant. Lastly, cyanotoxin detection challenges and strategies were presented.

We discovered target TP limits can be calculated through quantile regression analysis. The target TP limit in accordance with WHO drinking water alert level 1 in the Swedish trend lakes is suggested as 13 μg L^{-1}. Our study shows that TN / TP, preferably DIN / TP less than 10 indicates cyanobacteria peaks. We observed that most problematic lakes that experience intensive cyanobacterial blooms are in southern Sweden, where the lakes are eutrophic or hypereutrophic due to intensive land use. Clear seasonal patterns for cyanobacteria biomass and dominant conditions show that special attention should be paid in June to November regarding cyanobacteria risk. We also evaluated cyanotoxin screening tools such as ELISA or Lateral flow immunoassay (LFA) for indicating certain toxins. Advanced analytical tools such as LC–MS/MS are required for toxin profiles confirmation.

Research findings from this thesis can be used to update a local based CMT and applied as a workflow for water operators to improve their monitoring routines and develop their strategies. Management tools and MCYST test protocols are provided for practical application. On the one hand, cyanotoxins detection techniques and methods need to be further developed for the accurate identification of cyanobacterial toxins. On the other hand, instead of monitoring individual toxins, water managers can focus on monitoring cyanobacterial presence such as cell numbers or biomass. In the end, our work emphasizes again that nutrient control is the key to protect our drinking water from intensive cyanobacterial blooms.

Appendix 1: A protocol for MCYST test

This protocol is for total MCYST test in lake water by applying Beacon Microcystin Tube Kit (Beacon Analytical System Inc., 2019).

Step 1. *Sample collection*

1) Either grab samples or composite samples

Depending on the situation, a grab sample from a dense bloom might be enough from a boat or on the shore. PETG bottles are suggested. PETG was determined to be non-cytotoxic, and media stored in PETG bottles demonstrated proliferation and morphological characteristics comparable to control media.

2) Sample containers should be properly labelled. Gloves and proper clothes and shoes are recommended.

3) After sampling, immediately put the containers in a cool box with ice. Keep samples cooled, during shipping, and pending analysis at the laboratory.

Reference: Ohio EPA gives a clear guide of collecting grab and composite samples for cyanotoxins: https://www.youtube.com/watch?v=B2yLi1Bp0CY

Step 2. Testing water with algal cell suspension

1) Mix the water samples taken from the lake and mix it vigorously to make sure the cells are homogeneously suspended.

2) Transfer 1 ml or 1.5 ml of the homogeneously mixed water sample to a clean microcentrifuge tube (1.5 ml size). More than 10 of these kinds of vials can be distributed. Store the rest of the vials for future testing in a frost-free freezer or -80^{o}C freezer.

3) Freeze the vial in the regular freezer (-18C) and thaw it and repeat three times

4) Centrifuge the vial in a microcentrifuge for 5 min at 10K rpm or any centrifuge which can pellet the algal mass.

5) Remove the supernatant for ELISA

Step 3. ELISA *microcystin test kit*

1) Read the instructions carefully:

   https://d6a597b0-184e-4af7-b02d-98845136bc9b.filesusr.com/ugd/c8f857_802c57424e624b7fb485b0fed25b6fcd.pdf

2) Bring all kit reagents and samples to room temperature (30 minutes).

3) Remove the required number of antibody coated tubes from the re-sealable foil bag. Place the tubes in a rack and label each with a sample or calibrator level. Be sure to re-seal the bag with the desiccant to reduce moisture exposure.

4) Prepare 1X wash solution by diluting the 100X concentrate (i.e. 5 mL of the 100X plus 495 mL of deionized water in a 500 mL wash bottle).

5) Dispense 500 µL of the Enzyme Conjugate into each tube. (Multi pipette / Eppendorf repeater is suggested to insure the same volume)

6) Add 500 µL of the Calibrators, Control and Samples into the appropriate tubes.

7) Be sure to use a clean pipette tip for each solution to avoid cross contamination.

8) Dispense 500 µL of the Antibody Solution into each tube. (Multi pipette / Eppendorf repeater is suggested to ensure the same volume and avoid time delay between samples)

9) Swirl the tubes rapidly to mix the contents.

10) Incubate the tubes for 20 minutes at room temperature.

11) Decant the contents of the tubes into an appropriate waste container. Flood the tubes completely with 1X wash solution, then decant. Repeat this wash step three times for a total of four washes. Invert the rack onto absorbent paper and tap out as much water as possible.

12) Add 500 µL of the Substrate to each tube. (Multi pipette / Eppendorf repeater is suggested to ensure the same volume and avoid time delay between samples)

13) Swirl the tubes rapidly to mix the contents.

14) Incubate the tubes for 20 minutes at room temperature.

15) Add 500 µL of the Stop Solution to each tube in the same order of addition as the Substrate. (Multi pipette is suggested to ensure the same volume and avoid time delay between samples) WARNING: Stop Solution is 1N hydrochloric acid. Handle with care.

16) Read the tubes with a spectrometer or tube reader at 450 nm within 20 minutes of stopping reaction. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm. (A portable colorimeter is suggested)

17) For screening purpose, standard solution 0 and 0.3 can be used to indicate the presence of microcystins; to quantify the Microcystin -LR equivalent, all standards should be utilized.

18) Use the four parameters algorithm provided by the company for calculating the quantity of the microcystin

Step 4. *Spike and recovery*

To verify if the value below 0.3 ppb is valid, one can mix 500µl standard 1ppb or 2ppb with 500µl sample and then take 500 µl mixture for analysis; If the measured value of the mixture is within the range of 70 % to 120 % of the expected value ((value need to be verified + standard)/ 2), then the value needs to be verified as valid.

For targeting very low toxin concentration, there are two options;

1) Use the solid phase to concentrate the toxins in the water samples such as applying Solid Phase Adsorption Toxin Tracking (SPATT) (Roué, Darius and Chinain, 2018); 2) Collect algal cells and test the cell extracts which will have higher concentration than the water samples. Example below is given on how to handle samples to separate water and algal cells separately and extract toxin individually:

1) Mix the cells and water sample well.
2) Transfer 1.0-1.5 ml into a microcentrifuge tube. More than 10 can be distributed and frozen for future use.
3) Centrifuge 5 min at 10K rpm to pellet the cells from the water.
4) Remove the water for later testing
5) Use the freeze and thaw technique to break up the cells. Since the volume is quite small, the cycles will be short.
6) Use 1.0 ml PBS to resuspend the cells and extract the toxins.
7) Centrifuge again and test the supernatant as above.

If you do microcystin analysis for finished drinking water, quenching samples is necessary. A residual disinfectant, e.g., chlorine, should be quenched immediately upon sampling. Sodium thiosulfate or ascorbic acid are commonly used as quenching agents and their appropriateness can be specific to the analytical method selected to meet the monitoring data quality objectives. You need to test the pH to check if you need to quench the samples. For testing of samples using ELISA, the recommended range for sample pH is 5 to ≤ 7, as a pH of less than 5 can produce matrix interference.
https://www.abraxiskits.com/wp-content/uploads/2018/01/Anatoxin-a-Sample-Collection-and-Preservation-Sheet-012218.pdf

Useful materials:

1. How to proceed with microcystin tube test, the process is like Aflatoxin ELISA Tube Kit provided by Beacon Analytical Systems Inc. https://www.youtube.com/watch?v=XkoSiWVb1js
2. Sample extraction of Intracellular microcystins: https://www.youtube.com/watch?v=Oqgco26yXnY
3. How to analyse Microcystin-LR equivalence using ELISA:

   video 1: https://www.youtube.com/watch?v=NvDs7VHtp-I&t=45s

   video 2. https://www.youtube.com/watch?v=XkoSiWVb1js

8. References

Allen, J. F., Thake, B. and Martin, W. F. (2019) 'Nitrogenase Inhibition Limited Oxygenation of Earth's Proterozoic Atmosphere', *Trends in Plant Science*. doi: 10.1016/j.tplants.2019.07.007.

Amano, Y. *et al.* (2008) 'Prediction of Microcystis blooms based on TN:TP ratio and lake origin', *TheScientificWorldJournal*. doi: 10.1100/tsw.2008.89.

American EPA (2019) *Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems*. Available at: https://www.epa.gov/sites/production/files/2019-07/documents/cyanobacteria_and_cyanotoxins_fact_sheet_for_pws_final_06282019.pdf.pdf (Accessed: 27 August 2019).

Anfossi, L. *et al.* (2018) 'Multiplex lateral flow immunoassay: An overview of strategies towards high-throughput point-of-need testing', *Biosensors*. doi: 10.3390/bios9010002.

Beacon Analytical System Inc. (2019) *Microcystin Tube Kit*. Available at: https://docs.wixstatic.com/ugd/c8f857_802c57424e624b7fb485b0fed25b6fcd.pdf (Accessed: 1 April 2019).

Bhattacharyya, D. S. *et al.* (2015) 'Cyanobacteria and cyanotoxins in the World: Review', *International Journal of Applied Research*, 1, pp. 563–569.

Brack, W. *et al.* (2019) 'Let us empower the WFD to prevent risks of chemical pollution in European rivers and lakes', *Environmental Sciences Europe*. doi: 10.1186/s12302-019-0228-7.

Buratti, F. M. *et al.* (2017) 'Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation', *Archives of Toxicology*. doi: 10.1007/s00204-016-1913-6.

Carvalho, L. *et al.* (2013) 'Sustaining recreational quality of European lakes: Minimizing the health risks from algal blooms through phosphorus control', *Journal of Applied Ecology*. doi: 10.1111/1365-2664.12059.

Chorus, I. (2012) 'Current approaches to Cyanotoxin risk assessment, risk management and regulations in different countries', *Compiled and edited by Dr.Ingrid Chrous, Federal Environemnt Agency, Germany*, p. 174.

Chorus, I. and Bartram, J. (1999) *Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management*. World Health Organization. doi: 10.1046/j.1365-2427.2003.01107.x.

Chow, C. W. K. *et al.* (1999) 'The impact of conventional water treatment processes on cells of the cyanobacterium Microcystis aeruginosa', *Water Research*. doi: 10.1016/S0043-1354(99)00051-2.

Department of Aquatic Sciences and Assessment (2019) *Trend lakes | Externwebben*, *Swedish University of Agricultural Sciences*. Available at: https://www.slu.se/en/departments/aquatic-sciences-assessment/environment/environmental-monitoring-of-lakes-and-watercourses/trend-lakes/ (Accessed: 5 November 2019).

Dillon, P. J. *et al.* (2009) 'Managing aquifer recharge and discharge to sustain irrigation livelihoods under water scarcity and climate change', *IAHS-AISH Publication*. doi: Cited By (since 1996) 2\nExport Date 9 July 2012\nSource Scopus.

Dolman, A. M. *et al.* (2012) 'Cyanobacteria and cyanotoxins: The influence of nitrogen versus phosphorus', *PLoS ONE*. doi: 10.1371/journal.pone.0038757.

Dolman, A. M. and Wiedner, C. (2015) 'Predicting phytoplankton biomass and estimating critical N: P ratios with piecewise models that conform to Liebig's law of the minimum', *Freshwater Biology*, 60(4), pp. 686–697. doi: 10.1111/fwb.12518.

Du, X. *et al.* (2019) 'The diversity of cyanobacterial toxins on structural characterization, distribution and identification: A systematic review', *Toxins*. doi: 10.3390/toxins11090530.

European Commission (2019) *Commission welcomes provisional agreement to improve the quality of drinking water and the access to it*, *Press release*. Available at: https://ec.europa.eu/commission/presscorner/detail/en/ip_19_6830 (Accessed: 6 January 2020).

Eva Willén, B. (2007) *VÄXTPLANKTON I SJÖAR*. Available at: http://webstar.vatten.slu.se/IMA/Publikationer/internserie/2007-06.pdf (Accessed: 4 November 2019).

Filstrup, C. T. and Downing, J. A. (2017) 'Relationship of chlorophyll to phosphorus and nitrogen in nutrient-rich lakes', *Inland Waters*. doi: 10.1080/20442041.2017.1375176.

Folster, J. *et al.* (2014) 'The Swedish monitoring of surface waters: 50 years of adaptive monitoring.', *Ambio*. Sweden, 43 Suppl 1, pp. 3–18. doi: 10.1007/s13280-014-0558-z.

Fox, J. (2008) *Applied regression analysis and generalized linear models (2nd ed.)*, *Applied regression analysis and generalized linear models (2nd ed.).* Hamilton, ON, Canada: McMaster University. doi: 10.1111/j.1537-2995.2009.02111.x.

Ger, K. A. *et al.* (2016) 'The interaction between cyanobacteria and zooplankton in a more eutrophic world', *Harmful Algae*, pp. 128–144. doi: 10.1016/j.hal.2015.12.005.

Grützmacher, G. *et al.* (2002) 'Removal of microcystins by slow sand filtration', *Environmental Toxicology*, 17(17), pp. 386–394. doi: 10.1002/tox.10062.

Grützmacher, G. *et al.* (2005) 'Are there limits to cyanobacterial toxin (microcystin) elimination by sand passage ?', *Proceedings of the International Symposium on Management of Artificial Recharge*.

Grützmacher, G. *et al.* (2010) 'Microcystin elimination during sediment contact', *Environmental Science and Technology*. doi: 10.1021/es9016816.

Grützmacher, G., Bartel, H. and Chorus, I. (2007) 'Cyanobakterientoxine bei der UferfiltrationCyanobacterial toxins in bank filtrate. Under which conditions is their elimination reliable?', *Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz*. doi: 10.1007/s00103-007-0161-6.

Guiry, M. D. (1995) 'Algae: A Taxonomic Survey', *Phycologia*. doi: 10.2216/i0031-8884-34-3-252.1.

Hansson, L. A. *et al.* (2007) 'Cyanobacterial chemical warfare affects zooplankton community composition', *Freshwater Biology*, 52(7), pp. 1290–1301. doi: 10.1111/j.1365-2427.2007.01765.x.

Huang, L. *et al.* (2016) 'Effects of internal loading on phosphorus distribution in the Taihu Lake driven by wind waves and lake currents', *Environmental Pollution*, 219, pp. 760–773. doi: 10.1016/j.envpol.2016.07.049.

Ibelings, B. W. *et al.* (2014) 'Current approaches to cyanotoxin risk assessment and risk management around the globe', *Harmful Algae*, 40, pp. 63–74. doi: 10.1016/j.hal.2014.10.002.

iNaturalist Netværk (2019) *Woronichinia (cyanoScope)*. Available at: https://www.inaturalist.org/guide_taxa/700578 (Accessed: 7 March 2019).

Issa, A. A.-S., Abd-Alla, M. H. and Ohyama, T. (2014) 'Nitrogen Fixing Cyanobacteria: Future Prospect', in *Advances in Biology and Ecology of Nitrogen Fixation*. doi: 10.5772/56995.

Izaguirre, G. *et al.* (1982) 'Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems', *Applied and Environmental Microbiology*.

Jacquemot, P. *et al.* (2016) *An Investigation on Separation Effects of Discfilter as Pre-treatment at Vomverket, Sweden*, *Sydvatten AB, Report 2016*. Lund.

Janssen, E. M. L. (2019) 'Cyanobacterial peptides beyond microcystins – A review on co-occurrence, toxicity, and challenges for risk assessment', *Water Research*. doi: 10.1016/j.watres.2018.12.048.

Jeppesen, E. *et al.* (2009) 'Climate Change Effects on Runoff, Catchment Phosphorus Loading and Lake Ecological State, and Potential Adaptations', *Journal of Environment Quality*, 38(5), p. 1930. doi: 10.2134/jeq2008.0113.

Jin, C. *et al.* (2018) 'Quantification of cyanobacterial cells via a novel imaging-driven technique with an integrated fluorescence signature', *Scientific Reports*. doi: 10.1038/s41598-018-27406-0.

Kansas Department of Health and Environment Bureau of Water (2011) *Water Quality Standards White Paper-CHLOROPHYLL-A CRITERIA FOR PUBLIC WATER SUPPLY LAKES OR RESERVOIRS ISSUE Should Kansas adopt chlorophyll-a criteria for public water supply lakes?*

Karlsson, S. C. (2015) *Simulating Water and Pollutant Transport in Bark, Charcoal and Sand Filters for Greywater Treatment*. University of Agricultural Sciences: Uppsala, Sweden. Available at: https://pub.epsilon.slu.se/12417/1/ciuk_karlsson_s_150630.pdf (Accessed: 9 July 2019).

Kävlingeåns vattenvårdsförbund (2018) *Provtagnings- och analysprogram*. Available at: http://kavlingeans-vvf.com/undersokningar/provtagnings-och-analysprogram/ (Accessed: 23 April 2018).

Kiviranta, J. *et al.* (1991) 'Production and biodegradation of cyanobacterial toxins - a laboratory study', *Archiv für Hydrobiologie*.

Koenker, R. and Hallock, K. F. (2001) 'Quantile regression', *Journal of Economic Perspectives*. doi: 10.1257/jep.15.4.143.

Kreakie, B. J. *et al.* (2015) 'Predicting related indices of cyanobacteria blooms in lakes based on nutrient inputs and ecosystem attributes . Table Of Contents : Executive Summary ':, pp. 1–8.

Lahti, K. *et al.* (2001) 'Occurrence of microcystins in raw water sources and treated drinking water of Finnish waterworks', in *Water Science and Technology*. IWA Publishing: London, UK.

Larsson, J. *et al.* (2014) 'Picocyanobacteria containing a novel pigment gene cluster dominate the brackish water Baltic Sea', *ISME Journal*. doi: 10.1038/ismej.2014.35.

Lee, S. and Lee, D. (2018) 'Improved prediction of harmful algal blooms in four major South Korea's rivers using deep learning models', *International Journal of Environmental Research and Public Health*. doi: 10.3390/ijerph15071322.

Lehman, A. *et al.* (2013) *JMP ® for Basic Univariate and Multivariate Statistics Methods for Researchers and Social Scientists*. Second Edi. Cary, North Carolina, USA: SAS Institute Inc. Available at: http://support.sas.com/publishing/pubcat/chaps/65761.pdf (Accessed: 15 May 2018).

Livsmedelsverket (2018) *HANDBOK DRICKSVATTENRISKER Cyanotoxiner i dricksvatten*. Uppsala. Available at: www.livsmedelsverket.se/publicerat-material/. (Accessed: 6 February 2019).

Lu, X. *et al.* (2011) 'Groundwater Recharge at Five Representative Sites in the Hebei Plain, China', *Ground Water*. doi: 10.1111/j.1745-6584.2009.00667.x.

Ma, J. *et al.* (2015) 'Controlling cyanobacterial blooms by managing nutrient ratio and limitation in a large hyper-eutrophic lake: Lake Taihu, China', *Journal of Environmental Sciences (China)*. doi: 10.1016/j.jes.2014.05.042.

Martens, S. (2017) 'Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis', *Advances in Oceanography and Limnology*. doi: 10.4081/aiol.2017.7221.

Mcleod, C., Burrell, S. and Holland, P. (2015) *Review of the currently available field methods for detection of marine biotoxins in shellfish flesh*. Available at: https://pdfs.semanticscholar.org/6cfc/2767ba50831a484efd30b3200f965116b9b0.pdf (Accessed: 31 January 2019).

Megdal, S. B. and Dillon, P. (2015) 'Policy and economics of managed aquifer recharge and water banking', *Water (Switzerland)*. doi: 10.3390/w7020592.

Meriluoto, J. *et al.* (2018) *Expert opinion*. Available at: https://drive.google.com/file/d/1Pu-ovYTso0VkF-mgblbXFzr6cN9ihX8e/view?usp=sharing (Accessed: 21 September 2019).

Meriluoto, J., Metcalf, J. S. and Codd, G. A. (2017) 'Selection of Analytical Methodology for Cyanotoxin Analysis', in *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*. John Wiley & Sons, Ltd, pp. 309–312. doi: 10.1002/9781119068761.ch32.

Nabout, J. C. *et al.* (2013) 'How many species of Cyanobacteria are there? Using a discovery curve to predict the species number', *Biodiversity and Conservation*. doi: 10.1007/s10531-013-0561-x.

Ngo, D. H. *et al.* (2012) 'Biological activities and potential health benefits of bioactive peptides derived from marine organisms', *International Journal of Biological Macromolecules*. doi: 10.1016/j.ijbiomac.2012.06.001.

O'Neil, J. M. *et al.* (2012) 'The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change', *Harmful Algae*, 14, pp. 313–334. doi: 10.1016/j.hal.2011.10.027.

Orihel, D. M. *et al.* (2012) 'High microcystin concentrations occur only at low nitrogen-to-phosphorus ratios in nutrient-rich Canadian lakes', *Canadian Journal of Fisheries and Aquatic Sciences*. doi: 10.1139/F2012-088.

Paerl, H. W. (2008) 'Chapter 10: Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater–marine continuum', in Hudnell, H. K. (ed.) *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*. Springer, p. 22. doi: 10.1007/ 978-0-387-75865-7.

Paerl, H. W. and Huisman, J. (2008) 'Blooms like it hot', *Science (New York, N.Y.)*, 320(5872), pp. 57–58. doi: 10.1126/science.1155398.

Paerl, H. W. and Otten, T. G. (2013) 'Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls', *Microbial Ecology*. doi: 10.1007/s00248-012-0159-y.

Pekar, H. *et al.* (2016) 'Fast, rugged and sensitive ultra high pressure liquid chromatography tandem mass spectrometry method for analysis of cyanotoxins in raw water and drinking water-First findings of anatoxins, cylindrospermopsins and microcystin variants in Swedish source wa', *Journal of Chromatography A*. Elsevier B.V., 1429(January), pp. 265–276. doi: 10.1016/j.chroma.2015.12.049.

Qi, M. *et al.* (2012) 'Development of Models for Predicting the Predominant Taste and Odor Compounds in Taihu Lake, China', *PLoS ONE*. doi: 10.1371/journal.pone.0051976.

Rantala, A. *et al.* (2006) 'Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin synthetase gene E (mcyE) PCR and associations with environmental factors', *Applied and Environmental Microbiology*. doi: 10.1128/AEM.01058-06.

Reynolds, C. S. (2006) *The ecology of phytoplankton*, *The Ecology of Phytoplankton*. doi: 10.1017/CBO9780511542145.

Roué, M., Darius, H. T. and Chinain, M. (2018) 'Solid phase adsorption toxin tracking (Spatt) technology for the monitoring of aquatic toxins: A review', *Toxins*. doi: 10.3390/toxins10040167.

Sabart, M. *et al.* (2010) 'Spatiotemporal Variations in Microcystin Concentrations and in the Proportions of Microcystin-Producing Cells in Several Microcystis aeruginosa Populations', *Applied and Environmental Microbiology*. doi: 10.1128/AEM.02531-09.

Sanseverino, I. *et al.* (2017) *Cyanotoxins: methods and approaches for their analysis and detection*. Available at: http://publications.jrc.ec.europa.eu/repository/bitstream/JRC106478/kjna28624enn.pdf.

SMHI (2019a) *Svenskt vattenarkiv*. Available at: https://www.smhi.se/klimatdata/hydrologi/svenskt-vattenarkiv (Accessed: 20 April 2018).

SMHI (2019b) *Vattenwebb*. Available at: http://vattenweb.smhi.se/ (Accessed: 20 April 2018).

Smith, V. H. (1983) ‘Low Nitrogen to Phosphorus Ratios Favor Dominance by Blue-Green Algae in Lake Phytoplankton’, *Science*, 221(4611), pp. 669–671. doi: 10.1126/science.221.4611.669.

Steffen, M. M. *et al.* (2014) ‘Status, causes and controls of cyanobacterial blooms in Lake Erie’, *Journal of Great Lakes Research*, pp. 215–225. doi: 10.1016/j.jglr.2013.12.012.

Stewart, I. and Falconer, I. R. (2008) ‘Cyanobacteria and cyanobacterial toxins’, *Oceans and Human Health: Risks and Remedies from the Sea*.

Sundh, U. B. and Lindberg, T. (2014) *PST saxitoxin i dricksvattentäkt Riskvärdering*. Available at: https://www.livsmedelsverket.se/bestall-ladda-ner-material/sok-publikationer/artiklar/2018/2018-pst-saxitoxin-i-dricksvattentakt.

Svenskt Vatten (2019) *Produktion av dricksvatten*, *Fakta om vatten*. Available at: http://www.svensktvatten.se/fakta-om-vatten/dricksvattenfakta/produktion-av-dricksvatten/ (Accessed: 1 April 2019).

Svirčev, Z. *et al.* (2019) ‘Global geographical and historical overview of cyanotoxin distribution and cyanobacterial poisonings’, *Archives of Toxicology*. doi: 10.1007/s00204-019-02524-4.

Svrcek, C. and Smith, D. W. (2004) ‘Cyanobacteria toxins and the current state of knowledge on water treatment options: a review’, *Journal of Environmental Engineering and Science*. doi: 10.1139/s04-010.

Swedish Agency for Marine and Water Management (2014) *Vattenanknuten recipientkontroll*. Available at: https://www.havochvatten.se/hav/uppdrag--kontakt/vart-uppdrag/regeringsuppdrag/regeringsuppdrag/vattenanknuten-recipientkontroll-2014.html (Accessed: 20 April 2018).

Swedish University of Agricultural Sciences (2019) *List of accredited analytical methods at the geochemical laboratory*, *Ackred.nr.1208 Provning ISO/IEC 17025*. Available at: https://www.slu.se/globalassets/ew/org/inst/vom/laboratorier/ackrediterade-vattenanlysmetoder.pdf.

Taylor Enterprises (2019) *Change-Point Analyzer*. Available at: https://variation.com/product/change-point-analyzer/ (Accessed: 3 December 2019).

Tielemans, M. W. M. (2007) ‘Artificial recharge of groundwater in The Netherlands’, *Water Practice and Technology*. IWA Publishing, 2(3). doi: 10.2166/wpt.2007.064.

Tokodi, N. *et al.* (2018) ‘Screening of cyanobacterial cultures originating from different environments for cyanotoxicity and cyanotoxins’, *Toxicon*. doi: 10.1016/j.toxicon.2018.09.001.

United States Environmental Protection Agency (2019) *Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems*. Available at: http://water.epa.gov/scitech/drinkingwater/dws/ccl/ (Accessed: 30 June 2019).

Urrutia-Cordero, P., Ekvall, M. K. and Hansson, L. A. (2016) ‘Local food web management increases resilience and buffers against global change effects on freshwaters’, *Scientific Reports*. Nature Publishing Group, 6(February), pp. 1–9. doi: 10.1038/srep29542.

US EPA (2012) *Aquifer recharge (AR) and aquifer storage & recovery (ASR)*. Available at: http://water.epa.gov/type/groundwater/uic/aquiferrecharge.cfm (Accessed: 21 March 2019).

US EPA (2019) *Guidelines and Recommendations*. Available at: https://19january2017snapshot.epa.gov/nutrient-policy-data/guidelines-and-recommendations_.html (Accessed: 26 August 2019).

Utermöhl, H. (1958) 'Zur Vervollkommnung der quantitativen Phytoplankton-Methodik', *SIL Communications, 1953-1996*. doi: 10.1080/05384680.1958.11904091.

Westrick, J. A. *et al.* (2010) 'A review of cyanobacteria and cyanotoxins removal/inactivation in drinking water treatment', *Analytical and Bioanalytical Chemistry*, 397(5), pp. 1705–1714. doi: 10.1007/s00216-010-3709-5.

WHO (2003) *Cyanobacterial toxins: Microcystin-LR in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality*. Geneva. Available at: http://www.who.int/water_sanitation_health/dwq/chemicals/cyanobactoxins.pdf (Accessed: 23 April 2018).

Willén, T. and Mattsson, R. (1997) 'Water-blooming and toxin-producing cyanobacteria in Swedish fresh and brackish waters, 1981-1995', *Hydrobiologia*, pp. 181–192. doi: 10.1023/A:1003047019422.

World Health Organization (2015) *MANAGEMENT OF CYANOBACTERIA IN DRINKING-WATER SUPPLIES : Information for regulators and water suppliers*, *Geneva, Switzerland*. WHO: Geneva, Switzerland. Available at: https://apps.who.int/iris/handle/10665/153970.

Xie, L. *et al.* (2003) 'The low TN:TP ratio, a cause or a result of Microcystis blooms?', *Water Research*. doi: 10.1016/S0043-1354(02)00532-8.

Xu, H. *et al.* (2015) 'Determining critical nutrient thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake Taihu, China', *Environmental Science and Technology*. doi: 10.1021/es503744q.

Yang, Z., Kong, F. and Zhang, M. (2016) 'Groundwater contamination by microcystin from toxic cyanobacteria blooms in Lake Chaohu, China', *Environmental Monitoring and Assessment*. doi: 10.1007/s10661-016-5289-0.

Zamyadi, A. *et al.* (2013) 'Species-dependence of cyanobacteria removal efficiency by different drinking water treatment processes', *Water Research*. doi: 10.1016/j.watres.2013.02.040.