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DOI (link to publication from Publisher):
10.5278/vbn.phd.eng.00019

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

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BY
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DISSERTATION SUBMITTED 2017

AALBORG UNIVERSITY
DENMARK

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by

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Dissertation submitted in 2017

Dissertation submitted: October, 2017

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Department: Department of Civil Engineering

ISSN (online): 2446-1636
ISBN (online): 978-87-7210-085-2

Published by:
Aalborg University Press
Skjernvej 4A, 2nd floor
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

Printed in Denmark by Rosendahls, 2017

CV (*extract)*

PhD study related training:

- July 23rd – December 15th, 2014 – 5 months stay at Trent University in Canada in relation with the PhD study. Main activities: phytoplankton identification and enumeration in urban stormwater retention pond samples.
- Freshwater Algal Identification course with professors David M. John and Brian A. Whitton in Durham, England (July, 2013).
- Advanced Course in Freshwater Algal Identification with professors David M. John and Brian A. Whitton in Durham, England (July, 2015).
- Phytoplankton Enumeration Methods course with Maria Temponeras "Planktontax", Denmark (2014 – 2015).

Knowledge dissemination in conferences with oral presentations:

- June 4th – 8th, 2017 – SFS 2017 Annual meeting in Raleigh, North Carolina. Presentation title: "Planktonic algae abundance and diversity in urban stormwater ponds and natural shallow lakes" (comparison between Danish stormwater ponds and Danish natural shallow lakes).
- September 21st – 25th, 2015 – Ecology at the Interface – 13th EEF and 25th SItE joint conference in Rome, Italy. Presentation title: "Benthic Diatom Abundance and Diversity in Urban Stormwater Ponds" (Comparison among 9 Danish ponds).
- May 10th – 13th, 2015 – Wetlands – indicators of the changing environment in Bled, Slovenia. Presentation title: "Phytoplankton diversity in urban stormwater ponds compared to natural shallow lakes" (comparison between Danish stormwater ponds and Danish natural shallow lakes).
- September 14th – 18th, 2014 – International Wetlands Conference in Huesca, Spain. Presentation title: "Planktonic algae abundance and diversity in urban stormwater ponds and natural shallow lakes" (comparison among Danish stormwater ponds, Canadian stormwater ponds and Danish natural shallow lakes).
- September 7th – 12th, 2014 – 13th ICUD - International Conference on Urban Drainage in Kuching, Malaysian Borneo. Presentation title: "Photodegradation of three stormwater biocides".

List of Scientific Papers:

- Minelgaite, G., Nielsen, A.H., Pedersen, M.L., Vollertsen, J., 2015. Photodegradation of Three Stormwater Biocides. Urban Water Journal, 14, 1, 53-60, DOI: 10.1080/1573062X.2015.1076489.
- Minelgaite, G., Frost, P.C., Xenopoulos, M.A., Stephansen, D.A., Pedersen, M.L., Vollertsen, J. Planktonic algae abundance and diversity are similar in urban stormwater ponds of different geographic locations, and natural shallow lakes. *Not yet published and not yet decided where to submit to. The title is subject to change.*
- Minelgaite, G., Van Alst, N., Stephansen, D.A., Bollmann, U., Bester, K., Pedersen, M.L., Nielsen, A.H., Vollertsen, J. Catchment characteristics and pollutants affect the benthic diatom abundance and diversity in urban stormwater ponds. *Not yet published and not yet decided where to submit to. The title is subject to change.*
- Stephansen, D.A., Minelgaite, G., Simon, M., Rudelle, E.A., Pedersen, M.L., Vollertsen, J. Stormwater Phytoplankton Community Response to Biocide Contamination – A Microcosm and Mesocosm study. *Not yet published and not yet decided where to submit to. The title is subject to change.*

Other scientific papers and reports:

- Bollmann, U.E., Minelgaite, G., Schlüsener, M., Ternes, T., Vollertsen, J., Bester, K., 2016. Leaching of Terbutryn and Its Photodegradation Products from Artificial Walls under Natural Weather Conditions. *Environ. Sci. Technol.*, 2016, *50* (8), pp 4289–4295. DOI: 10.1021/acs.est.5b05825.
- Vollertsen, J., Bester, K., Rudelle, E.A., Bollmann, U., Minelgaite, G., Stephansen, D.A., Nielsen, A.H., Pedersen, M.L. 2013-2016. Biocider i urbane småsøer – effekt og skæbne. Danish Environmental Protection Agency project MST- 667-00151.
- Poulsen, T.G., Minelgaite, G., Bentzen, T.R., Andreasen, R.R., 2013. Minor Losses During Air Flow into Granular Porous Media. Water, Air and Soil Pollution, 224:1666. DOI 10.1007/s11270-013-1666-2.

ENGLISH SUMMARY

Stormwater pollution is a widely recognized issue, especially in urban areas. The runoff water can carry a variety of contaminants emerging from human activities, such as the materials used for maintenance of houses and gardens, traffic, or industries. To protect the natural environment, different stormwater management practices come into action. Stormwater retention ponds are among the most commonly implemented ones due to their numerous benefits. Also, recently they receive wide attention as potential means to support biodiversity in urban environments, despite common elevated contamination levels in their water column and sediment.

In this study I investigated stormwater ponds from the pollutant, as well as from the ecosystem point of view. The former was addressed through a photodegradation study of three common stormwater pollutants of high environmental significance – the biocides carbendazim, terbutryn, and diuron. Biocides were also included in the study of the basis of the aquatic food chain – phytoplankton and benthic diatoms – where stormwater pond phytoplankton communities were compared to those of natural shallow lakes. Ponds of different catchment types, receiving varying amounts of biocide input, were selected to analyze and compare the benthic diatom communities. Finally, the response of phytoplankton communities to environmentally relevant biocide contamination was tested conducting microcosm and mesocosm experiments.

The photodegradation study provided some evidence that the degradation of the three selected stormwater biocides by natural sunlight does not contribute significantly to their concentration reduction in the water column. The phytoplankton analysis showed that stormwater ponds are very rich in taxa and they are somewhat similar to natural shallow lakes, with respect to the counted numbers and estimated biovolumes at the investigated taxonomic level. The diatom diversity and abundance examination revealed some significant differences among ponds from different catchment types, namely, highway and industrial or residential catchments. This could be due to higher loads and variety of pollutants that are received by the ponds located in industrial or residential areas. The measured water column biocide concentrations clearly distinguished highway ponds from the others. Nevertheless, a clear relationship between measured water column biocide concentrations and identified diatom communities could not be found. Lastly, the conducted microcosm and mesocosm study did not show any clear phytoplankton community response to the applied biocide treatments either.

DANSK RESUME

Regnvandsforurening er et stort og anerkendt problem, især i byområder. Overløbsvand kan viderebringe en lang række kontaminanter fra menneskelige aktiviteter, såsom vedligeholdelsesmaterialer til hus og have, trafik eller industri. For at beskytte miljøet er der forskellige metoder til at håndtere regnvandsforurening, der kan komme i brug. Regnvandsbassiner er blandt de mest anvendte metoder, på grund af deres mange fordele. Desuden har de for nylig fået fokus som hjælp til at støtte biodiversiteten i byområder, på trods af forhøjede forureningsniveauer i deres vandkolonner og sediment.

I denne afhandling har jeg undersøgt regnvandsbassiner både for forureningskilder og for økosystemets synspunkt. Første del blev undersøgt gennem analyser af lysnedbrydelsen af tre kendte forureningskilder med stor miljømæssig indflydelse i regnvandsbassiner; biociderne carbendazim, terbutryn og diuron. Biocider var også med i undersøgelsen af grundlaget for vandige fødekæde – fytoplankton og bentiske kiselalger – hvor tilstedeværelsen af fytoplanktonkolonier i regnvandsbassiner blev sammenlignet med tilstedeværelsen i søer. Bassiner med forskellige tilstrømningsforhold, og som modtager forskellige mængder af biocider, blev udvalgt til videre analyse og til sammenligning af tilstedeværelsen af kolonier af bentiske kiselalger. Til sidst blev fytoplankton koloniers reaktion på forskellige biocid kontaminanter undersøgt ved mikrokosmer og mesokosmer forsøg.

Forsøg viste at nedbrydelsen af de tre udvalgte regnvandsbiocider med sollys ikke bidrager signifikant til reduktion af deres koncentration i vandkolonnen. Fytoplankton analysen viste at regnvandsbassiner er meget rige på taxa og de er i nogen grad sammenlignelige med overfladiske søer med hensyn til antal og estimerede biovolumener under det undersøgte taksonomiske niveau. Undersøgelsen af kiselalgers mangfoldighed og forekomst afslørede signifikante forskelle blandt bassinerne med forskellige opsamlingsmetoder, især blandt opsamling fra veje og industri eller beboelse. Dette kunne skyldes højere belastning og variation af forurening i bassiner som modtager regnvand fra industri- eller beboelsesområder. Den målte biocidkoncentration i vandkolonerne adskilte vist klar forskel på vej-bassiner og de andre. Dog kunne en klar sammenhæng mellem målt biocidkoncentration i vandkolonnen og identificerede kolonier af kiselalger ikke findes. Til sidst viste de udførte mikrokosmo og mesokosmo undersøgelser ikke at biocider har en klar effekt på fytoplankton kolonier.

PREFACE

This thesis has been submitted for assessment in partial fulfillment of the PhD degree. The thesis is based on the submitted or published scientific papers which are listed above. Parts of the papers are used indirectly in the extended summary of the thesis. As part of the assessment, co-author statements have been made available to the assessment committee and are also available at the Faculty.

This PhD study was carried out at Aalborg University, Department of Civil Engineering, in the period from September 2012 till October 2017. The PhD study period also included a 5 months stay at Trent University, Department of Biology, in Peterborough (Ontario), Canada. One joint study resulted in a written manuscript ready to submit for publication.

The thesis is based on one published paper and three scientific manuscripts which are briefly discussed in the extended summary of the thesis. Additional details are given in the paper and manuscripts.

ACKNOWLEDGEMENTS

I am very lucky to have had this opportunity to do a PhD study at Aalborg University in Denmark. I am grateful to the university for providing me with a scholarship to do research here. Also, thank you for the Danish Environmental Protection Agency, who have funded part of the study and made it possible to be accomplished. These years did not only provide me with a great amount of knowledge and skills, but also helped me to grow personally.

Firstly, I would like to say thank you to my supervisor Jes Vollertsen for all his hard work guiding me through these challenging years of my PhD study. I am grateful for his never ending optimism, quick tackling of any obstacles, for his constructive criticism, as well as encouragement and belief in me and my work. And most of all, I am grateful for his ability to always find a bright side of all matters. Also, thank you to my co-supervisor Morten Lauge for his help dealing with microscopy laboratory related questions and tasks, as well as valuable discussions regarding my scientific publications.

I am also very grateful to all my research group colleagues for being a wonderful team and for their help and support during the 5 years of my PhD. I am especially grateful for Jytte Dencker and Asbjørn Haaning Nielsen for their patience with all my questions, troubles and doubts, and for their professional assistance regarding my laboratory tasks. I would also like to address a great thank you to Nikki, Marta and Elise for their help with laboratory work and sample analysis, and great moments together throughout those years spent in the laboratory and while sharing the office.

Especially great thank you to Diana, who has been not only a great colleague and partner in projects, but also a very good friend, always supporting me and helping to cope with obstacles which crossed my way while doing the PhD. Thanks to my office-mate Emil for the help and friendship which made the start of my PhD so much easier to handle. Also, a great thank you to Maria Temponeras, who was not only my phytoplankton teacher, but also a great colleague helping to solve my work related doubts and difficulties.

Thank you to my colleagues in Canada, where I have spent 5 great months doing part of my PhD – my supervisor Paul Frost for an opportunity to visit and collaborate with his laboratory, and for all his research group who were always so kind, friendly and understanding.

Also, thank you for Røskva, Evelina, Lorenzo, Rune, Mads, Priscila, Fan, Bruno, Ulla, Violeta, Darius, Artūras and Laima and so many other wonderful people whom I have met in Denmark and who have shared with me their friendship, insights into philosophical, as well as other matters of life and work.

And last but not least, I am very grateful to all my family and my husband for their kind patience and support through all these years of my PhD, and for always being there for me during the good moments, as well as the very difficult ones.

Thank you to all for making my years in Denmark full of so many great and irreplaceable experiences and for making this exciting PhD research accomplishable.

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CHAPTER 1. INTRODUCTION

Increasing urbanization is an inseparable part from today's world. According to the United Nations Human Settlements Programme (UN-Habitat) World Cities Report (2016), the number of large cities and megacities present in 2015 has doubled in the last 20 years. By 2015, 54% of the world's population was registered as residents of urban areas. On the one hand, the growth of cities is a sign of progress and opportunities. It creates new jobs, offers better quality of life and enhances the development of our societies, as well as technological progress (WCR, 2016). Well-planned infrastructure has reduced the transportation times remarkably even between further destinations.

On the other hand, next to all the commodities available in urban areas, many challenges have to be faced as well. Some of the most important ones are the adverse effects caused to our environment. Various industrial processes and means of transport are the sources of a number of pollutants, such as heavy metals, polycyclic aromatic hydrocarbons (PAHs), biocides, suspended particulate matter, and etcetera. Present at elevated concentrations, they can have harmful effects on terrestrial or aquatic ecosystems, as well as human health (Hvitved-Jacobsen *et al.*, 2010; Cahill, 2012) Moreover, according to WCR (2016), "by 2030, global demand for energy and water is expected to grow by 40 and 50 per cent respectively". As a consequence, through overexploitation of resources, urban areas may lose their biodiversity and the services which healthy ecosystems can provide. Hence, willing to reduce or eliminate the harm that urbanization involves, new greener technologies and best pollution management strategies need to be addressed and implemented (Cahill, 2012; Rossmiller, 2014).

One of the areas experiencing a harmful impact of urbanization is water resources. Growth of cities, construction of new roads and highways, densely constructed neighborhoods and paved areas increase the pollution pressure on the natural environment. Wet weather conditions not only ease pollutant transport to the surrounding environment, but may also lead to flooding of residential areas. To prevent and control the undesirable stormwater induced flooding and pollution, a number of management practices are implemented depending on the purpose and location (Hvitved-Jacobsen *et al.*, 2010). Some of the most common ones are stormwater retention ponds, which among others are reviewed in the following sections.

1.1. STORMWATER MANAGEMENT AND TREATMENT

A need for stormwater management was first acknowledged when the urban development became the cause of flooding events in the adjacent environments. Increases of the built environment resulted in a reduced amount of pervious surfaces which together with an insufficient drainage system resulted in floods of varying degrees. As a solution, detention basins were implemented as a common runoff management practice. That way, stormwater volume, magnitude and frequency issues were controlled reducing the pressure on the urban environments (Cahill, 2012).

In time, not only stormwater volume, but also stormwater quality became a question of importance, raising a need for more advanced detention basin implementation and management strategies (Cahill, 2012). City growth and development is closely related with pollution. Depending on the land use and activities present in an area, runoff may contain a range of pollutants resulting in varying degrees of environmental impact. Among the most common ones, suspended solids, heavy metals, pesticides, oils, greases, inorganic nitrogen and phosphorus compounds, oxygen demanding materials, are listed (Parkinson and Mark, 2005; Eriksson *et al.*, 2007).

When implementing stormwater management strategies, a great deal of attention has to be paid not only to stormwater volume and pollutant control, but also to design criteria recognizing site-specific needs and limitations. Over the years, stormwater management became a practice dependent on a variety of established local, regional or national guidelines, standards and regulations (Sage *et al.*, 2015).

Among others, the use of vegetation, buffer zones and infiltration came into practice (Cahill, 2012), which nowadays are widely-recognized means for stormwater management. Some of the best management practices (BMPs) also include constructed wetlands, which act as ecosystems with shallow waters and dense vegetation, where a number of physical, chemical, and biological processes proceed. In addition, runoff after storm events can be directed to specially implemented infiltration basins (Malmqvist, 2000).

Keeping in mind the variety of existing stormwater management and treatment facilities, stormwater retention ponds are one of the most recognized and widely investigated best management practices (Hvitved-Jacobsen *et al.*, 2010). In a number of studies, they are acknowledged not only from flood control, but also from pollution mitigation, as well as from the ecological point of view (France, 2002). Well documented design guidelines assure a proper implementation of such facilities adapted to the particular local stormwater management needs and the urban area conditions (SMPDM, 2003; SWPWMG, 2009; WSUDG, 2017).

Recently, BMPs are becoming "tools" for the low-impact development (LID) (Cahill, 2012), using which, "water is managed to reduce built-area impacts and promote natural water movement within an ecosystem or watershed" (Rossmiller, 2014). Incorporation of LID in the new land developments or rebuild of the existing cities is becoming a way towards a sustainable development (Cahill, 2012).

1.1.1. STORMWATER RETENTION PONDS

Stormwater retention ponds are one of the most widely acknowledged and implemented stormwater management practice. Such ponds, also called wet detention ponds, are man-made shallow water bodies. They are implemented with an intention to detain stormwater to mitigate downstream hydraulic impacts and to retain pollutants collected from the catchment area before they reach the surrounding environment (SWPWMG, 2009). *Picture 1-1* illustrates examples of the ponds located in Denmark and Canada.

Picture 1-1 Stormwater retention ponds. The picture on the left illustrates a pond in Denmark (Jutland), while the one on the right shows a pond in Canada (Peterborough) with several inlet pipes.

A stormwater pond has a permanent water volume (often 1 – 1.5 m depth) and an additional storage volume, which is present after a storm event. A schematic of a typical pond is shown in *Figure 1-1*. Depending on the wet weather periods and the catchment size for which a pond is constructed, runoff water is retained in a basin for a specific period of time (often on average 1-4 weeks). This way, stormwater pollutants are retained in a pond through sedimentation, degradation, and other processes before the stormwater flows out to the natural environment.

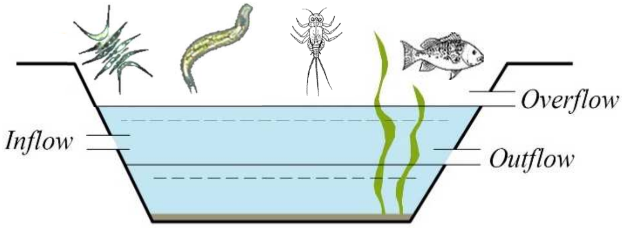


Figure 1-1 A schematic of a typical stormwater retention pond. In time, these ponds become inhabited by different organisms, such as phytoplankton, zooplankton, macroinvertebrates, plants and etc.

Stormwater ponds are built based on different design principles, depending on the purpose of each case and the best features in order to avoid possible problems, such as e.g., eutrophication or stagnant water. Also, these water bodies may be implemented to resemble a natural water body, attractive to different living organisms to inhabit. The size of the pond can be based for instance, on the impervious surface area of the catchment, on the pollutant removal efficiency and the mean storm events or the dry period characteristics among storm events (Hvitved-Jacobsen *et al.,* 2010).

Additionally, stormwater ponds are being addressed as means to attract different types of living organisms into urban areas (Greenway, 2010; Hassal and Anderson, 2015). With an increasing urbanization, we face a need not only to mitigate pollution, but also to implement more green areas and prevent the biodiversity loss in the cities. Among other stormwater control measures, ponds may contribute to the provision of a number of ecosystem services, such as greenhouse gas regulation, recreation, education, aesthetics, biodiversity, etcetera (Moore and Hunt, 2012). In order to implement a stormwater pond in an area of interest, which fulfills its purpose and the needs of the society, many aspects have to be considered, among which occurrence of potential runoff pollutants is of a great importance. The most relevant and more widely researched ones are discussed in the following subsection.

1.1.2. STORMWATER POLLUTANTS

With rainwater, a number of substances can reach stormwater ponds located in the catchment area. Different particulate and dissolved materials originate from households, industrial processes or traffic and accumulate on pavements, streets, and roofs. Heavy metals, PAHs, organic pollutants, inorganic nitrogen and phosphorus compounds, and suspended solids are some of the most commonly addressed pollutants in the urban environment (Parkinson and Mark, 2005; Eriksson *et al*., 2007)). The concentrations, at which they enter a pond, vary depending on the storm event, as well as on the pond location, or in other words, the catchment type of a pond (Chiandet and Xenopoulos, 2016).

Depending on the types and concentrations of the pollutants in stormwater ponds, their presence may lead to unwanted consequences, such as eutrophication, aesthetic deterioration of a pond, as well as toxic effects on organisms inhabiting them (Parkinson and Mark, 2005; SWPWMG, 2009). In order to better understand and manage the occurring pollutant related issues, a number of studies have researched stormwater ponds from the pollutant accumulation and removal efficiency point of view (e.g., Comings *et al.*, 2000; Istenič *et al.,* 2011; Borne, 2014; Sønderup *et al.,* 2016).

Comings *et al.* (2000) reported a reduction of phosphorus, total suspended solids, and removal of the majority of analyzed metals in two wet detention ponds. Another study by Istenič and the co-authors (2011) noted that PAHs concentrations decreased in the outlet compared to the inlet of seven investigated stormwater retention ponds. Borne (2014) proposed a new floating treatment wetland strategy for stormwater retention ponds, which proved to increase the efficiency of total phosphorus removal. A large number of stormwater ponds (66) were investigated in the study of Sønderup *et al.* (2016). The authors found that nitrogen, phosphorus and particulate fractions of organic matter are retained better in the young ponds (less than 5 years) compared to the older ones.

Trace elements (Frost *et al*., 2015), heavy metals in pond sediments (Liebens, 2001; Egemose *et al*., 2015) and their bioaccumulation through the aquatic food chain (Søberg *et al*., 2015; Stephansen *et al*., 2016) have been well-examined by different authors as well. The authors reported trace element variation among different ponds and heavy metal pollution dependency on the catchment type, as well as higher metal concentrations in pond invertebrates compared to natural lakes.

Within the variety of common stormwater pollutants discussed above, biocide contamination in stormwater runoff is a major topic of discussion in the latest years as well (Burkhardt *et al*., 2007; Jungnickel *et al*., 2008; Bollmann *et al*., 2014). Their significance and use is briefly discussed in the following subsection.

1.1.3. EMERGING IMPORTANCE OF BIOCIDE POLLUTION

The need to prolong the lifetime of different products and materials and to protect them from the attacks or growth of unwanted organisms has led to higher use of chemical substances, such as biocides. At present, the EU biocide regulation 528/2012 describes 22 biocidal product types, known as disinfectants, preservatives, pest control or antifouling agents. This rule ensures their proper and safe use “with a high level of protection of both human and animal health and the environment” (HSE, 2017). Biocides are widely used as preservatives of various construction materials, as algaecides or fungicides in paint or façade coatings (Schoknecht *et al*., 2009). Some of the most commonly used ones are the algaecides terbutryn, diuron and irgarol, as well as the fungicide carbendazim.

Although biocides provide us with numerous benefits, they are increasingly receiving attention as potential pollutants. Occurring in surface waters they may lead to possible adverse effects on living organisms (Wittmer *et al.,* 2010; Chen *et al.*, 2014). Painted building façades and roofs exposed to rainfall have been shown to leach biocides at significant concentrations (Jungnickel *et al.,* 2008; Burkhardt *et al.,* 2012; Bollmann *et al.,* 2014).

Depending on the biocidal compound, they can have different fate in the environment: for instance, biodegradation, photodegradation or sorption to particles (Thomas and Brooks, 2010). The final concentration of the compound reaching natural water bodies may induce changes in the ecosystem, for example, with regard to its community composition and biodiversity (Daam *et al.,* 2010; McMahon *et al.,* 2012). As a group of biocidal products, algaecides are specifically targeted to kill algae, and their occurrence in surface waters may lead to adverse effects and changes in phytoplankton communities (Huertas *et al.*, 2010; Gregorio *et al.,* 2012). Being the basis of the aquatic food chain (John *et al.,* 2011), phytoplankton diversity and abundance influence the presence of the higher organisms feeding on them. Shifts in phytoplankton communities may alter the whole aquatic food web. Hence, there is a need to investigate the effects of biocidal products at environmentally realistic concentrations and draw recommendations regarding the allowance and regulation of their release to the environment.

1.2. STORMWATER RETENTION PONDS AS AQUATIC ECOSYSTEMS

Stormwater retention ponds are primarily engineered to retain runoff and contribute to pollutant removal in stormwater before it enters the natural environment. Nevertheless, even though pollutants accumulate in ponds at significant concentrations, over years these ponds become attractive to different species of wildlife, such as macrophytes, macroinvertebrates and even amphibians (Scher and Thiery, 2005; Le Viol *et al.,* 2009; Le Viol *et al.,* 2012; Greenway, 2010; Hassal and Anderson, 2015). In their study, Søberg *et al.* (2015) and Stephansen *et al.* (2016) concluded that stormwater ponds provide sufficient habitats for a diversity of invertebrates despite higher metal concentrations in the pond sediments and invertebrates themselves. Different plants, birds, and even fish find stormwater retention ponds as habitats (*Picture 1-2)*.

Picture 1-2 Stormwater retention ponds in Denmark: left side – Silkeborg, right side - Lemming. Plants, fish, birds, and etc. find them as habitats.

Recently more and more focus is also being placed on the value of stormwater ponds as man-made ecosystems, which become a part of the urban environment. Being implemented in urbanized and industrialized areas, which are somewhat limiting the numbers of natural habitats for wildlife, stormwater retention ponds could as well be considered as means to increase biodiversity in such environments (Le Viol *et al.,* 2012; Hassal, 2014; Hassal and Anderson, 2015; Hill *et al.*, 2017). Pond networks (their connectivity) also help species move and spread in the urbanized areas (Hassal, 2014). In fact, some studies found somewhat comparable macroinvertebrate diversity to that of natural water bodies (Vermonden *et al.,* 2009; Hassal and Anderson, 2015; Stephansen *et al.*, 2016).

Stormwater pond suitability for wildlife can be expected to differ depending, e.g., on construction parameters, loads of pollutants and maintenance. Investigations taking a look at individual constituents of a stormwater ecosystem may lead to a better understanding of its performance and contribution to biodiversity (Hassal, 2014). Only a few studies have, for instance, looked at the basis of the aquatic food chain – the phytoplankton (Olding, 2000; Olding *et al.*, 2000; Vincent and Kirkwood, 2014). The analysis of phytoplankton habitat preferences and behavior could give a better understanding of possible relationships between the phytoplankton and the stormwater pond construction characteristics, as well as the consequential composition of higher organisms of the ecosystem.

1.2.1. STORMWATER PHYTOPLANKTON

Phytoplankton, or in other words microscopic water column algae, are the basis of the aquatic food web, which is developed depending on their abundance and diversity (John *et al.,* 2011). In situations where considerable amounts of nutrients reach stormwater retention ponds with prolonged water residence times, often an excessive growth of these organisms occurs. This leads to the well-known eutrophication problem, a process which contributes not only to an unpleasant look of the water body, but also to odors released from the decaying organic matter (Toet *et al.*, 1990). An example of *Microcystis* cyanobacteria formed surface bloom, observed in a Danish shallow lake is illustrated in *Picture 1-3*.

Picture 1-3 Microcystis formed surface bloom in a shallow lake in Denmark (Poulstrup sø, Poulstrup).

Additionally, cyanobacteria blooms in stratified or stagnant waters might lead to toxin release by certain species of e.g., *Microcystis*, *Anabaena,* etcetera (Sandgren, 1988). This poses hazards to invertebrates and vertebrates feeding on them, as well as human health (Chorus and Bartram, 1999; WHO, 2003).

Algae are also considered good water quality indicators, providing information about long-term water quality or short-term environmental changes (Bellinger and Sigee, 2010). For instance, at an increasing phytoplankton biomass, dominance of cyanobacteria indicates eutrophic conditions in the system. Among the different groups of algae (e.g. filamentous green, cyanobacteria, chrysophytes, eyglenophytes), diatoms are especially widely used, for instance, for flowing water quality assessment (Kelly *et al.*, 1998; Gonzalo and Fernandez, 2012). They grow attached to macrophytes or stones or are associated with sediment and reflect the recent conditions of the location.

A better understanding of phytoplankton composition and its possible relationships with stormwater pond construction parameters or the catchment type and land use, could lead to a better management of stormwater ponds and help preventing, for instance, unwanted algae blooms.

1.2.2. STORMWATER POLLUTANTS AND THEIR EFFECTS ON LIVING ORGANISMS

Studying stormwater retention ponds from the ecosystem point of view, it becomes particularly important to investigate the effects of stormwater pollutants on the organisms living there. A number of studies have been conducted testing pond water or sediment toxicity. For instance, Wium-Andersen *et al.* (2011) studied heavy metals and PAHs toxicity on the algae species *Selenastrum capricornutum.* No correlations between these contaminants and toxicity were identified. Karlsson *et al.* (2010) conducted a study of heavy metal toxicity on bacteria *Vibrio fischeri*, and found that stormwater heavy metal concentrations were not toxic to the bacteria, while sediment concentrations were. This indicates the importance of pollutant accumulation in the sediments of stormwater treatment facilities and their possible toxicity towards sediment associated organisms. Snodgrass *et al.* (2008) tested pond sediment toxicity to the early development stages of several amphibian species, and concluded that the effect is dependent on species tolerance towards the pollutant.

Considerable concentrations of different biocides have been measured in runoff water as well (Bollmann *et al.,* 2014). This raises a concern of the adverse effects that they might have on the aquatic organisms. As an example, a freshwater microcosm study done by Cuppen *et al.* (2000) showed significant effects of the biocide carbendazim on macroinvertebrate communities, such as changes in abundance of different taxonomic groups. Zimba *et al.* (2002) tested the biocide diuron effects on pond ecosystems and found some alterations in phytoplankton communities and zooplankton populations. A great deal of studies have also addressed terbutryn and irgarol (algaecides) induced effects on phytoplankton communities (Struve *et al.,* 1991; Mohr *et al*., 2008; Gregorio *et al.,* 2012). Depending on the compound and its concentration, no major effects or shifts in community structure were observed.

Based on the evidence documented by numerous research works, stormwater retention ponds appear to be attractive environments to inhabit to a diversity of fauna and flora species, despite considerable levels of different contamination present there. Knowledge on the interaction between the biotic part and contaminants of the pond could help to better manage those systems, contributing both to pollution mitigation as well as contribution to a greener and more sustainable urban environment.

CHAPTER 2. STUDY AIM AND OBJECTIVES

A great deal of recent environmental concern is related with environmental contamination by biocidal products. One of the most common groups of these substances is herbicides and algaecides, specifically targeted to control the growth of undesired plants and algae. Their leaching to the environment with runoff may lead to impairment of natural ecosystems, provoking possible changes already at the very basis of the food chain. As the major runoff volume is usually directed to and detained in stormwater retention systems, the inflowing biocide concentrations may have significant impacts on the stormwater ecosystem itself.

Therefore, the raised **aim** of the present study was to investigate stormwater retention ponds as man-made ecosystems with a focus on parameters, which distinguish them from natural lakes.

The object of the investigation was the **phytoplankton and the benthic diatom communities**, with **the main parameter of differentiation between natural and man-made water bodies being biocides** from the built environment, specifically targeted to control algae.

To achieve the aim, the study was divided into several research questions, which were answered completing the particular objectives.

> **Research question 1:** Biocides in stormwater ponds: is the photodegradation process significantly contributing to biocide transformation in the water column?

To answer this, the **first objective** was to investigate the fate of biocides in stormwater retention ponds, addressing one of the fate pathways – a photodegradation process of the selected three common stormwater biocides: carbendazim, diuron and terbutryn.

> **Research question 2:** Phytoplankton diversity and abundance in stormwater ponds: are stormwater phytoplankton communities comparable to those of natural shallow lakes?

To answer this, the **second objective** was to compare the phytoplankton composition in selected stormwater retention ponds and natural shallow lakes, analyzing the diversity and abundance with respect to the sampling months and the geographical locations of these water bodies.

Research question 3: Diatom communities in stormwater pond sediment: do they reflect biocide contamination and stormwater retention pond catchment type?

To answer this, the **third objective** was to examine benthic diatom communities, with respect to their genera diversity and abundance, in stormwater retention ponds from different catchment types and receiving varying loads of biocide pollution.

Research question 4: Biocide contamination and stormwater phytoplankton: does the stormwater phytoplankton community composition reflect biocide contamination at environmentally realistic concentrations?

To answer this, the **fourth objective** was to look into stormwater phytoplankton communities with respect to their diversity and abundance, carrying out controlled laboratory microcosm and outdoors mesocosm studies with applications of environmentally realistic concentrations of selected common biocides: carbendazim, diuron, terbutryn, and irgarol.

CHAPTER 3. METHODOLOGY

The PhD study was carried out setting a focus on 4 main topics, one per each research question:

- Photodegradation of stormwater biocides
- Stormwater phytoplankton diversity and abundance
- Benthic diatom diversity and abundance in stormwater retention ponds
- Stormwater phytoplankton community composition and abundance in response to biocide contamination

Each topic was addressed developing experimental setups, sampling plans and laboratory protocols for the work with the selected chemical substances and the microscopic analysis of phytoplankton and benthic diatom communities.

3.1. BIOCIDE PHOTODEGRADATION STUDY

Two experimental setups were prepared, where photodegradation of selected compounds (carbendazim, diuron and terbutryn) was tested under 254 nm UV light in the laboratory, as well as under natural sunlight outdoors. With the UV light study it was intended to test the photodegradation kinetics of selected compounds, as "most biocides absorb light at relatively short UV wavelengths" and "direct photodegradation of biocides by sunlight is expected to be, in most cases, of only limited extent" (Sakkas *et al.*, 2006). The laboratory chamber with UV-C lamps and the outdoors setup located under natural sunlight are shown in *Picture 3-1* and *Picture 3-2*.

Picture 3-1 Laboratory photodegradation study setup.

Picture 3-2 Outdoors photodegradation study setup.

The photodegradation under UV-C light was tested in demineralized water and collected water from a stormwater pond. The process under natural sunlight was tested only in demineralized water, but in 3 seasons – winter, spring, and summer – under different sunlight intensity. Further details about the setups along with a thorough explanation of the experimental procedures, equipment and measurements can be reviewed in **Paper I**.

3.2. PHYTOPLANKTON SAMPLING SITES, STRATEGIES AND SAMPLE ANALYSIS

Phytoplankton composition was investigated in six stormwater retention ponds (Danish and Canadian), and three natural shallow lakes (Danish). The water bodies were sampled in May, July and September. The collected samples were analyzed for selected water quality parameters. The phytoplankton communities of each pond and lake were analyzed using a microscope and described with respect to their diversity, abundance and biovolume. The locations of the ponds and lakes are illustrated in *Figure 3-1,* and the sampled water bodies are shown in *Picture 3-3*.

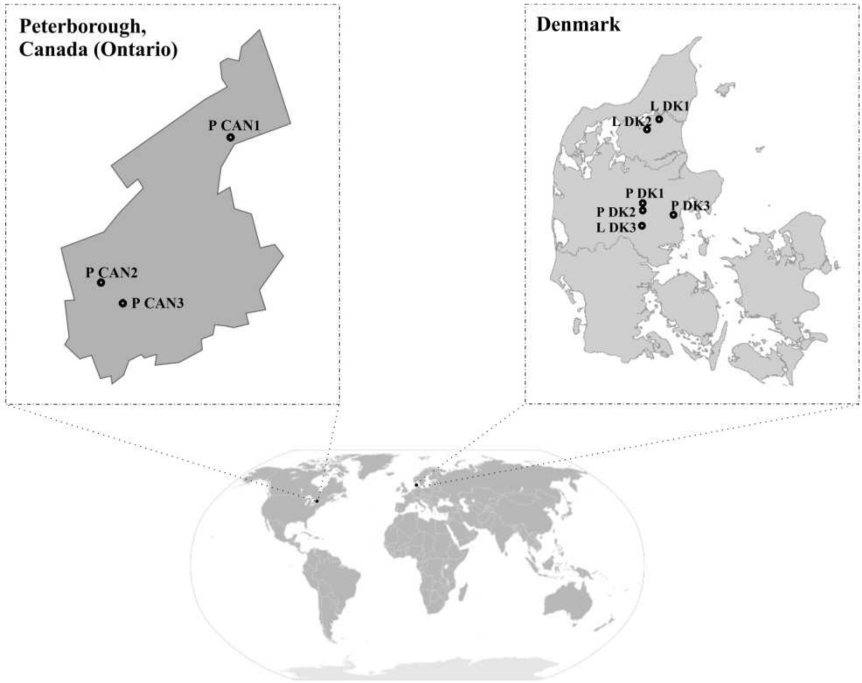


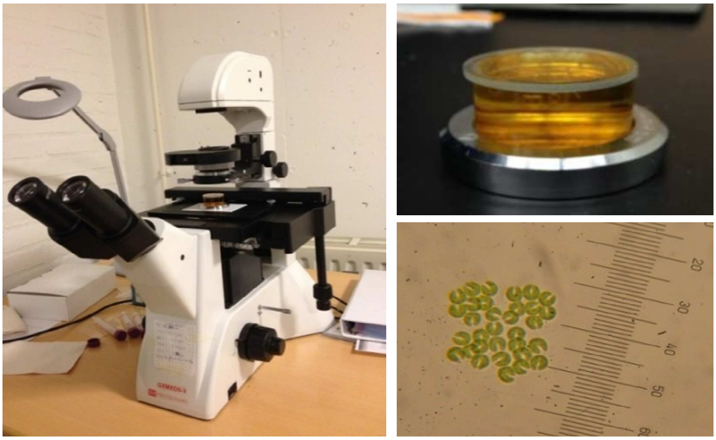
Figure 3-1 Locations of the investigated water bodies in Denmark and Canada: letter L indicates lake, letter P – pond, abbreviations DK and CAN - Denmark and Canada respectively.

Picture 3-3 The investigated Canadian and Danish stormwater retention ponds and Danish shallow lakes: letter L indicates lake, letter P – pond, abbreviations DK and CAN - Denmark and Canada respectively.

The water quality parameters conductivity, pH, dissolved oxygen, and temperature were measured in lakes and ponds while sampling, at 5 locations of each water body using field multimeters *(Picture 3-4)*. Nitrogen, phosphorus, and chlorophyll-*a* were analyzed in the laboratory. More detailed descriptions of each water body, sampling, and laboratory procedures can be found in **Paper II**.

Picture 3-4 Field sampling for phytoplankton and measurements of water quality parameters: conductivity, pH, dissolved oxygen, temperature.

The phytoplankton of each pond and lake was described by taxa identification and counting by inverted microscopes using sedimentation chambers (*Picture 3-5*). Taxonomic identification was done down to genus level, as much as the expertise confidence allowed. If genus identification was not possible, organisms were ascribed to higher taxonomic groups or unknown organisms. For each pond and lake, all the identified taxa were grouped under the major phyla – *Cyanophyta, Chlorophyta, Euglenophyta, Chrysophyta, Dinophyta, Bacillariophyta, Xanthophyta, Cryptophyta,* and *Haptophyta.* This was done to be able to easier compare all the water bodies at the same taxonomic level, because the organism variability and chaotic behavior is much higher and more unpredictable at lower taxonomic levels (Cottingham, 1996). Detailed identification and enumeration procedures are described in **Paper II and Paper IV**.



Picture 3-5 Inverted microscope, sedimentation chamber and green algae Kirchneriella colonies.

Several representatives from the most commonly observed phytoplankton phyla are shown in *Picture 3-6*: a) cyanobacteria *Microcystis* colonies, b) euglenophyte *Euglena*, c) green algae *Closterium*, d) dinoflagellate *Ceratium*.

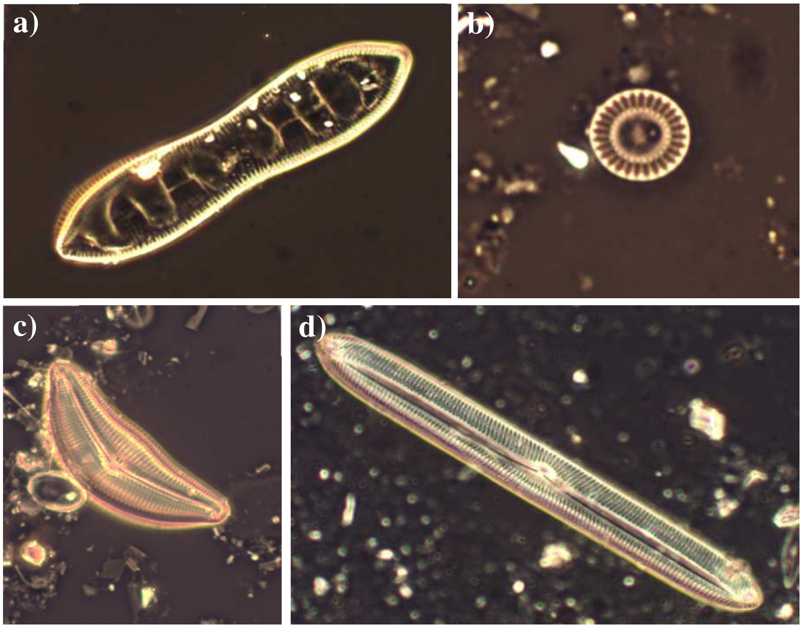
Picture 3-6 Representatives from the most commonly observed phytoplankton phyla: a) Microcystis – Cyanophyta (blue-green algae), b) Euglena – Euglenophyta, c) Closterium – Chlorophyta, d) Ceratium – Dinophyta.

3.3. DIATOM SAMPLING AND SAMPLE ANALYSIS

For the diatom study, 9 stormwater ponds from a range of catchment types were selected and sampled in a similar manner as phytoplankton. At 5 locations of each water body a sediment sample was extracted with a coring system (*Picture 3-7*). At the same time, water temperature, pH, dissolved oxygen, and conductivity were measured onsite, and samples collected for nitrogen, phosphorus and chlorophyll-*a* analysis were processed in the laboratory. The stormwater ponds were also sampled to determine the water phase biocide concentrations with an intention to identify a possible relationship between diatom communities and the level of biocide contamination in a pond. The top 1 cm of sediment from each location was extracted and preserved with ethanol until further sample treatment, permanent slide preparation and analysis. The prepared diatom slides were studied for diatom taxonomic composition with an upright microscope. An exemplification of permanent diatom slide collection is given in *Picture 3-7*, and several identified diatom genera are shown in *Picture 3-8*.

Laboratory procedures for permanent slide preparation, and different pond characteristics are described in detail in **Paper III**.

Picture 3-7 Sediment core for benthic diatom sampling and prepared permanent diatom slides.

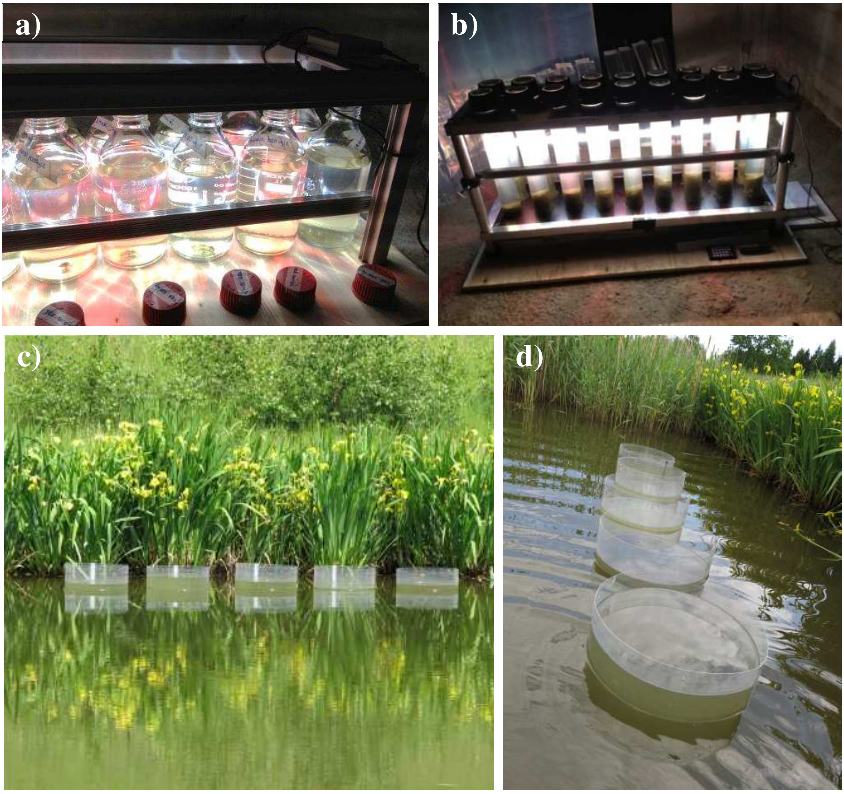


Picture 3-8 Several representatives of the identified diatom genera: a) Cymatopleura, b) Cyclotella, c) Cymbella, d) Pinnularia.

3.4. MICROCOSM AND MESOCOSM STUDIES

The response of stormwater phytoplankton to contamination by the selected biocides (carbendazim, diuron, terbutryn and irgarol) was investigated through the implementation of 3 experimental setups: two laboratory microcosm and one outdoors mesocosm setup. The microcosm studies were conducted first with an isolated water phase, and later including both water phase and sediment compartments. The setups are shown in *Picture 3-9*. In order to determine the response of the phytoplankton communities, they were looked at both from the taxonomic and abundance points of view.

The biocides were dosed into each microcosm and mesocosm at different environmentally realistic concentrations, and the water samples were collected at selected time intervals. The taxonomic phytoplankton composition and abundance was compared among different samples at the end of each experiment. Detailed description of the setups, sampling procedures, applied biocide concentrations and their measurements, as well as phytoplankton identification and enumeration procedures are given in **Paper IV**.



Picture 3-9 Setups of the microcosm and mesocosm study: a) water phase laboratory microcosm study, b) sediment core laboratory microcosm study, c) and d) outdoors mesocosm study.

CHAPTER 4. RESEARCH OUTCOMES

The PhD study resulted in four scientific papers, where four main research topics were addressed in order to answer the initially raised questions:

- Fate of biocides in stormwater retention ponds – is photodegradation process a significant contributor to the decrease of water column biocide concentrations?
- Phytoplankton diversity and abundance in stormwater retention ponds – are these man-made systems comparable to natural shallow lakes from a biodiversity perspective?
- Benthic diatom diversity and abundance in stormwater retention ponds – is the diatom community composition related with the characteristics of a pond, catchment type, and does it indicate the level of biocide contamination?
- Biocide contamination and stormwater phytoplankton – do biocides applied at environmentally realistic concentrations induce changes in phytoplankton abundance and community composition?

The results of the photodegradation study (**Paper I**) of the three selected biocides – carbendazim, diuron, and terbutryn – indicated that at natural sunlight conditions this process is not a significant contributor to the water column concentration reduction of these compounds. For the experiments run in three different seasons – spring, summer and winter – only in the warmest months (June-July) some decrease of terbutryn and diuron (7%) concentration was observed over two weeks of exposure. On the other hand, UV-C light experiments showed that diuron and terbutryn were rapidly degradable following 1st order kinetics, while carbendazim remained stable during the experimental time interval. It was also observed that photodegradation depends not only on a compound itself, but also on other factors, such as, for instance, physical and chemical water parameters. This aspect was, however, not further developed in the present study. Overall, a decrease in water column biocide concentration is likely governed by processes other than photodegradation, such as for instance, biodegradation, adsorption to particles, etc. (Thomas and Brooks, 2010).

The **Paper II** aimed to compare stormwater retention ponds with the natural shallow lakes from a biodiversity point of view, with the focus on the basis of the aquatic food chain – the phytoplankton. The results revealed that the phytoplankton taxa diversity in the investigated Canadian and Danish ponds were at least as diverse as that of the studied lakes. Phytoplankton numbers and biovolume varied greatly in all water bodies throughout the sampling period. A multivariate analysis carried out at the selected phyla taxonomic level did not indicate any apparent separation between natural and man-made ecosystems. Little difference was found between stormwater ponds from the investigated geographic locations as well, which provides some indications of the worldwide urban homogenization phenomenon (Groffman *et al.*, 2014). Overall, the main findings of the study suggest that despite substantial higher contamination present in stormwater ponds, they are capable of providing habitats exploited by a variety of phytoplankton, which communities bear somewhat resemblance to small lakes.

Stormwater pollutants are not only retained in the water column, but to a high extent they accumulate in pond sediment (Karlsson *et al.,* 2010; McNett and Hunt, 2011). In the **Paper III,** a study of benthic diatom communities was conducted in stormwater ponds from different catchment types and receiving varying loads of biocide contamination. Diverse diatom communities were found in the sediments of highway, residential, industrial, and mixed residential/industrial ponds, indicating the potential of stormwater ponds to serve as habitats for a diversity of organisms. The benthic diatom communities of highway ponds, by the conducted statistical tests, were notably distinguished from the ones identified in ponds from other catchment types. Such result indicated that catchment properties, including types of pollutants, affect the resulting organism diversity and abundance in the ponds. Some indications were observed that diatom community composition reflects the degree of biocide contamination in a pond, although a clear relationship could not be defined.

To investigate in more detail whether the stormwater biocide contamination could be reflected in the organism community composition, a separate study was prepared, once again setting the focus on the basis of the aquatic food web – phytoplankton (**Paper IV**). Single applications of environmentally significant concentrations of commonly used biocides carbendazim, diuron, terbutryn and irgarol were carried out in microcosm and mesocosm experimental setups. The response of the phytoplankton communities was examined at the end of the selected effect period (10 or 15 days), with respect to their taxonomic composition, abundance, and biovolume. Depending on the test setup complexity and the environmental variable control, a different community response was observed. Smallest taxa abundance and biovolume variations were observed for the single phase (water) microcosm setup, while the variability increased when the sediment-water phase interaction was included in the microcosms, as well as when the study was performed outdoors in mesocosms. A conducted multivariate analysis and statistical tests did not reveal any significant differences in phytoplankton community composition among the different biocide treatments in any of the studies. Only the biocide terbutryn applied at its highest concentration showed indications of induced community changes, as taxa diversity reduction was observed in all microcosms and mesocosm experiments. Also, in the cosms treated by irgarol, an increase in *Chlorophyta* abundance and biovolume with an increase in irgarol concentration was observed throughout the three studies. The overall result, obtained from the micro- and mesocosms, suggested that either the applied biocide concentrations are not toxic for the stormwater phytoplankton communities, or that continuous exposure to the biocides present in the investigated pond induced a phytoplankton tolerance to their contamination at environmentally realistic concentrations. Additional studies with phytoplankton communities from ponds of different catchment types, receiving varying loads of biocides, could help to get a deeper insight into phytoplankton-biocide relationship. Phytoplankton analysis in different seasons of the year, when other environmental variables are present or absent at varying degrees, could help identifying the factors and the conditions influencing the community behavior in response to biocide contamination as well.

CHAPTER 5. CONCLUSIONS

The PhD study was divided into four main research questions, which were answered following the established objectives. The following conclusions were drawn for each question:

Research question 1: Biocides in stormwater ponds: is the photodegradation process significantly contributing to biocide transformation in the water column?

The photodegradation study of the three common stormwater biocides, namely, carbendazim, diuron and terbutryn, has shown that the concentrations of these biocides are not significantly reduced by the natural sunlight radiation. Processes, other than photodegradation, likely govern biocide contamination reduction in the water column.

Research question 2: Phytoplankton diversity and abundance in stormwater ponds: are stormwater phytoplankton communities comparable to those of natural shallow lakes?

Stormwater and natural shallow lake phytoplankton identification has revealed that pond taxonomic diversity is at least as high as that of lakes. Taxa enumeration and biovolume estimation showed a high variability among water bodies and sampling periods. The conducted statistical analysis did not show any significant differences between ponds and lakes, indicating some similarities between the man-made and natural water bodies. Little difference was found between Canadian and Danish stormwater ponds as well, indicating an urban homogenization phenomenon. Overall, this study provided evidence that despite higher contamination levels compared to lakes, stormwater ponds serve as suitable habitats and in time are colonized by diverse phytoplankton communities.

Research question 3: Diatom communities in stormwater pond sediment: do they reflect biocide contamination and stormwater retention pond catchment type?

The stormwater benthic diatom identification and enumeration showed that diverse diatom communities inhabit these man-made water bodies. The applied statistical analysis demonstrated that the diatom communities present in highway pond sediments are significantly different from those of residential and industrial ones. This provided some evidence that different diatom taxa inhabit ponds depending on their environmental conditions, such as water quality parameters, pollutants and their concentrations, and etc., which at the same time depend highly on the catchment where the pond is located. Although a weak relation of diatom communities with several measured biocide concentrations was found, clear diatom diversity – a clear biocide contamination relationship – could not be established.

Research question 4: Biocide contamination and stormwater phytoplankton: does the stormwater phytoplankton community composition reflect biocide contamination at environmentally realistic concentrations?

A high variability of numbers and biovolumes of stormwater phytoplankton was found among the microcosm and mesocosm samples treated with biocides carbendazim, terbutryn, diuron and irgarol at environmentally relevant concentrations. No apparent changes or statistically significant differences among the analyzed phytoplankton communities were observed for the applied treatments. Only the highest concentration of terbutryn showed a reduction of taxa diversity in all the conducted microcosm and mesocosm experiments, indicating its possible toxicity towards the stormwater phytoplankton. Also, in the cosms treated by irgarol, an increase in *Chlorophyta* abundance and biovolume with an increase in irgarol concentration was observed throughout the three studies. The lack of apparent stormwater phytoplankton community response to biocide contamination could be related with their tolerance to these compounds, induced by the continuous exposure to runoff biocides frequently loaded into the examined pond.

CHAPTER 6. FUTURE PERSPECTIVES

Ecosystem investigations are complex and challenging, consisting of a high number of variables, such as water chemistry, weather conditions, trophic system, and etc. To be able to conduct the desired tests, certain assumptions and simplifications have to be made. Thus, to supplement and evolve the results obtained in the present PhD study, there are a number of possibilities for future investigations, combining a larger number of variables, which were not addressed yet. For instance:

- Investigations at species level. Each phytoplankton species is very unique with its particular range of environmental preferences. Further research could help to get a deeper insight into the diversity and dynamics of stormwater phytoplankton.

- Functional diversity. A number of studies next to the taxonomic phytoplankton diversity acknowledge the importance of their functional diversity. Such analysis could contribute to the understanding of phytoplankton community composition from a broader perspective.

- Continuous monitoring. Stormwater ponds are very dynamic systems compared to natural lakes, as they have short water residence times and frequent influx of different contaminants. Compared to grab sample analysis, continuous frequent monitoring of species and water quality parameters could be more beneficial. Larger data sets could aid the establishment of connections between the phytoplankton communities and the environmental conditions.

- Larger data sets. A larger number and variety of stormwater ponds and natural lakes could be investigated in order to see whether the variability among water bodies holds or whether they would form groups depending on their catchment type, morphological features, measured different pollutant concentrations, and etc.

- Urban homogenization. This phenomenon has been studied by several researchers as well. Investigations of a wider variety of ponds from different geographic locations could also broaden the knowledge of stormwater phytoplankton communities and their behavior.

- Contaminant analysis. A variety of contaminants of different origin are present in stormwater ponds, each with its unique influence on the living organisms. Investigations of their fate and effects could also supplement the knowledge of the behavior of stormwater phytoplankton communities.

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ISSN (online): 2446-1636
ISBN (online): 978-87-7210-085-2

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