MASTER THESIS

BIOCIDES IN STORMWATER POND SEDIMENTS

SORPTION, BIODEGRADATION AND MICROBIAL INHIBITION

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Environmental Engineering I Spring 2014

AALBORG UNIVERSITY
STUDENT REPORT

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Synopsis:

Biocides used as antifoulants in paints and renders are leached off of building facades by rain and thereby end up in natural environments. It can be assumed such compounds can be harmful to the ecosystems in the receiving waters, but that they could also be degraded by the microbial activity in the sediments of the waterbodies, especially in stormwater ponds.

A series of tests was performed on sediments from two stormwater detention ponds, one in Aarhus and one in Silkeborg. The potential of these sediments for sorption and biodegradation of biocides Diuron, Terbutryn and Cybutryn was tested in a suspension experiment. Sorption was also studied in intact sediment cores. Additional tests were conducted to measure the possible inhibition caused by the biocide dosing to the microbial populations present in the samples. The results were plotted in graphs to estimate the reduction rates, and used to calculate the log K_D and log K_{oc} values for the samples. The results indicated, amongst other things, that the biocides can be degraded in the sediments, and showed a varying degree of microbial inhibition by at least one of the tested biocides. The project can also be seen as a part of preparatory studies for a bigger collaboration project by Aalborg University and Aarhus University on the same topic.

Preface

This report is a Master's Thesis in Environmetal Engineering, Section of Chemistry, Biology and Environment at Aalborg University. The project was carried out as short dissertation in the spring of 2014 under the supervision of Jes Vollertsen.

I want to thank the supervisor, as well as the assistant supervisor Asbjørn Haaning Nielsen, for their guidance during the project. I also want to express my gratitude to Elise Rudelle, Greta Minelgaite and Diana Stephansen for their invaluable help with the experiments.

Reading guide

The project is divided into 7 main chapters: Introduction, Definitions, Background, Methods, Results, Discussion and Conclusions. Each part contains a number of chapters and each chapter contains a number of sections and subsections. Any additional information is gathered in the Appendix at the end of the report. Literature will be referred to throughout the report, and an alphabetical list of literature sources is collected in the bibliography. The references are listed by the Harvard Method. Figures and tables are numbered according to the chapter in which they occur. Equations are specified by a number in a bracket and they are numbered in the same way as the figures and the tables.

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1 Introduction

Urban sprawl has been an ongoing phenomenon for the past few decades and it does not show any signs of stopping. As a result, an increasing amount of land area is covered by artificial, impermeable surfaces. This leads to a variety of problems that of late have become a cause of concern. The most obvious problem is the physical alteration of the water cycle, since the natural infiltration of water to the ground is reduced, causing flooding of streets, overflowing sewers and inhibiting groundwater recharge. In addition, the surfaces are a source of numerous pollutants, either deposited by atmospheric pollution, leachates from cultivated land, animal faeces, but also additives used in surface materials. A range of organic compounds, such as flame retardants, are added to surface materials to make them more resistant to the surrounding conditions.

One subgroup of such additives is biocides, which are added to paints and other coating materials. These are effectively leached off when the surfaces are new and the runoff water can therefore have significant concentrations of the said biocides [Burkhardt et al., 2012]. In comparison to the biocides being released from cultivated lands, the urban release of biocides is often overlooked as minor. However as these compounds are often led with rainwater in a centralized manner to detention ponds, the concentrations may accumulate and effects in the receiving waters can be magnified. The significance of urban biocide sources has also been pointed out in a study by Wittmer et al. [2010], claiming that pesticides from urban sources are as significant in magnitude as those from rural applications.

Biocides are added to paints and other surfacing material in order to inhibit algal, fungal or bacterial growth. When these compounds end up in stormwater ponds, they can have a detrimental effect on the ecosystems present in the pond, as well as in the downstream receiving waters. This should be a cause for action, also because the biocide concentrations in natural environments will likely be restricted by legislative means [Vollertsen, 2013]. In the context of the Water Frame Directive, the European Commission has suggested that the yearly average concentrations of Diuron, Terbutryn and Cybutryn in heavily modified freshwater bodies should not exceed 200 μg/L, 65 μg/L and 2.5 μg/L respectively.

Although there are ways to reduce the leaching at source to some extent, the sources as such cannot be totally removed. Therefore it is necessary to look into how the spread and accumulation of the pollutants in the receiving waters could be controlled, and their possible effects thereby reduced.

On entering the stormwater ponds, biocides are partially sorbed to the particles and settled to the bottom. They are therefore partly held back in the pond sediment, and the amount released to receiving natural waters should be notably lower than the incoming amount [Hvitved-Jacobsen et al., 2010]. It can be assumed that biocides are also removed to some extent by the biological activity in the sediments as tehy provide a substrate for the degrading organisms. This study aims to make investigations of the degradation potential in two stormwater ponds, one in Aarhus and one in Silkeborg. The location of the ponds are pointed out on the map in figure 1.1.

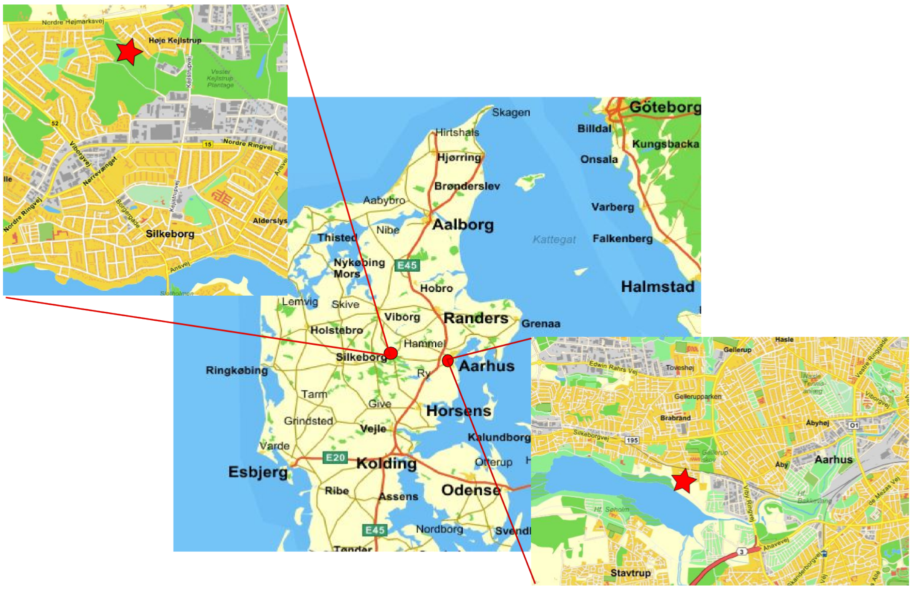


Figure 1.1: Locations of the stormwater ponds included in this study. Pictures courtesy of Krak.dk [2014].

The Aarhus pond is approximately one year older than that in Silkeborg, and therefore accumulation of sediment in Aarhus can be slightly higher. However a more likely reason for a higher level of organic matter in the Aarhus pond is the illicit inflow of wastewater it receives. This can make a difference regarding the removal of biocides, as their sorption is found to be directly proportional to the amount of organic matter in the sediment.

This project can be seen as a preparatory study for a bigger project, titled *Biocider i urbane småsøer - effekt og skæbne*. It is a collaboration between Aalborg University and Aarhus University and aims to study the fate of four biocide compounds - Terbutryn, Cybutryn, Diuron and Carbendazim - in several stormwater ponds, as well as their effects on the ecosystem of the ponds [Vollertsen, 2013]. In the project at hand, however, the focus is on three compounds, namely the herbicides Diuron, Terbutryn and Cybutryn, whereas Carbendazim was left out for practical reasons. The work done in the thesis project can serve as guideline and reference data for the experiments, analyses and method development in the main project.

Problem statement

The problem statement for the project is formed to be in line with the statement for the research project *Biocider i urbane småsøer* . Therefore the following hypotheses can be set up:

- It is assumed that Diuron, Terbutryn and Cybutryn are biodegraded to some extent in the sediments of stormwater ponds.
- It is assumed the organic fraction of the sediment, and thereby sorption and degradation, are higher in the Aarhus pond.
- It is assumed that high levels of these said pollutants can be harmful for the ecosystems in the stormwater ponds.

It can be summarised, that the purpose of this project is to study the sorption and the further removal of Terbutryn, Cybutryn and Diuron in the pond sediments by microbial degradation, as well as the possible inhibition of microbial activity they might cause.

To facilitate the laboratory analysis, the samples are spiked with higher concentrations of biocides than what would typically occur under natural circumstances. Therefore a test is conducted to measure the possible inhibition of biological activity caused by addition of the said compounds. The sorption and degradation potentials are studied with suspended sediment samples from Aarhus and Silkeborg stormwater ponds. Sorption is also measured in intact core samples from both lakes.

2 Acronyms and Definitions

There are some important ecotoxicological definitions used to describe the chemicals in the study, as well as some laboratory equipment, substances or procedures, that are referred to with abbreviations and acronyms, or their names being otherwise not generally known. These definitions are written out and their meaning explained in table 2.1 on the following page.

Table 2.1: Definitions for the symbols and abbreviations used in the study.

Abbreviation	Definition
Acetonitrile (CH_3CN)	An organic solvent of medium polarity. The hyperpure variant used for analysis is >99.8 % and anhydrous
MilliQ	A brand of deionised, hyperpure water
HPLC	High Precision Liquid Cromatograph
SPE	Solid Phase Extraction
K_{ow}	The Octanol-Water partitioning coefficient. The ratio of a substance concentrations in octanol and aqueous phases respectively [Food and Agriculture Organisation of the United Nations, 2014].
K_d	The distribution coefficient [mL/g]. Ratio of the concentration between sediment [mg/kg] and water phase [mg/L] at equilibrium.
K_{oc}	The Organic carbon-Water partitioning coefficient. The ratio of K_d and the sediment organic fraction.
Microcosm	An experimental model set-up representing the characteristics of an ecosystem for ecotoxicological studies [U.S. Department of Health & Human Services, 2014].
DT_{50}	Degradation half-life, the time after which 50 % of the initial substrate concentration is expected to have been removed by transformation [Food and Agriculture Organisation of the United Nations, 2014].
EC_{50}	Median Effective Concentration is the concentration required to produce a pre-defined effect in 50 % of the studied population in a given time [U.S. Department of Health & Human Services, 2014]. A sub category of EC_{50} is LC_{50}.
LC_{50}	Median Lethal Concentration, or lethal concentration 50 %, is the dosage of a chemical expected to kill 50 % of the tested population in a given time [U.S. Department of Health & Human Services, 2014].
PNEC	Predicted No Effect Concentration, concentration that is expected to cause no adverse effects to populations in the natural environment [U.S. Department of Health & Human Services, 2014].
TS	Total solids, the dry weight of the sediment (after 12 hours in 105°C)
VS	Volatile Solids, the amoun of organic matter in the sediment, determined by loss on ignition for 1 h in 500°C.
FS	Fixed Solids, total solids – volatile solids
OM	Organic Matter, here equivalent to VS

3 Background

3.1 Biocides in Stormwater

Until recently, stormwater runoff has been considered as unproblematic water and has therefore received little or no treatment. In many cases stormwater is still lead directly either into natural waters or to the sewer system, where it can cause sewer overflow and overloading of the wastewater treatment plants. However, the impermeable or semi-permeable man-made materials in urban areas are sources for a great variety of pollutants that can get leached away during rain events and cause consequent harm either on the natural environment or to human health. Lately the concern of pollutants that the stormwater carries along has gained more visibility, as more and more studies have been conducted on the matter.

Urban stormwater pollution can originate from building materials and surfactants, lawns and gardens, vehicle parts, oil and traffic emissions [Burkhardt et al., 2011]. When only biocides are considered, the sources can be divided into roughly two: urban gardens and lawns and antifoulants used on buildings. Biocides from gardens include mostly pesticides, and it can be simplified that the biggest source of algae-, herbi- and fungicides are building surface materials.

The concentrations and types of biocide used naturally depend on the manufacturer and the type of surface material used. In general paints usually contain double the amount of biocides than render materials do [Burkhardt et al., 2012], the concentrations varying from 0.1 g/kg to 2 g/kg [Burkhardt et al., 2011]. Often a combination of three to five biocides is required for effective protection, adding up to as high as 0.5 % biocide content in surface materials. Based on this Burkhardt et al. [2011] estimated, that in the case of Switzerland, 50-100 tons of biocides are used in new paints and renders yearly. Adding this to the established fact, that leaching from the facades is highest when the surfaces are new, it can be concluded that urban biocide use poses a real risk of pollution in the receiving waters.

This was also confirmed by Wittmer et al. [2010], who claim that the rate of urban leaching is relatively high, so that the resulting loading of biocides to a combined catchment were of the same magnitude from both agriculture and urban use. This even though the rate of biocide application in urban areas is relatively low in comparison to agricultural use.

In a study of the dynamics of biocide leaching from building façades by wind driven rain (WDR) were inspected, Burkhardt et al. [2012] confirmed that the leaching is highest when the surface material is new, and reaches a stable level after some time. The leaching is also dependent of the structure of the surface material, as well as the area, material and orientation of the wall, wind speed and direction, rain intensity, splashing and evaporation etc., making the estimation of biocide leaching very complicated. Bollman et al. [2013] noted, that the pollutant loads in stormwater during storm events were more than 10-times higher than during average rainfall events. Another study also made by Burkhardt et al. [2011] measured the concentrations in runoff from real urban areas. They found that all the biocides in the receiving stream were detected in concentrations exceeding their predicted no-effect concentration (PNEC) values.

The focus on this project was on three biocides, Diuron, Terbutryn and Cybutryn, all of which are used as algaecides [Bollman et al., 2013]. They have been included in several biocide-related studies due their wide use and persistence [Burkhardt et al., 2012].

Most of the studies on the degradation of these biocides have been conducted in soil or the water phase, due to their use as agricultural pesticides and leaching to ground or surface waters. Not as much is know of their degradation in sediments. In some cases the water-sediment half-life DT_{50} has been estimated to be 60 days for Diuron /table 3.1.1) and 48 for Terbutryn (table 3.1.2 on page 10), whereas no value for Cybutryn was to be found.

All these biocides bear the environmental risk-clause stating they are ”very toxic to aquatic organisms” and as such can cause long-term adverse effects in the aquatic environment [Sigma Aldrich, 2014b], [Sigma Aldrich, 2014c], [Sigma Aldrich, 2014a]. Diuron and Terbutryn are also on the European Union priority list of possible endocrine disruptors [European Comission, 2000]. Less data was available of Cybutryn, which is excluded from several databases, such as the IUPAC Pesticide Footprint database [IUPAC, 2014]. This is in contrast of its apparent ecotoxicological importance, with lowest PNEC values [Bollman et al., 2013] and the lowest suggested limit values [European Commission, 2013] of all the three biocides. The comparison of the properties of these three chemicals is therefore not always equal because of missing data.

All the biocides used in this study were manufactured by Sigma-Aldrich as part of their Fluka analytical standards-series and are described in more detail below.

3.1.1 Diuron

Diuron (N-(3, 4-dichlorophenyl)-N, N-dimethylurea) is a herbicide that prevents the production of ATP and fixation of CO_2 in plants. It is very commonly used in agricultural weed control. Other uses of Diuron include vegetation control in commercial and industrial areas, as well as algaecides in artificial waterbodies [Moncada, 2011].

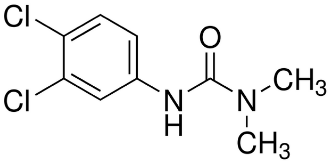


Figure 3.1: Chemical structure of Diuron

Table 3.1: Selected properties of Diuron. Some of the values can vary depending on the source. For clarification of the abbreviations, see table 2.1 on page 6.

Formula	$C_9H_{10}Cl_2N_2O$
Molar mass [g/mol]	233.09
Water solubility [mg/L] [1]	42
Log K_{ow} [2]	2.67
Vapour pressure [mPa] [1]	1.15×10^{-03}
pKa	not applicable
PNEC [ng/L] [2]	20
EC_{50}, algae [mg/L] in 72 h [1]	0.0027
EC_{50}, Daphnia Manga [mg/L] in 48 h [3]	1.4
Water–sediment DT_{50} [d] [1]	48

[1] [IUPAC, 2013a]
[2] [Bollman et al., 2013]
[3] [Sigma Aldrich, 2014b]

Due to its low K_{ow}, see table 3.1.1, it does not adsorb effectively to sediments. According to Moncada [2011], the most common way to biodegrade Diuron is by microbes. On the contrary, photolysis is not common in aqueous environments. Of the three biocides, Diuron has the highest water solubility and is therefore more likely to leach off in higher concentrations. This was confirmed in a laboratory test made by Burkhardt et al. [2011], where leaching rates of Diuron were twice as high than those of Terbutryn and Cybutryn.

According to a test made by Tixier et al. [2000], degradation of Diuron by fungi results in the appearance of two metabolites which were tested to have a higher toxicity than their parent compound.

3.1.2 Terbutryn

According to Extension Toxicology Network [1996], Terbutryn is a selective herbicide that belongs to the triazine-group and works by inhibiting photosynthesis. One of the uses of Terbutryn is as an aquatic herbicide used for controlling algal growth in for example fish ponds. It is also mentioned to be readily sorbable to soils with high organic or clay content [Extension Toxicology Network, 1996]. Its half-life in soil has been reported to be between two to four weeks [Royal Society of Chemistry Information Systems, 1994], whereas in sediment it can take up to two months [IUPAC, 2013b].

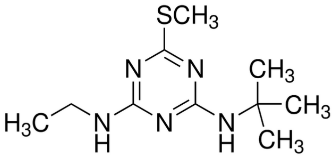


Figure 3.2: Chemical structure of Terbutryn

Table 3.2: Selected properties of Terbutryn. Some of the values can vary depending on the source. For clarification of the abbreviations, see table 2.1 on page 6.

Formula	$C_{10}H_{19}N_5S$
Molar mass [g/mol]	241.36
Water solubility [mg/L] [1]	25
Log K_{ow} [2]	3.77
Vapour pressure [mPa] [1]	0.13
pKa at 25°C [1]	approx. 4.3
PNEC, algae [ng/L] [2]	34
EC_{50}, algae [mg/L] [3]	2,4 - 2,9
EC_{50}, Daphnia Manga [mg/L] in 48 h [1]	> 2.66
Water-sediment DT_{50} [d] [1]	60

[1] [IUPAC, 2013b]
[2] [Bollman et al., 2013]
[3] [Sigma Aldrich, 2014c]

Terbutryn is very toxic to aquatic life [Sigma Aldrich, 2014c] and a suspected endocrine disruptor [European Comission, 2000]. Due to its low vapour pressure and water solubility (see table 3.1.2) Terbutryn is not volatile, nor does it readily dissolve in water.

3.1.3 Cybutryn

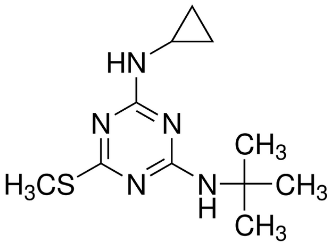


Figure 3.3: Chemical structure of Cybutryn

Cybutryn is a photosynthesis inhibiting algaecide, often referred to by its trade name Irgarol. Unlike Diuron, Cybutryn is used mainly on building surfaces or vessels as antifoulant, instead of agricultural land. It is very harmful to aquatic life, but has no known endocrine disrupting qualities [Sub-Group on Review of the Priority Substances List, 2011]. It has been reported, that degradation of Cybutryn in sediments would be between 100 to 200 days under aerobic concentrations [Sub-Group on Review of the Priority Substances List, 2011], making it the hardest of the three biocides to degrade.

Table 3.3: Selected properties of Cybutryn. Some of the values can vary depending on the source. For clarification of the abbreviations, see table 2.1 on page 6.

Formula	$C_{11}H_{19}N_5S$
Molar mass [g/mol][1]	253.37
Water solubility [mg/L][2]	7
Log K_{ow} [3]	4.07
Vapour pressure [mPa] [4]	0.088
pKa at 25°C [5]	4.12
PNEC [ng/L] [3]	1
EC_{50}, algae [mg/L] in 70 h[1]	0.002
LC_{50}, Daphnia Manga [mg/L] in 48 h [2]	2.4-8.3

[1] [Sigma Aldrich, 2014a]

[2] [Sub-Group on Review of the Priority Substances List, 2011]

[3] [Bollman et al., 2013]

[4] [SinoHarvest, 2013]

[5] [Vollertsen, 2013]

Alike to Diuron and Terbutryn, Cybutryn is also non-volatile. Its high log K_{ow} value should make it the most readily sorbable while being the least water soluble. According to Bollman et al. [2013], its PNEC value is the lowest of all three and therefore possibly a notable environmental concern.

4 Methods

4.1 Overview of the Experiments

In total three different types of laboratory experiment set-ups were made to study the effect and behaviour of the biocides: one for finding out if there is inhibition of biological activity, one for sorption and degradation in suspended sediments, and one for sorption to sediments in intact cores.

Table 4.1: An overview of the experiments made during the project. For tests 1A-3 only Aarhus sediment was used. Tests 4A-5 were conducted using sediments from both Aarhus and Silkeborg.

Experiment name	Experiment Description
	Inhibition of biological activity
Test 1A	Comparison of inhibition by different biocides, first run
Test 1B	Comparison of inhibition by different biocides, second run
Test 2	Comparison of the effects of different biocide concentrations
Test 3	Effect of exposure to daylight on inhibition
	Experiments with suspended sediments
Test 4A	Sorption and degradation of biocides in suspended sediments, first run
Test 4B	Sorption of and degradation of biocides in suspended sediments, second run
	Experiments with intact core samples
Test 5	Sorption of biocides into intact sediments in cores

To facilitate the referring to a particular experiment, an overview of all experiments with their names and descriptions is presented in table 4.1. The methodology of these experiments will be explained in the following subchapters.

4.2 Inhibition of Biological Activity

As the purpose of the studied biocides is to prevent algae, fungi or weeds from growing in unwanted places, it is fair to assume the microbial ecosystem in the investigated stormwater pond samples will also be affected in some way by the addition of biocides. This was especially of concern in the current study, where it was envisioned to use a higher dosage than would occur in nature even under special circumstances.

All the biocides used in the experiments are known for their algaecidal qualities, and hence it is highly likely that some algae inhibition will happen with high dosages. The effects of the biocides on the degrading organisms however are not known, and therefore it was unsure whether such dosage might also have a negative effect on the degradation rate.

To shed light on this matter, a series of inhibition tests were carried out. The approach was to measure the oxygen uptake of the spiked samples and compare them to blank samples. A lower rate of oxygen uptake would indicate that the biocides have affected the organisms, some of which could also be involved in the biodegradation.

4.2.1 Comparison of Biocides

The first test was made with all the four biocides included in the main project, as mentioned in chapter 3.1 on page 7. That is to say, in addition to Diuron, Terbutryn and Cybutryn, also Carbendazim was included. In total two similar inhibition experiments, 1A and 1B, were carried out (see table 4.1 on the previous page).

The tests were conducted with only Aarhus sediment for practical reasons. BOD-bottles were used as vessels because they can be closed airtight. With the sediment added, the volume of the bottles was approximately 105 mL. The biocides were added as a solution made in acetonitrile. A description of the experiment process is given in the following list.

1. 20 g of Aarhus sediment was added to six BOD-bottles each. A small amount of pond water was added to prevent sediments from drying. The sediment samples were then aerated by shaking for an hour.
2. The bottles were filled up with aerated pond water and their oxygen contents were measured.
3. Four of the samples were spiked each with a different biocide solution of concentration 5 mg/L, and two were left blank.
4. The bottles were covered with tinfoil to prevent photosynthesis, and placed on a shaker.
5. The oxygen content in the samples was measured at 30-60 minute intervals during three hours. The oxygen contents were measured also the following morning.

Figure 4.1 shows the test vessels together with the degradation experiment vessels. The measurements were done with a 140 μm needle-type optical fiber microsensor manufactured by PreSens. It is non-invasive and thereby does not consume oxygen itself, but it can be sensitive to organic solvents [PreSens, 2014].

Figure 4.1: The BOD–bottles used for inhibition experiments (in the front) and the vessels used for degradation experiments (at the back), all placed on a shaker.

4.2.2 Dose-Response of a Specific Biocide Compound

Another experiment, hereby called Test 2, was made in order to find out if the sediment oxygen uptake is dependent on the amount of biocides added. The test was made using five different concentrations of only one biocide, as well as a blank sample. The concentrations used for the degradation experiments were in the range of 1-5 mg/L. In addition to these it was though interesting to see if lower concentrations than those used would still cause inhibition. Therefore the biocide concentrations chosen for the inhibition experiment were 5 mg/L, 2.5 mg/L, 1 mg/L, 0.5 mg/L and 0.1 mg/L.

In total six vessels were prepared by weighing approximately 20 g of sediment to each of them. The samples were filled with water and aerated as described for tests 1A and 1B in chapter 4.2.1 on the facing page. Similarly to the previous tests, the vessels were covered in tinfoil to prevent photosynthesis.

One vessel was left as a blank, and the remaining five were spiked with different concentrations of a chosen biocide at a 10 minute interval. The oxygen saturation in the vessels was then measured approximately every 30 minutes.

4.2.3 Inhibition of Photosynthetic Activity

As this experiment only shows the overall oxygen uptake, it cannot be specified which of the organisms, if any, get inhibited. Therefore in test 3 the effect of daylight on the samples was studied. If the oxygen concentration would start to rise with exposure to light, this would indicate that at least not all the algae has been killed off. If no change in the oxygen uptake rate is seen, it can be concluded that because no photosynthesis occurs, the biocide has caused inhibition of the algae.

This experiment was a continuation to test 2, carried out as described in section 4.2.2. After three hours of measuring, the samples were exposed to daylight. Two measurements were taken during the exposure, after 1.5 and 2.5 hours. The rates of decrease before and after daylight exposure will be compared in the final graphs, as demonstrated by the example in figure 4.2.

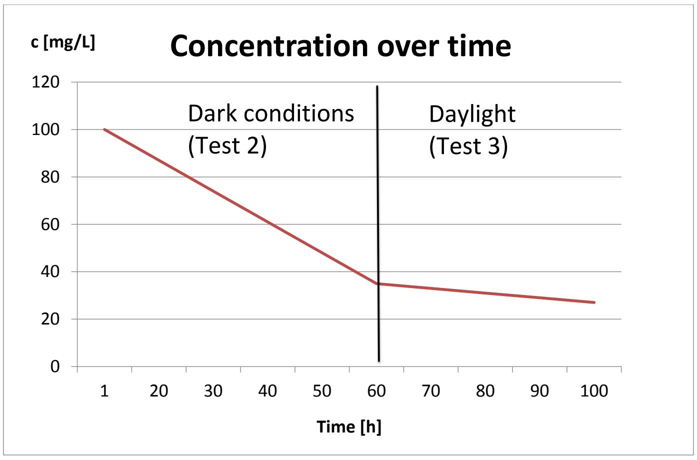


Figure 4.2: A fictional example of the interpretation of the results from test 2 and test 3.

4.3 Sorption and Degradation in Suspended Sediments

4.3.1 Theoretical Background on Sorption and Degradation in Sediments

The sorption of pesticides to soils or sediments is known to be highly dependent of the soil organic carbon, as was also confirmed by e.g. Kah [2007]. The octanol-water partitioning coefficient log K_{ow} is the ratio of the concentration in octanol to the concentration in water, and thereby describes the hydrophobicity of the substance [Pirika, 2011]. The higher the value is, the more readily the compound leaves the water phase. Log K_{ow} is used to predict the behaviour of pesticides and their distribution between water and soil. The log K_{ow} values for the biocides used in this study have been calculated with software from the U.S. EPA, as used by [Bollman et al., 2013] and presented in their respective tables in section 3.1.

Sorption kinetics for neutral compounds are driven only by their hydrophobicity. However, some biocides belong to ionisable herbicides, which means they are weakly acidic or basic and can be ionised in the range of pH present in soil [Wauchope et al., 2002], lower soil pH typically increasing sorption [Kah, 2007]. One such ionisable group is triazines, here represented by Terbutryn and Cybutryn with pKa values of 4.3 and 4.12 respectively. If the soil pH is close to the biocide pKA values, it could cause changes in their log K_{ow} values and thereby mobility [Wauchope et al., 2002]. However as the pH in the sediments is close to that of water, and therefore much higher than the pKa values of Terbutryn and Cybutryn, it should not have an effect on the sorption of the said biocides.

The sediment is a heterogenous material and therefore its capacity for sorption and biodegradation can also vary significantly throughout the pond. The sediments used for the experiments were a mixture of core samples from several locations from the ponds, shown in figures 4.4(a) and 4.4(b). The results represent therefore an average of the existing degradation potentials, that vary throughout the pond. In natural circumstances the sediment would be more still and the access of degrading bacteria to the biocides is more limited, the degradation rate therefore likely being lower than in suspension.

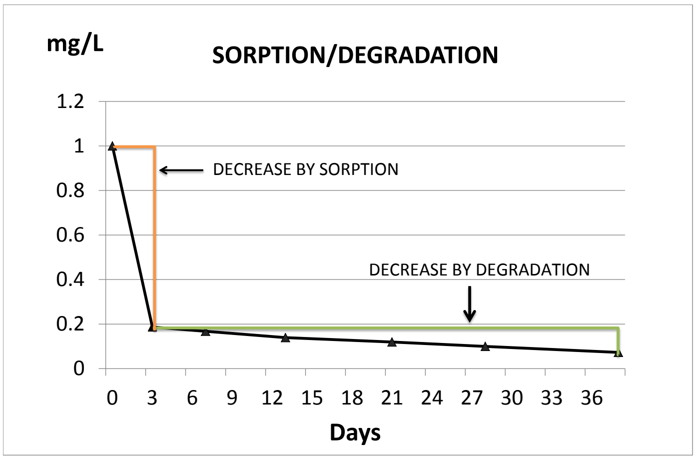


Figure 4.3: A fictional example of the division to sorption and degradation in the concentration curves from tests 4A and 4B.

Similar suspension studies made before by Rudelle [2014] have shown that the biocides are sorbed to particles during the first 24 hours, causing a considerable fall in concentration. After this time period sorption can be considered complete, and any decrease in concentrations would be an indication of degradation. Therefore the tests were continued as biodegradation tests after the first measurement at 24 hours. Figure 4.3 shows an example of how the resulting plot is expected to look like. It should be able to divide it into two clear parts: a considerable fall in concentration after one day caused by sorption, followed by a lower rate of decrease caused by degradation.

4.3.2 Experiment Set-up

To assess the potential of the sediments for sorption and further removal of biocides, two runs (tests 4A and 4B, see table 4.1 on page 13) of suspension experiments were carried out. The experiments were chosen to be performed in suspension to find out the sorption and degradation potential of the sediments, as well as to get results faster than what was expected with intact core samples. Mixing the sediment with water increases the available adsorption area on the particles. Thereby the contact between the biocides that are in solution in the waterphase, and the sediment particles becomes easier, facilitating faster adsorption and further biological removal of the studied compounds.

The sediments used for the experiments were mixtures of several core samples take in several locations near the inlet of each pond. The sampling locations are depicted in figures 4.4(a) and 4.4(b).

(a) Aarhus

(b) Silkeborg

Figure 4.4: The locations where the cores were collected. Each number represent the respective intact core sample.

The suspension experiments were carried out in 1000 mL glass bottles placed on a shaker and protected from light by black plastic covers. The amount of sediment for test 4A was 80g, covered with 500 mL of pondwater. For test 4B, the amount of sediment was reduced to 60 g and the amount of water was reduced to 300 mL. The differing parameters between Tests 4A and 4B are explained in table 4.2. The samples were aerated with compressed air to exchange the airphase, but not conducting any oxygen into the water phase to minimize evaporation. However the possibility of evaporation cannot be excluded, but is assumed low and thereby unimportant. The switch from centrifuging and filtering to double centrifuging was done based on a test, explained in the Appendix in section A.3 on page A2.

The general process of the experiment is explained in the list below. The purpose was to measure with a smaller interval in the beginning to get a grasp of how fast the degradation might be, and then continue with a sample once a week. The sample vessels are shown in figure 4.1 on page 14.

1. A pre-defined amount (check table 4.2 for details) of mixed sediment was weighed into 1000 mL bottles. Three bottles were prepared for both Aarhus and Silkeborg. The bottles were covered with black plastic to prevent photosynthesis.
2. 500/300 mL of unfiltered pond-water was measured and added to the bottles. Then, the volume required by the biocide solution was subtracted by pipetting. This was done in order to keep the final volume and thereby concentrations accurate.
3. The samples were spiked with a mixture of the three biocides dissolved in acetonitrile. Three different concentrations, 5 mg/L, 2.5 mg/L and 1.0 mg/L, were used, one for each sample from each pond.
4. A subsample of approximately 1-2 mL was collected from each bottle on certain days (check table 4.2 for details) after the spiking.
5. The subsamples were collected with Pasteur pipettes and centrifuged in a vial for 5 minutes at 2500 rpm.
6. The supernatant of the subsamples was removed with a Pasteur pipette and filtered with a glassfiber filter (Test 4A) or further centrifuged in Teflon vials for 10 minutes at 13 000 rpm (Test 4B).
7. The final subsamples were analysed on the day of sampling by using the High-precision liquid chromatograph (from now on HPLC).

Table 4.2: Changing parameters in degradation experiments.

	Test 3A	Test 3B
Sediment mass [g]	80	55
Water volume [mL]	500	300
Wat:Sed – ratio	6.3	5.5
Sampling days	1,3,6,10,14	1,3,7,14,21,28
Sampling method	Centrifuge + filter	Double centrifuge
Additional aeration	no	yes

The project at hand was done as short dissertation, so certain simplifications in the procedures had to be made in order to fit it into the limited time frame of the project. To facilitate faster analyses, high start concentrations of the biocides were used. Unlike in the main project, *Biocider I urbane småsøer*, a fourth compound, Carbendazim was intentionally left out from this investigation. This is because it is only soluble in methanol and nevertheless the solubility remains low. Combining this fact to the high concentrations needed in the experiments would have increased the volume of Carbendazim solution added to each sample bottle to become too high. For a 500 mL volume and concentration 5 mg/L, the amount of Carbendazim solution would be 13 mL, adding up to almost 3 % of the entire volume. This much methanol would jeopardize the reliability of the experiment and Carbendazim was therefore left out. This also brought on an additional advantage, as the long retention time of Carbendazim in the HPLC was eliminated and the time needed to analyse one sample was reduced from 30 to 19 minutes.

A realistic and yet a high value for incoming biocides would be in the range of a few hundred μg/L [Burkhardt et al., 2012]. However as the concentration decreases, it eventually becomes too low for the HPLC to detect without extensive work on sample preparation. The latter requires an additional step of up-concentrating by Solid Phase Extraction (SPE), which is rather time consuming. Thus it was decided to use higher concentrations to skip this step and proceed straight to the HPLC.

The ideal case of studying degradation in the sediment would be to close the mass-balance by extracting the biocides deposited in the sediment. This however is more complicated than extraction from the water phase, and therefore often ignored even in scientific studies [Wauchope et al., 2002].

4.3.3 Dry Matter, Organic Matter, K$_D$ and K$_{oc}$

As the organic fraction of the sediment plays an important part in the sorption of the biocides, it was necessary to determine the total solids (TS) for the dry matter and volatile solids (VS) for the organic matter of each sediment used for the experiments. The determination method is explained in the Appendix in section A.1 on page A1.

The TS and VS values were then used to calculate the K$_D$ and log K$_{oc}$ [mL/g] values for each sample. K$_D$ is the distribution coefficient between water and soil phases. K$_{oc}$ is also called the organic carbon-water partition coefficient and it determines the ratio of the adsorbed and dissolved chemical concentrations and the fraction of organic matter in the soil or sediment [Wauchope et al., 2002]. The K$_D$ and the K$_{oc}$ were calculated with equations 4.2 and 4.3 respectively.

$$C_s = \frac{V(C_i - C_e)}{m_s} \tag{4.1}$$

$$K_D = \frac{C_s}{C_e} \tag{4.2}$$

$$K_{oc} = \frac{K_d * 1000}{OC} \tag{4.3}$$

Where

C_s	Concentration of biocide sorbed in the sediment at equilibrium	[mg/kg]
V	Water volume	[mL]
C_i	Initial concentration of biocide in the water phase	[mg/L]
C_e	Concentration of biocide dissolved in the water phase at equilibrium	[mg/L]
m_s	Mass of sediment	[g]
K_D	Distribution coefficient	[mL/g]
K_{oc}	Organic carbon-water partitioning coefficient	[mL/g]
OC	Fraction of organic matter in the sediment	[g/kg]

4.4 Sorption to Sediment in Intact Cores

Studying degradation was assumed to be very slow in a non-suspended microcosm and was deemed not possible to study in the given time frame. The focus was instead put on the adsorption in immobile sediment instead. This experiment seeks to point out the difference in the rate of sorption in intact microscosms, compared to those found in suspension experiments by Rudelle [2014], as well as to the sorption in suspension in test 4A and 4B. Variation between the cores will be expected as the sediment material is heterogenous and varies throughout the pond.

Intact sediment cores present a microcosm model of the natural environment, and as such are more alike to real conditions in the pond than the suspension experiment. However, the cores also have their limitations. In these intact cores the sediment stays essentially immobile as in the nature, but there is no flow of water that would occur in the ponds.

Three intact core samples were collected near the inlet of each pond. The locations where the samples were taken were the same also used for the suspension experiment. Each cores was numbered according to the location. The numbers can be seen in figures 4.4(a) and 4.4(b) on page 17. The core samples were taken with steel cores to minimise adsorption to the core itself.

In total three cores were taken from both Silkeborg and Aarhus ponds. As the cores contained a different amount of sediment, the distance of the sediment layer from the top of the core was measured, and the water level adjusted to 10 cm (total water volume 196 mL). This however was not a very accurate measurement, since the depth of the water column was defined by feeling with a metal measuring tape, and determining where the bottom starts was at times challenging because of the soft sediments.

The core samples were placed in a temperature controlled room, where the temperature was held at approximately 20°C. Unlike in the suspension experiment, only one concentration of biocides was used to compare the differences between the cores. The samples were spiked with a mixture of the biocides dissolved in acetonitrile to achieve an initial concentration of 2.5 mg/L. Subsamples taken were approximately 1.5-2 mL in volume, and were collected with muffled volumetric pipettes.

During the experiment, the cores were kept in the dark and each core was aerated with an aquarium pump. The set-up is presented in figure 4.5. To prevent evaporation, the cores were covered with tinfoil, but evaporation was noted to happen nevertheless. However, as explained in chapter 3.1 on page 7 the pesticides are not volatile and should therefore not evaporate.

Figure 4.5: Set – up of the sorption experiment.

Subsamples were taken after 1, 4, 8, 24, 30 and 48 hours. During this time the samples were aerated continuously with aquarium pumps. The sampling was continued on days 4, 7 and 16 of the trial to see if the concentrations still continued to decrease. During this time the samples were aerated only periodically to reduce evaporation.

4.5 Sediment Sampling

The sampling for the first rounds of suspension and toxicity experiments was carried out on 10.3.2014 and for the second round on 7.4.2014. The cores for sorption experiment were taken on 15.4.2014.

The sediment used for the experiments was collected with sediment cores, and the sampling itself was done with a core sampling device. In some cases in shallow water the cores were collected manually, as shown in figure 4.6. The greatest advantage of taking a core samples is that the sediment remains intact. When the core has been extracted, the biologically active top layer of the sediment column can be separated. Because the surface of the sediment is disturbed less than with grab sampling, very little biofilm should be lost in the process.

The sediment material was collected from three different locations in Aarhus, and three different spots in Silkeborg, as was pointed out earlier in figures 4.4(a) on page 17 and 4.4(b) on page 17. The Silkeborg pond is divided into three sections by reeds. A sample was planned to be taken also in the second section of the Silkeborg pond but there was a sufficiently thick sediment layer only in the first section closest to the outlet.

1. The core is pressed in the sediment and closed with a stopper to create a vacuum.
2. The core is raised with the sediment inside and plugged underwater.
3. Water from on top of the sediment is decanted using a rubber tube and a syringe.
4. The sediment is pushed up with a piston, and the few (approx. two) topmost centimeters are collected with a spoon.
5. The sample is then stored in a special purpose sample bag and the process repeated as many times as necessary.

The collection of the intact core samples followed the same pattern, with the exception that the sampling core was made of stainless steel, and the sediment was left in the core. Because the metal cores were not compatible with the sampling device, the cores where extracted by hand, using a rubber plug to create the vacuum. The excess water was removed in the laboratory before the start of the experiment with a volumetric pipette, in order to have the same volume of water in each core.

A possibly interesting area for sampling would have been right in front of the inlet and in the middle of the pond, since it can be assumed these areas contain the majority of the settled organic matter. Because of the build-up of the light and soft sediments, the pond bottom became difficult to walk on, thus it was not possible to get samples from said areas with the present equipment.

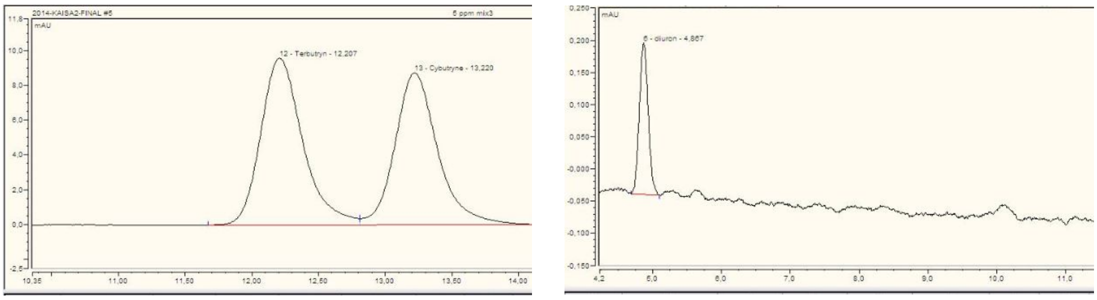
4.6 High Precision Liquid Cromatograph HPLC

The analyses for the degradation experiment consist of preparation by filtering and/or centrifuging (see table 4.2) and a High Precicision Liquid Cromatography (HPLC) analysis of the subsamples. The apparatus used for the purpose is a Dionex UltiMate ® 3000, a reversed phase HPLC The analysis was done running a program with an isocratic flow. The mobile phase consisted of 70 %

Figure 4.6: A core sample taken out manually.

HPLC -grade Acetonitrile and 30 % MilliQ. The pH of the eluent was adjusted to 4.0 pH, since this has been noted to improve the separation of the peaks of Terbutryn and Cybutryn, which have a very similar retention time and would otherwise appear almost simultaneously.

The method is not completely without fault. Figure (a) shows that the separation of the peaks of Terbutryn and Cybutryn is not complete, even with the eluent adjusted to pH 4. This causes some uncertainty in the final results based on the area of the peak. For reasons not clear, the baseline of the UV-aqcuisition showed often some background noise and a decreasing trend, as shown in figure (b).



(a) **(b)**

Figure 4.7: Screenshots of the HPLC UV–detection, showing the unseparated peaks of Terbutryn and Cybutryn (a) and the noise on the baseline (b).

To find out the real retention times and areas of Terbutryn and Cybutryn, a sample containing a 5 ppm concentration of each of them were analysed separately. The results are shown in table 4.3.

The retention times kept changing as the standard solution and the sample age kept growing. But what can be compared is the ratio of the peak areas of Terbutryn and Cybutryn in the separate samples and in the standards. It seems that due to the incomplete separation the area of Cybutryn in the samples has been overestimated. This does not make a huge difference when converting the areas to amounts. Therefore the used HPLC-program was deemed sufficient for the purpose as the concentrations found with this method nevertheless followed a decreasing trend, but the uncertainties should be borne in mind when looking at the results.

Table 4.3: The retention times and peak areas of separate 5 ppm samples of Terbutryn and Cybutryn and the peak areas of a mixed standard solution from experiment day 1.

	Retention time	Peak area, 5 ppm test	Peak area, 5 ppm standard
Terbutryn	11.593	3.6203	3.6393
Cybutryn	12.500	2.9483	3.3312
$\frac{Cybutryn}{Terbutryn} \times 100$		88.3 %	91.5 %

5 Results and Discussion

5.1 Inhibition of Biological Activity

The measurement results from tests 1-3 were plotted into graphs as oxygen concentration in water [mg/L] over time [min]. The rate of oxygen uptake was defined by the slopes of the linear trendline. The results for each experiment are presented and discussed in sections 5.1.1 – 5.1.3.

5.1.1 Comparison of Inhibition by Four Biocides

Both the tests 1A and 1B showed similar results, and only those of test 1B will be presented here. The rates of decrease in oxygen uptake seem to indicate that all the biocides caused some level of inhibition. As can be seen in table 5.1, the oxygen uptake rates of Terbutryn and Diuron show only slight deviation from that of the blank sample. Cybutryn seemed to show slightly greater inhibition considering oxygen consumption, its inhibition accounting for 65 % of the uptake rate of the blank average (figure 5.2). This can also be observed from the plotted results in figure 5.1 on the next page. The inhibition by Diuron, Terbutryn and Cybutryn was nevertheless not complete, as the O_2 concentrations in the samples were practically at 0 mg/L the following morning (see table 5.1).

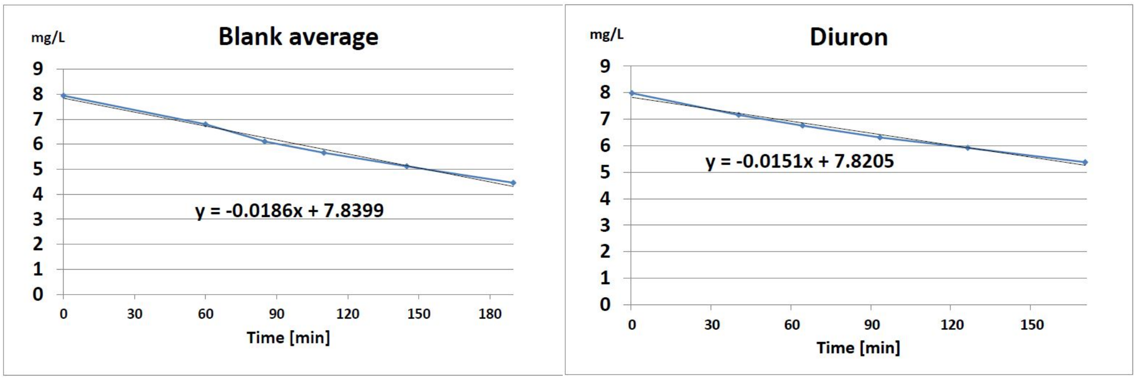
Table 5.1: Decrease in oxygen concentration in the waterphase of the sample vessels as concentration over time, as well as the final O_2 concentration on the following morning.

	Blank average	Diuron	Terbutryn	Cybutryn	Carbendazim
Rate -[C/t]	0.0186	0.0151	0.0120	0.0064	0.0038
Final O_2 concentration [mg/L]	0.03	0.04	0.03	0.1	3.54

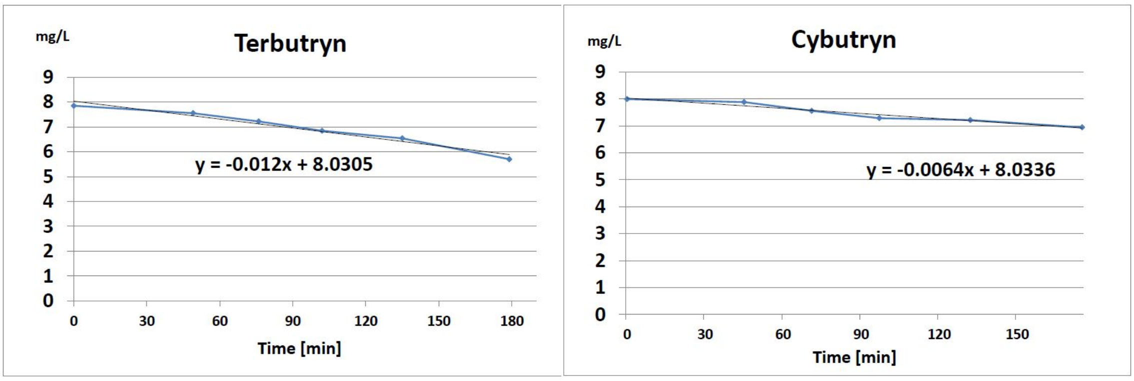
According to the results Carbendazim seemed to have the greatest effect on the oxygen consumption. The amount inhibition occuring in each sample in comparison to the blank average are presented in figure 5.2. The values were calculated by (5.1).

$$1 - \frac{Rate_{biocide}}{Rate_{blank}} \times 100 \tag{5.1}$$

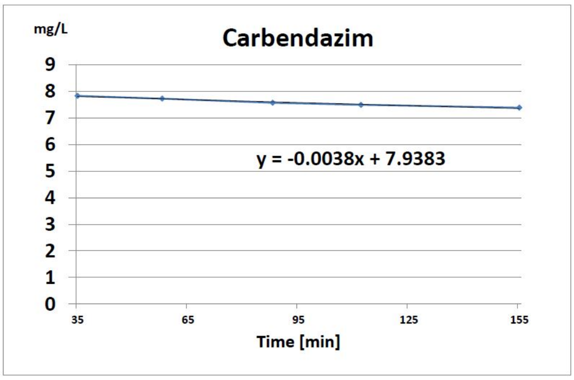
The rate of the fall in oxygen concentration in the sample containing Carbendazim is notably smaller than in any of the other samples, the inhibition accounting for approximately 80 % (figure 5.2). The sample containing Carbendazim was also the only one where the oxygen had not been consumed by the following morning (table 5.1).



(**a**) Blank (**b**) Diuron



(**c**) Terbutryn (**d**) Cybutryn



(**e**) Carbendazim

Figure 5.1: The decrease in O_2 concentration [mg/L] over time in the test samples. The decrease rate is defined by the slope of the linear trend line.

Based on these results it would be interesting to study further the dose-response of Carbendazim on oxygen uptake. But seen as Carbendazim is not in focus in this project, a dose-response test was performed with the second largest inhibitor Cybutryn instead, and the results will be studied in the following section.

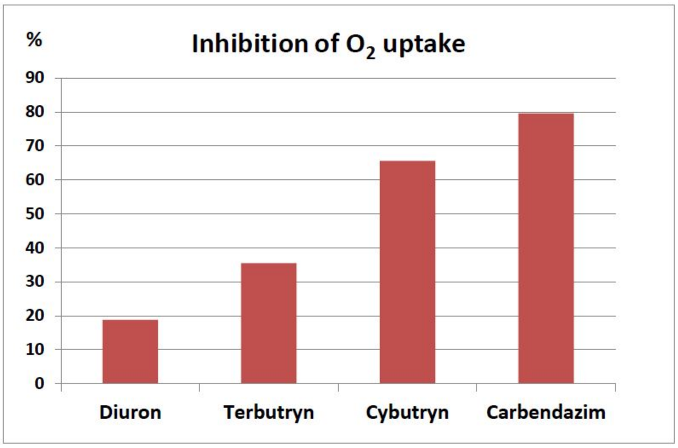


Figure 5.2: The amounts of the oxygen uptake inhibition for each spiked sample.

5.1.2 Inhibition by Different Concentrations of Cybutryn

The slopes of the oxygen uptake curves from different concentrations of Cybutryn are presented in table 5.2. This table also includes the rates from the continuation experiment test 3, which will be discussed in section 5.1.3.

In general the rates seemed to indicate that inhibition happened to some extent with all concentrations of Cybutryn. The plotted results are however presented separately in figure 5.3 on the next page. When comparing figures 5.3(a) and 5.3(b), the oxygen uptake of the sample with 5 mg/L biocide content seems indeed slower. However there are some discrepancies in the results, since the uptake rates do not grow linearilly when the biocide concentrations decrease (table 5.2). This might be due to natural variation in the sediment, and using duplicates or triplicates instead of only one sample might yield more consistent results. Also higher concentrations could be used to see more clearly the possible difference between the dosings.

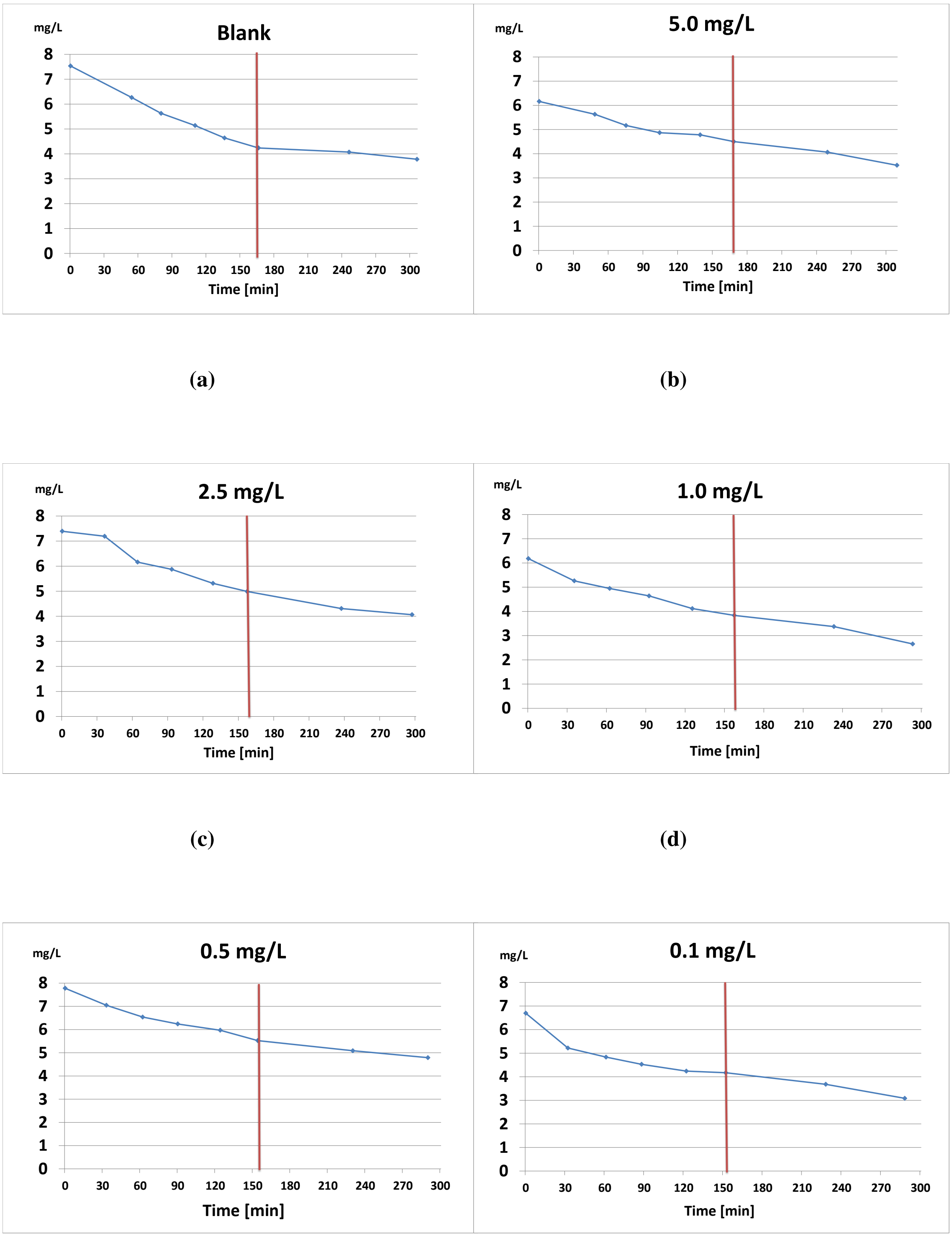
Table 5.2: Decrease in oxygen concentration in the Cybutryn sample vessels as concentration over time.

Rate -[C/t]	Blank	5 mg/L	2.5 mg/L	1 mg/L	0.5 mg/L	0.1 mg/L
Dark	0.02	0.0098	0.0164	0.0143	0.0138	0.015
Daylight	0.0032	0.0069	0.0067	0.0086	0.0054	0.0079
Total slope	0.0116	0.008	0.0117	0.011	0.098	0.0101

As Cybutryn is a herbicide with a known algaecidal qualities, it seems highly likely that the lower oxygen consumption in spiked samples was due to some inhibition of the micro-organisms. Its EC_{50} concentration for algae is as low as 2μg/L, and the used dosing 5 mg/L falls into the range of EC_{50} for bigger organisms (Dapnhia Manga) (see table 3.1.3 on page 11). If Cybutryn has effect on the degrading organism however remains unclear.

5.1.3 Inhibition of Photosynthetic Activity

Test 2 was continued as test 3 by uncovering the samples from tinfoil and placing them in daylight. The results did not show any increase in oxygen concentrations. However, a change can be seen in the rate of oxygen uptake rates before and after the exposure, as the slope levels out and oxygen uptake seems to get slower, as seen in table 5.2.



(a)

(b)

(c)

(d)

(e)

(f)

Figure 5.3: The decrease in O_2 concentration [mg/L] over time in the test samples. The red line depicts the samples being moved from the dark to daylight.

If these two measurements are plotted as continuum to the measurements from test 2, the slope of the total oxygen uptake curve (test 2 + test 3) decreases to some extent in all the samples (see table 5.2 on page 27). The curves are depicted in figures 5.3(a)-5.3(f). When looking at the graphs, the change is only clear in the the blank sample (figure 5.3(a)) and the sample with initial concentration of 0.5 mg/L (figure 5.3(e)).

The leveling out of the slope could refer to the daylight having some effect and the oxygen production slowly starting again. This again indicates that the algae in none of the samples was killed off completely. However, it must be noted, that the weather conditions at the time were not ideal, as there was no direct sunshine. In addition, it would be interesting to follow the development for a longer period of time to see if the oxygen concentrations would increase again.

5.1.4 General Discussion

There are some general uncertainties common to all the inhibition experiments. First, it was difficult to get the water in the vessels fully saturated, and this is why the initial level of saturation is different for all samples. Therefore the curves are not directly comparable, but the rates are comparable as the oxygen concentrations were much above the growth limiting conditions.

Second, in order to get more reliable results when using such small amounts of sediment, the sediment should be thoroughly homogenised. Also each sample should be triplicated or at least duplicated, and the results inspected as mean values of the replicates. Now in the lack of comparison, it is not possible to know if there has been any disturbance in the samples that might have endangered the results. Third, as the biocides were in solution in acetonitrile, one more sample spiked with only acetonitrile should be included to be completely sure of the effect of the solvent itself.

It can in any case be concluded, that the experiments indicate some level of inhibition by all the biocides. Carbendazim was clearly the greatest inhibitor, but when looking at only the three biocides used in the study at hand, Cybutryn seemed to exhibit the largest impact on oxygen uptake.

5.2 Sorption and Degradation in Suspended Sediments

The sorption in suspended sediments has previously been shown to reach equilibrium in 24 hours [Rudelle, 2014]. Therefore the first subsamples in tests 4A and 4B were taken 24 hours after the samples had been spiked with the biocides.

The results from tests 4A and 4B are in line with findings from sorption experiments by Rudelle [2014]. The expected curve of the plots was demonstrated in the methods with figure 4.3 on page 16, where the concentration in each sample falls drastically in the first 24 hours, after which the rate slows down. This phenomenon can also be seen from the plotted results in figure 5.6 on page 33 and figure 5.7 on page 35.

The rates of reduction for both sorption and degradation were defined by the regression line of the curves. A complete listing of the rates is given in table B.1 on page A3 (sorption) and B.2 on page A3 (degradation) in the Appendix. Also all the measurements of concentration done during the experiments are presented in figures B.1 on page A5 and B.2 on page A6.

5.2.1 Test 4A

In test 4A, sorption accounted for approximately 30 % decrease in Diuron concentrations to 60-70 % for Terbutryn and Cybutryn. As can be seen in table 5.3, the sorption rate was slightly higher in the samples containing Aarhus sediment. This supports the assumption of the Aarhus pond receiving more organic matter, since a higher organic content should correlate with higher sorption [Wauchope et al., 2002].

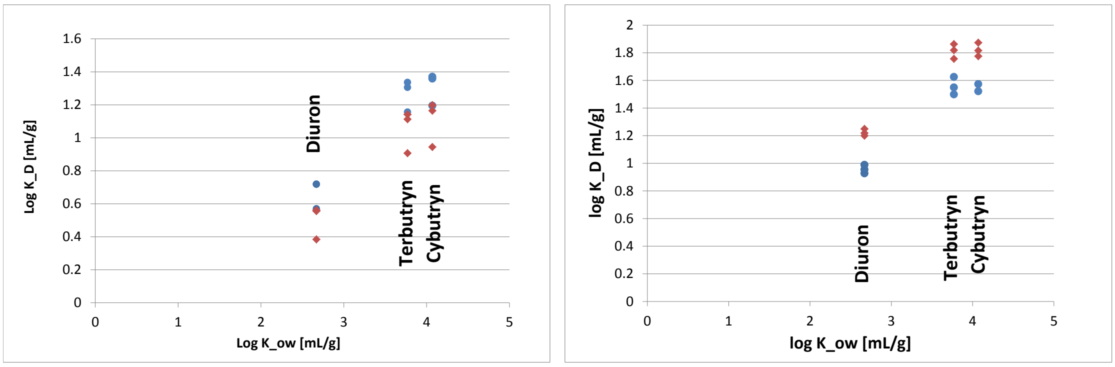
Table 5.3: The average reduction rate RR in % in the biocide concentrations for Aarhus (Aa) and Silkeborg (S) sediments by sorption in the first 24 hours, as well as the percentage of the Aarhus reduction rates of those in Silkeborg.

	Test 4A	m_{sed} =	80 g	Test 4B	m_{sed} =	55 g
	RR Aarhus	RR Silkeborg	$\frac{Aa}{S}$ ratio	RR Aarhus	RR Silkeborg	$\frac{Aa}{S}$ ratio
Diuron	32 %	28 %	114.3 %	46 %	55 %	84 %
Terbutryn	65 %	58 %	112.1 %	77 %	83 %	93 %
Cybutryn	67 %	60 %	111.6 %	77 %	83 %	93 %

Table 5.4: The amount of Dry Matter (DM) and Organic Matter (OM) of each sediment sample in Tests 4A and 4B.

		Test	4A			Test	4B	
	Mass [g]	DM [g]	OM [g]	OM [g/kg TS]	Mass [g]	DM [g]	OM [g]	OM [g/kg TS]
Aarhus 5	80.00	51.04	1.38	42.0	55.65	28.27	1.19	27.0
Aarhus 2.5	80.19	51.16	1.38	42.0	55.35	28.12	1.18	27.0
Aarhus 1	80.56	51.40	1.39	42.0	55.51	28.20	1.18	27.0
Silkeborg 5	80.02	60.34	1.27	28.0	56.00	22.29	0.62	21.0
Silkeborg 2.5	80.60	60.77	1.28	28.0	55.67	22.16	0.62	21.0
Silkeborg 1	80.55	60.73	1.28	28.0	55.62	22.14	0.62	21.0

The results are also in line with the biocide log K_{ow} values. Diuron has the lowest log K_{ow} value of the three, and is thereby expected to sorb the least. Figure 5.4 shows the dependency of the log value of the distribution coefficient K_D on the log K_{ow} value of the biocides in both tests 4A and 4B. Figure 5.5 on the other hand compares the dependency of the organic matter (log K_{oc}) and the log K_{ow} values. Both log K_D and log K_{oc} values were inversely proportional to the initial concentration, with the highest sorption in most cases witnessed in the samples of 1 mg/L start concentration.

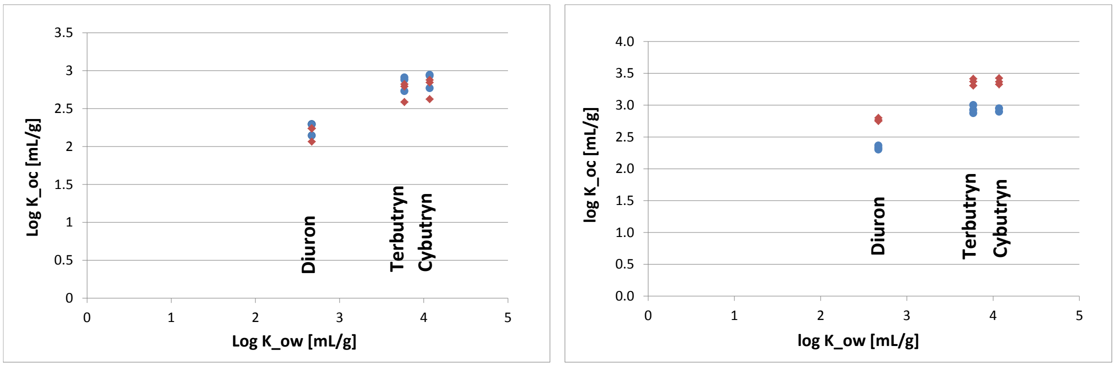


(**a**) Log K_D vs log K_{ow} 4A

(**b**) Log K_D vs log K_{ow} 4B

Figure 5.4: Comparison of the calculated log K_D values of the samples to the log K_{ow} values of the biocides. Blue colour indicates Aarhus sediments and red colour Silkeborg sediments.

The variation of sorption is larger with Terbutryn and Cybutryn, but a general trend of lower rates for Diuron is evident. The sorption rates were also dependent on the initial concentration of the biocide. The rates of reduction in % with the initial and final biocide concentrations for all samples are listed in table B.3 on page A4 of the Appendix.



(**a**) Log K_{oc} vs log K_{ow} 4A

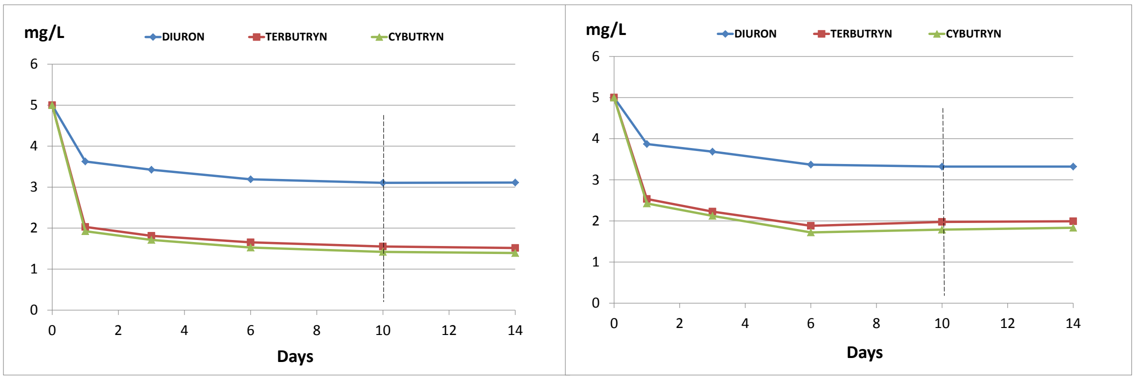
(**b**) Log K_{oc} vs log K_{ow} 4B

Figure 5.5: Comparison of the calculated log K_{oc} values of the samples to the log K_{ow} values of the biocides. Blue colour indicates Aarhus sediments and red colour Silkeborg sediments.

The headspace of the sample vessels was assumed to be sufficient, and therefore no additional aeration was performed. However, the odour from the samples indicated that some of the sediments had turned anaerobic somewhere between days 6 and 10. After this the samples were aerated in attempt to restore the oxic conditions. Two subsamples were taken after the samples became anaerobic, and the concentrations showed an increasing trend instead of decreasing. This is possibly due to the change in redox-conditions leading to desorption of biocides. This however did not occur in all the samples and to a lesser extent with Diuron. The effects of the anaerobic conditions on some of the samples can be seen in the plots in figure 5.6 on the next page.

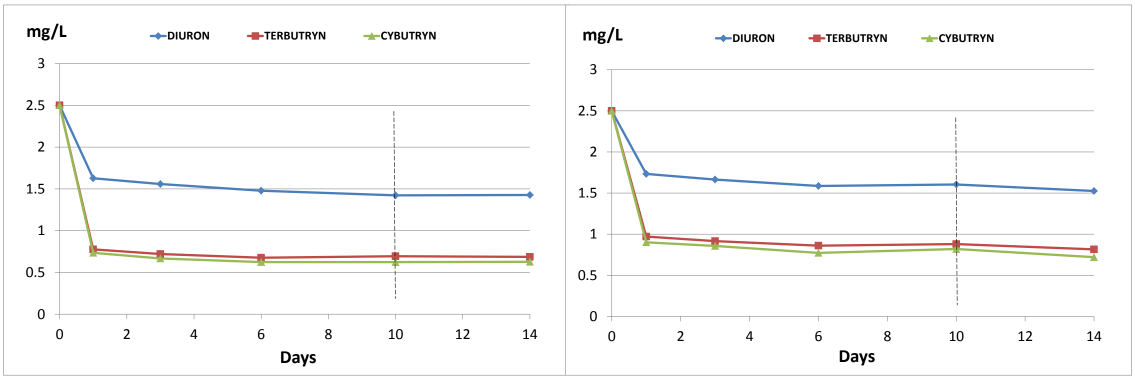
The disturbance caused by the oxygen deprived conditions varied from sample to sample, and also differences between different compounds were seen. For example, on the first day anoxia was noticed (day 10), the concentrations of Cybutryn became higher in five bottles out of six (as an example figure 5.6(b)). However at the same time, the Diuron concentrations in all bottles continued decreasing as before, see figure 5.6 on the facing page. There was no clear pattern as to when, which compound, pond or concentration the lack of oxygen would affect.

After the initial drop caused by sorption, the results from test 4A show very slow degradation during the first week. During the experiment, the fall in concentrations by degradations varied more depending on the initial concentration than the sorption did. Degradation accounted for approximately 15 % decrease for Diuron, 20 % for Terbutryn and 25 % for Cybutryn (table B.3 in Appendix). Also the degradation seemed to be slightly higher in the Aarhus sediments. The numbers however are not completely reliable due to the anoxic conditions causing increased concentrations in the last subsamples.



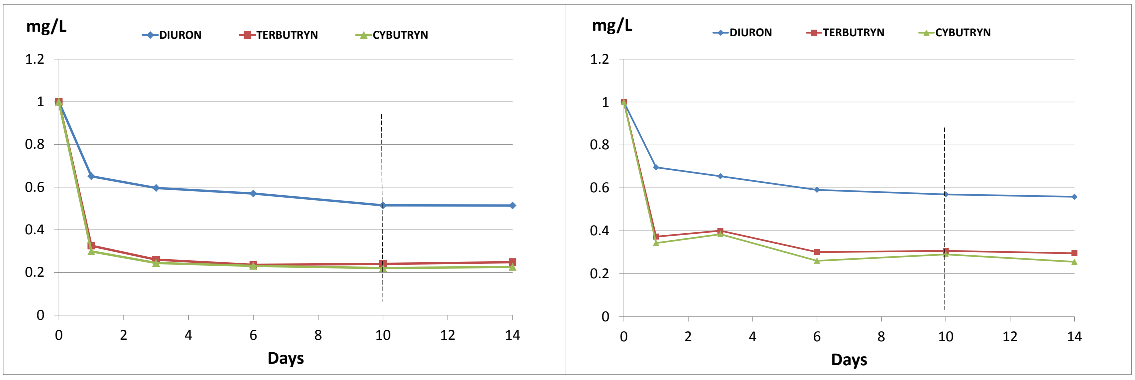
(a) Aarhus 5 mg/L

(b) Silkeborg 5 mg/L



(c) Aarhus 2.5 mg/L

(d) Silkeborg 2.5 mg/L



(e) Aarhus 1 mg/L

(f) Silkeborg 1 mg/L

Figure 5.6: The decrease in biocide concentration [mg/L] over time in suspended sediment samples of Test 4A. Any missing datapoints are caused by an invalid result from the HPLC-analysis. The dashed line marks the occurence of the anaerobic conditions and start of daily aeration.

5.2.2 Test 4B

Because of the samples turning anaerobic in test 4A, the amount of sediment was reduced for test 4B in order to mobilize and to reach a better penetration of oxygen. Also the water volume was reduced by 200 mL, leaving more headspace. As precaution the samples were also cautiously aerated.

Similarly to test 4A, the concentrations in the samples in test 4B kept decreasing slowly after the initial fall caused by sorption, as seen in figure 5.7 on the next page. This time however, the rate of sorption seemed to be higher in samples from Silkeborg, as can be seen from the rates in table 5.3 on page 30. Same can be observed in figure 5.4(b) on page 31. The dependance of sorption on organic matter seems to be likewise reversed in test 4B, where Silkeborg sediments showed higher sorption despite their lower OM content.

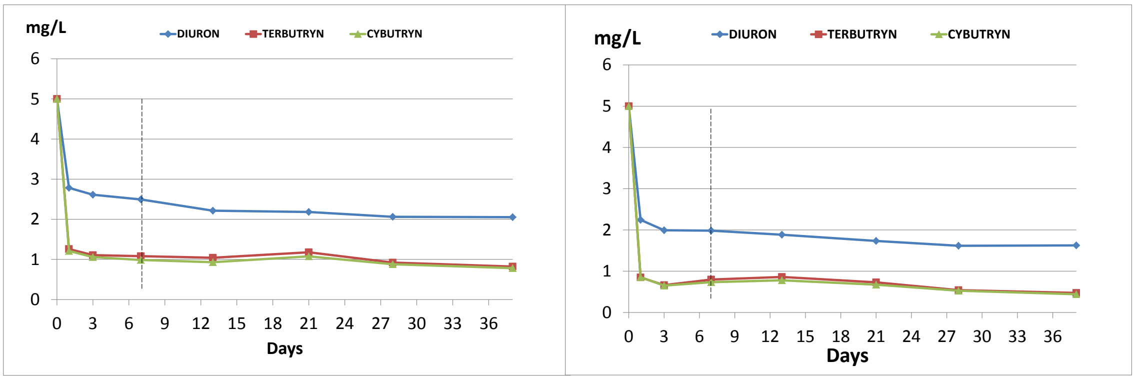
A possible explanation for this could be the amount of total solids (TS) in the sediment. When looking at the results from the dry matter determination (see table 5.4 on page 30), it seems that the TS content of Silkeborg sediments was higher in test 4A and lower in test 4B. This might cause the sediments with less TS weight to become more easily suspended, allowing more particle area for adsorption. This is also supported by the average sorption rate in % being higher during test 4B (table 5.3 on page 30), even though the total amount of sediment, and thereby the total amount of organic matter, was considerably less than in the test 4A (table 5.4 on page 30).

The total and relative amount of organic matter, however, in both tests was considerably higher in Aarhus sediments. It has also been studied that besides organic matter, also clay particles can act as effective adsorbant [Kah, 2007]. It is possible the Silkeborg sediments used in test 4B contained some clay that is used as isolation material in the pond, and therefore increase adsorption. It was tried to avoid including clay in the samples, but because of the thin sediment layer, the core samples containing some clay particles cannot be ruled out.

The efforts to aerate the samples proved to be too feeble, as some of the samples showed signs of anaerobic conditions after day 5 also in test 4B. The aeration was continued with a higher airflow on a daily basis and the experiment was carried on until day 38. In addition, the column for the HPLC was changed to a new one during the experiment, and the results for days 21, 28 and 38 might therefore be not directly comparable to results before that.

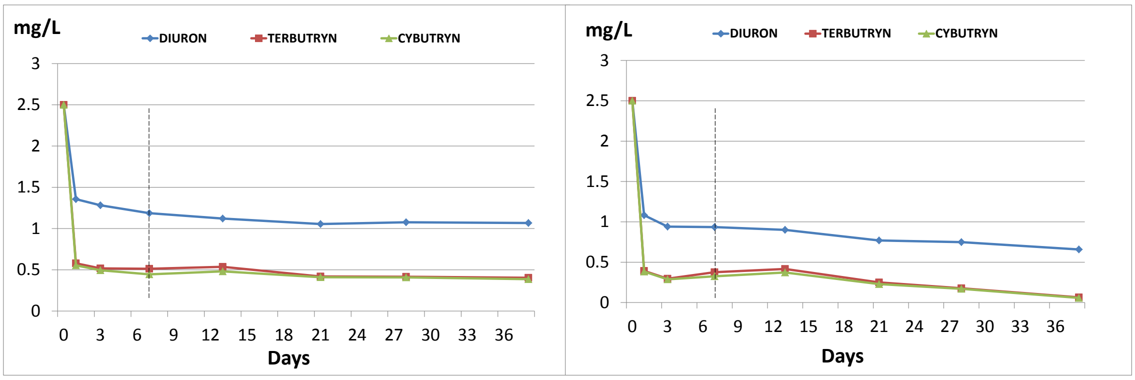
An explanation as to why the oxygen was used up after about a week, but not earlier, might be that the dosing of biocides may have initially killed some of the bacteria. However, those more resistant to the biocide survived and would multiply, hereby increasing the oxygen consumption. This should also result in the reduction of the biocides becoming faster, which was not the case. This again could be explained by the insufficient oxygen levels possibly causing desorption, which in turn would reverse the effect of degradation.

Similarly to test 4A, the results do not show a consistent pattern on which sediment or biocide is affected by the anoxia. After noticing the anoxic conditions, the concentrations of Terbutryn and Cybutryn still seemed to increase instead of decreasing in some of the vessels. For an example see days 13 in figure 5.7(b), where the concentrations of Terbutryn and Cybutryn still seem to increase after a week of daily aeration. In the case of Aarhus sample with initial concentration 5 mg/L (figure 5.7(a)), the first rise in Terbutryn and Cybutryn concentrations happened only after two weeks of daily aerating. By this the conditions would have been assumed to be aerobic.



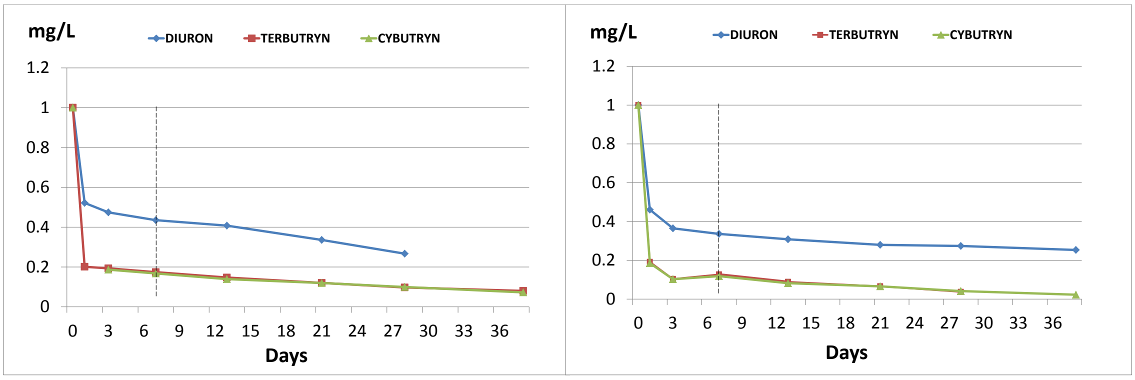
(a) Aarhus 5 mg/L

(b) Silkeborg 5 mg/L



(c) Aarhus 2.5 mg/L

(d) Silkeborg 2.5 mg/L



(e) Aarhus 1 mg/L

(f) Silkeborg 1 mg/L

Figure 5.7: The decrease in biocide concentration over time in suspended sediment samples of Test 4B. Any missing datapoints are caused by an invalid result from the HPLC-analysis. The dashed line marks the occurence of the anaerobic conditions and start of daily aeration.

There can be multiple explanations to such differences. One natural reason is that the compounds react differently to changes in redox conditions. Second, even though the sediment was mixed with the goal of homogenizing it, differences can still prevail between different bottles containing the same sediment, facilitating different growth patterns of the organisms. Another explanation is the possibility of fine particulate matter ending up in the samples despite centrifuging. The sediments seemed to become lighter and the particulate matter finer with continued shaking. Figure 5.8 shows beakers containing the water and sediment from the sample vessels after the experiment. On the time the picture was taken, the samples had been settling for one day, but some of them are nevertheless very turbid. This shows the increase in hard-to-settle fine particulate matter after long periods of shaking.

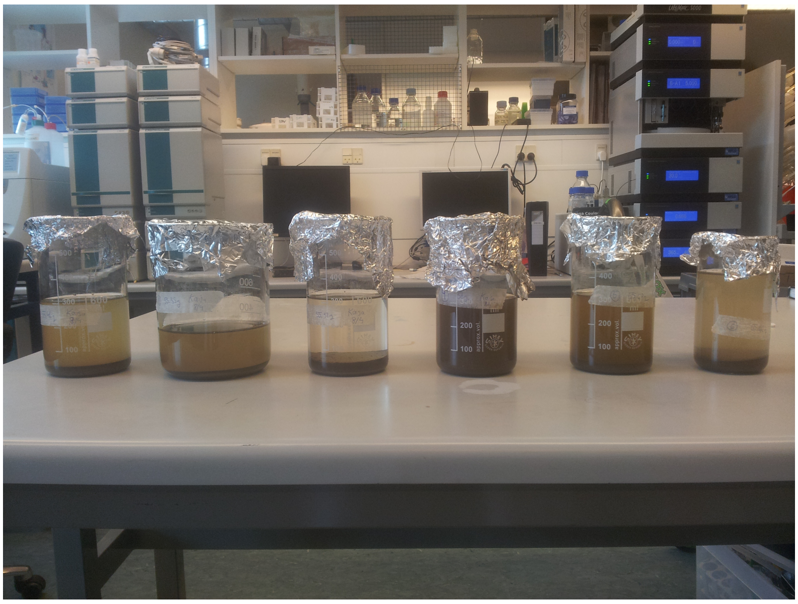


Figure 5.8: The samples of test 4B after 24 hours of settling. The samples are in decreasing order by concentration, with Aarhus sediments on the left and Silkeborg on the right.

The reduction of the biocides by degradation varied as much as 20-50 % for Diuron, 35-80 % for Terbutryn and 35-90 % for Cybutryn, with the higher end of the values found in Silkeborg samples and most often increasing with the decreasing start concentration. The exact values for each sample can be seen in table B.3 on page A4 in the Appendix.

Another way of looking at degradation is DT_{50}, the half-live values of the biocides. Table 5.5 presents the half-lives for those compounds that had reached 50 % of the concentration at equilibrium. Test 4A was too short-lasting for this to happen in any sample and is therefore left out of the table.

The results from test 4B show the half-life time for Terbutryn ranging from 11 to 27 days and to Cybutryn from 11 to 25. This indicates the degradation is dependent on the initial concentration, as the shortest DT_{50} is found in a sample with the lowest initial concentration. At the same time no half-lives were found for the samples with the highest concentration. As the amount of biomass in each sample is assumed to be approximately the same, it is natural higher concentrations will be degraded slower. However as there is no apparent microbial population growth that would compensate for the difference, it could be assumed the maximum degrading capacity of the sediment biomass has been reached.

As the experiment was made in suspension, the half-life of Terbutryn is less than half of that given by IUPAC [2013b] (60 days), which indicates of the difference in the rate of degradation between suspended and still sediments. It must be also noted, that the Silkeborg 1 mg/L was affected by the anoxic conditions causing the concentrations to rise. Had this not been the case, it seems very likely the DT_{50} values had been even shorter than they are now. No data was found for the expected DT_{50} for Cybutryn.

Diuron not reaching the half concentration even in a longer time period points to it being slowly biodegradable. According to IUPAC [2013a], the DT_{50} of Diuron is 48 days and thereby shorter than that of Terbutryn. The results also seemed to indicate that higher rate of sorption might facilitate a higher rate of degradation. As was mentioned earlier, the samples containing Aarhus sediments had lower sorption. According to the half-lives they also seemed to have notably less potential for degradation, with Terbutryn reaching its 50 % concentration in only one sample. Additionally, Diuron was sorbed the least of all the biocides, and did not reach its DT_{50} value in any sample. On the other hand, Terbutryn and Cybutryn showed higher sorption throughout the experiments and were the only ones to reach some of their 50 % concentrations. This is however only indicative, and to be able to conclude on the connection would require more tests with more compounds.

Table 5.5: The half-lives of the biocides in each sample in test 4B. A dash (-) indicates DT_{50} was not reached. Sample marked with x was missing analysis results so it was not possible to determine the half-life for Cybutryn.

DT_{50} [d]	Aarhus 5	Aarhus 2.5	Aarhus 1	Silkeborg 5	Silkeborg 2.5	Silkeborg 1
Diuron	-	-	-	-	-	-
Terbutryn	-	-	26	-	27	11
Cybutryn	-	-	x	-	25	11

It can be concluded that the sediments in both Aarhus and Silkeborg ponds clearly show potential for biodegrading the incoming biocides, although this is a slow process. The rates of degradation showed more variation between samples due to the heterogenous distribution of biomass. In general the degradation rates seemed to grow in the same order as sorption, with Diuron degrading the least and Cybutryn and Terbutryn decreasing notably faster, both more or less at the same rate. The potentials of the different sediments after one week in test 4A were more or less equal.

However as the degradation period is prolonged, as was the case in test 4B, Silkeborg showed higher capability for degradation. No definite conclusions cannot be drawn, since the results from DM determination show higher OM for Aarhus. However it seems in any case that in suspension a lesser amount of sediment plays a significant role in increased sorption as witnessed by higher sorption in test 4B with less sediment in general compared to test 4A. A higher rate of sorption also seemed to facilitate higher degradation. Series of new long lasting experiments are in order to confirm the difference in potentials.

5.3 Sorption of Biocides to Intact Sediment Cores

Possibly due to the difficulties in defining the exact water level in the cores, there was an error with the initial concentration of the biocides. The calculated concentration 2.5 mg/L was trusted to be correct and no samples were analysed immediately after spiking the cores. However, the concentrations in the subsamples one hour after spiking were in some cases as high as 4 mg/L (table 5.6), when they should have been < 2.5 mg/L. Therefore the initial concentrations at t = 0 were not known and have been excluded from the result plots.

As was discussed in section 5.2, the sorption in suspension reached equilibrium in 24 hours, but degradation was rather slow. It can thereby as a first approximation be assumed, that the decrease in biocide concentrations in the intact cores is caused by sorption only.

Sorption kinetics are often presented in isotherms, such as the Freundlich adsorption isotherm, which takes into account certain parameters of the process at equilibrium [U.S.EPA, 2014]. As it was not possible to carry on the experiment long enough to establish the equilibrium, no isotherm could be created. Hence the results are the presented as decrease of concentration in the waterphase, as seen in figures 5.10(a)-5.10(f).

Similarly due to lack of appropriate data at the time, it was not possible to calculate the log K_D or log K_{oc} values, as was done for experiments 4A and 4B. Instead, the % reduction in biocide concentration is used in comparison with the biocide log K_{ow} values (figure 5.9). This is not the ideal case, since the percentage of reduction is dependent on the mass of sediment and organic matter adsorbing it. However not knowing the weights, the approximate volumes of sediment in each core is used as a reference parameter for the amount of reduction, as shown in table 5.6.

Table 5.6: The initial concentrations c_i at hour 1 and final biocide concentrations c_f at hour 384 (day 16) in the waterphase. The rates of reduction (RR) by sorption for each sample is given in %. For average values for each core, see table B.4 on page A4.

		Aarhus 1	Aarhus 2	Aarhus 3	Silkeborg 1	Silkeborg 2	Silkeborg 3
V_{sed}	[mL]	196	255	137	118	157	167
Diuron	c_i [mg/L]	4.06	3.95	2.98	3.07	2.20	3.04
	c_f [mg/L]	0.90	0.85	0.75	0.85	1.64	0.98
	RR [%]	77.70	78.38	74.83	72.42	25.48	67.92
Terbutryn	c_i [mg/L]	3.67	3.52	2.70	2.82	2.04	2.65
	c_f [mg/L]	0.38	0.28	0.26	0.32	0.98	0.40
	RR [%]	89.62	92.17	90.25	88.58	51.96	84.70
Cybutryn	c_i [mg/L]	3.64	3.50	2.69	2.81	2.03	2.59
	c_f [mg/L]	0.35	0.25	0.24	0.30	0.95	0.38
	RR [%]	90.27	92.94	91.04	89.24	53.43	85.30

Similarly to the results from the suspension experiments, Diuron showed lowest sorption, whereas the sorption rates for Terbutryn and Cybutryn were higher and almost alike. Even though the core samples were collected in different locations in the ponds, the sorption curves in different samples from Aarhus follow a similar trend, whereas those from Silkeborg show more variation, albeit being more linear.

The reduction in the concentrations does not seem to be related to the volume of the sediment in the core, especially so in the cores from Silkeborg (table 5.6). A possible explanation to this might be the amount of clay contained in the cores. As was explained if section 5.2.2 on page 34, clay particles also enable sorption, and in Silkeborg the sediment over the clay layer can be only a few centimeters deep. Therefore the inclusion of clay in Silkeborg cores is highly likely, and the differences of sorption between the cores might be possibly due to variation of the amount of clay.

The reduction rates (5.6) by sorption in the intact cores seemed to be considerably higher, than those witnessed in the suspension experiments (table 5.3 on page 30). Also the concentrations in the end of the experiment were lower than those after 24 hours in the suspension experiment, when sorption was assumed to have reached equilibrium. This can be because cores contain more sediment than the suspension vessels, and can therefore facilitate greater sorption. Also as mentioned before, the possible inclusion of clay an also increase the soprtion. All the analysis results of concentrations during the sorption experiment are presented in table B.3 on page A7.

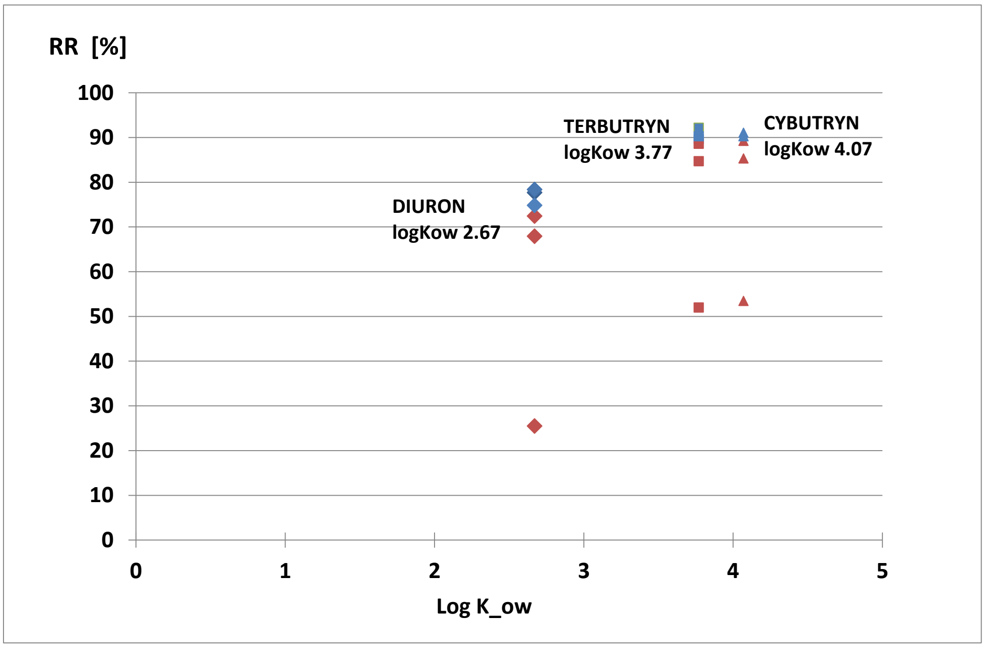
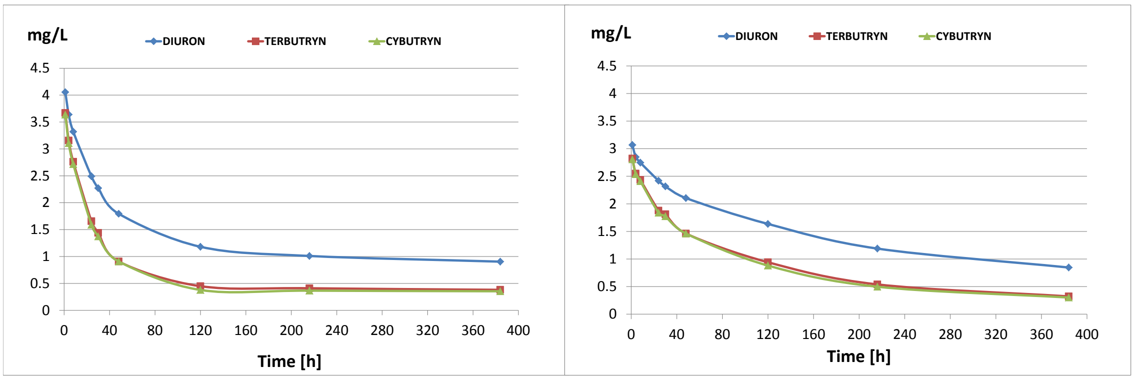


Figure 5.9: Reduction rate % of each biocide related to the compounds log K$_{oc}$ value. Blue colour indicates Aarhus sediments and red Silkeborg sediments.

The difference between the sorption of the compounds becomes clear, when relating their log K$_{ow}$ values to sorption rates, as seen in figure 5.9. The same trend could also be observed with sorption in suspension in section 5.2.

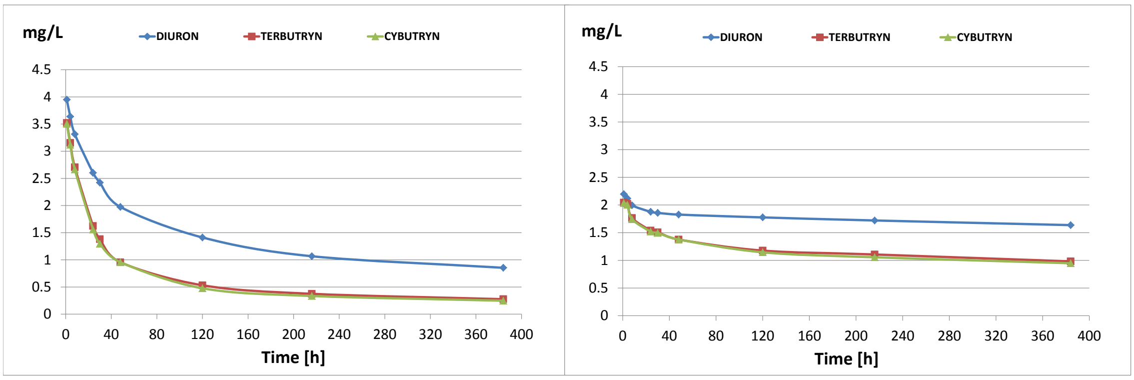
In average it can be summarized, that higher sorption occurred in the samples from Aarhus due to their assumably higher organic content. The Silkeborg 2 sample, however, exhibited lower sorption than the other cores from Silkeborg (table 5.6). This might be because explained by the sampling location closest to the inlet, but in an area on the side of the outlet pipe where there was possibly less build-up of organic matter. For technical reasons it is not possible to control the amount of sediment during sampling, so the different sediment amounts in the cores can also cause differences in sorption between the cores.

The evaporation caused by aeration could have been compensated for by adding water. This was deemed too difficult to do precisely enough, since measuring the water level already was challenging due to the non-transparent material. At the same time care should be taken not to overcompensate and fill in for the water taken out in subsamples, ending up diluting the solution.



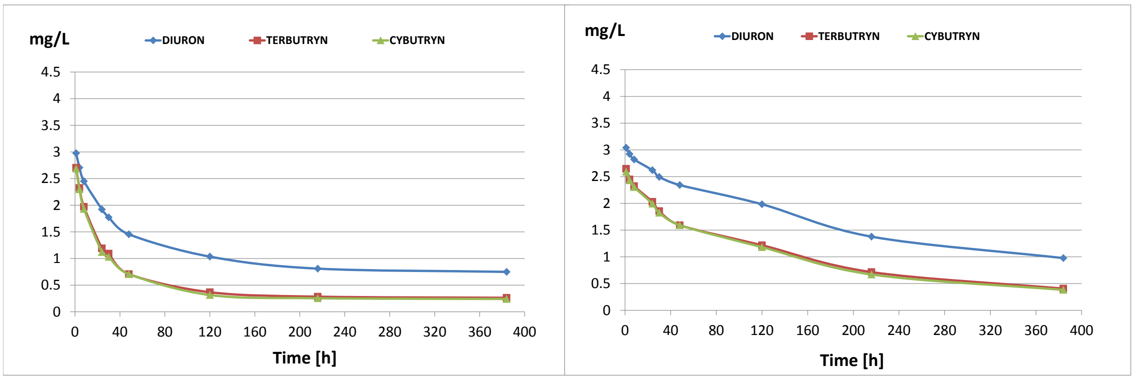
(a) Aarhus 1

(b) Silkeborg 1



(c) Aarhus 2

(d) Silkeborg 2



(e) Aarhus 3

(f) Silkeborg 3

Figure 5.10: The decrease in biocide concentration over time in intact sediment samples of Test 5.

6 Discussion

The inhibition studies seemed to show some changes in the uptake of oxygen when the samples were spiked by biocides. However a major drawback in these inhibition experiments was that it could not be distinguished whether it is algae, fungi or bacteria that gets inhibited. To make the test more reliable, triplicate, or at least duplicate samples of each biocide, as well as the blank samples, should be used, and the sediments thoroughly homogenised. The exposure of the samples to daylight in test 3 should have maybe been continued for a longer time to see if the difference in oxygen uptake rates would become more obvious. It might also be of interest to carry on two similar inhibition experiments, one in daylight and one in darkness and compare the results for photosynthesis inhibition.

The results from both suspension tests 4A and 4B show that Diuron has clearly the lowest rate of sorption, and therefore had higher concentrations also after degradation. This is again in line with findings made by Rudelle [2014], and relates also to the log K_{ow} values of the biocides. Terbutryn and Cybutryn had very similar reduction curves for both sorption and degradation. When looking at the numbers, Cybutryn was the fastest to adsorb, which was in line with its high log K_{ow} value. The half-lives for Terbutryn and Cybutryn were also very much alike, whereas degradation of Diuron was too slow for it to reach its DT_{50} concentrations in any occasion. Therefore it would seem the sorption for the used biocides was not strong enough to reduce the bioavailability of the substances, but on the contrary: higher sorption could facilitates the access of the degrading organisms to the biocides.

A slight difference was observed between both adsorption and degradation in sediments from Aarhus and Silkeborg. As the Aarhus pond receives illicit inflow of wastewater, it was assumed to contain more organic matter and thereby have a higher degradation potential. According to the results from the VS determination this seems valid, as the sediments from Aarhus had a higher organic matter content in both tests 4A and 4B. In addition, test 4A shows that the degradation rates for all the compounds in samples with Aarhus sediment were slightly higher than those with Silkeborg sediment.

On the second round however the situation seemed to be the opposite. A possible explanation to this might be the lower dry matter content in the Silkeborg sediment used in test 4B, making it easier to be held in suspension and increasing the amount of particle area for sorption. To minimise the uncertainty caused by any disturbance in the samples, more experiments are required in order to establish a difference between the potential of the two ponds.

Differences between the rates in single samples were at times significant, but due to different initial concentrations they were not directly comparable with each other. As was stated before concerning the inhibition tests, more replicas are necessary to minimize uncertainty and to get a statistically reliable averages of the result. Several repetition rounds alone are not sufficient, for as is seen with the tests 4A and 4B, the rates are quite different with sediments from different dates, even though the sampling areas are the same. It would be interesting to carry out experiments with sediments before and after a storm event for example to see if there is change in the sediment qualities. The differences between the samples can also be seen as demonstrating the heterogenic nature of the pond sediments and the unequal distribution of biomass.

The sediments in both tests 4A and 4B showed signs of anoxia during the first week of experiments. The inhibition test seemed to show slight inhibition at least by Cybutryn, so it is possible they would have effect on the degrading organisms. Nevertheless the inhibition would only be partial, and the population would grow stronger in a few days, causing an increase in oxygen consumption. The effects of anaerobic conditions on the sorption and degradation as such would be an interesting topic for further studies. As for now, it can only be assumed that the sudden increase in biocide concentrations following the lack of oxygen were caused by the biocides starting to desorb from the sediments.

An alternative explanation to the fluctuations in concentrations after the two first weeks can be the particulate matter getting finer as time passes. This is suspected also, because no smell referring to anoxia was noted at that time. This is only true for test 4A, where the sampling was done by double centrifuging as opposed to centrifuging and filtering on the first round. It was noted that after some weeks, the particles in the subsamples seemed to become more difficult to sediment by centrifuge. This could be due to the relatively vigorous shaking of the samples causing the big particles to break into finer material that is harder to settle. If any of this fine matter would end up in the analysis vials, it would naturally increase the concentrations in the given sample. The problem lies in adjusting the shaking, so that the particles would stay intact and the sediments suspended.

The suspension scenario includes several uncertainties that need acknowledgment. One is the ratio of sediment and water, that had to be chosen arbitrarily. The bottles also required additional aeration despite the relatively large headspace in the bottles. Therefore suspension experiments cannot be recommended for long term degradation studies. A microcosm study would have been a more nature-like environment to conduct the study in, but this was excluded for scheduling reasons.

Even though degradation in intact samples is expected to be slower to that in suspension, intact samples are a more natural-like environment and thereby the result likely to be more reliable. The intact core samples also gave more linear results, and due to the continuous aeration, there was no interference of anoxic conditions.

The equilibrium of sorption in suspension was found to occur after 24 hours, but for intact samples it is not known. Therefore more frequent subsampling is in order if the line between sorption and degradation needs to be established. To get more accurate results, glass or other see-through cores should be used. The metal cores were difficult to operate because they were not transparent, which complicates the estimation of sediment and water level, and cause therefore a major source of uncertainty in the results.

Because of the different layers of the intact core sample, no averaging TS or VS determination was made for the sediments in test 5. If the amount of dry matter, organic matter as well as the clay fraction could be found layer by layer in the cores, it would be easier to relate the sorption to the change in parameters in each core. The ideal closing off for the mass balance would be to extract the biocides from the sediment at different depths to see how deep and in how big concentrations the biocides can be sorbed.

7 Conclusions

Considering the amount of biocides used in new surface materials each year, as well as the fact that the leaching is highest when the surfaces are new, it can be concluded the potential effects of urban biocide leaching should not be ignored.

According to the inhibition tests, the dosages of biocides seemed to show a varying range of effect on the sediment oxygen uptake. Cybutryn seemed to have some effect on oxygen uptake also in lower concentrations, but daylight exposure indicated that even the highest concentrations did not eliminate all algae. The inhibition by Diuron, Terbutryn or Cybutryn was not considered great enough to complicate the degradation experiments using the same concentrations as were tested in the inhibition experiments.

In possible future studies more focus should be put on Carbendazim, that was clearly showing the highest inhibition. However, the concentrations used were higher than would occur in the natural environments, and it was not possible to define which micro-organisms got inhibited. Further studies on ecotoxicity with lower concentrations and a pre-determined microbial population would be preferable instead.

A connection between the biocide log K_{ow} value and rate of sorption was confirmed, with Terbutryn and Cybutryn sorbing almost twice as much in suspension as Diuron. However the dependence of sorption on the organic fraction was not unanimously proven. The organic content of Aarhus sediment was determined to be higher than that in Silkeborg, and in two tests out of three, the biocides were adsorbing more efficiently in Aarhus samples. It seemed however that in suspension tests a smaller mass of the total sediments, and thereby better suspension, was more defining for sorption affinity than the sediment organic fraction. In intact cores the sorption was slower, but also seemed to result in a higher percentage of the biocides sorbed, since the cores contained more sediment for the biocides to adsorb to. Possible clay particles in the cores may also have positively affected the sorption.

Based on the results from the suspension experiments it can be summarised that Diuron, Terbutryn and Cybutryn can all be degraded in the pond sediments. The results also seemed to indicate that a higher rate of degradation might be related to a higher level of sorption, however confirming this would require additional studies. The half-lives of Terbutryn and Cybutryn varied according to the initial concentration, with lower concentrations degrading faster. This can possibly be seen as indication of the sediment biomass having reached its maximum degradation capacity in the samples with higher biocide concentrations. However the results are unanimous with the reduction rates in that Terbutryn and Cybutryn seem to be the fastest to break down.

It can also be concluded that the suspension studies includes some uncertainty concerning the parameters such as shaking velocity and amount of mass. Therefore any further investigations on sorption or degradation should be done as microcosm experiments, due to their better resemblance to natural conditions as well as the problematics of sustaining a long term suspension experiment. More experiments are required in order to establish the sorption and degradation potentials of each pond and the relation of sorption to the organic matter content. It is also recommendable to homogenise the sediments and use duplicates or triplicates with each used biocide concentration to reduce the uncertainty. If more intact core experiments are to be done, the cores should be of transparent material for practical reasons, and the sediment in each core analysed for TS, VS and clay fraction afterwards. Ideally, the extraction of biocides in the sediment would be essential to confirm the mass balance.

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Appendix

A Analytical Methods

A.1 Dry Matter DM and Volatile Solids VS

As the sediment is a naturally very wet matter, it is important to distinguish how much of the mass of sediment used in the experiments is actually soil. Therefore a test was carried out to find out the total solids (TS) of the sediments and its partitioning into volatile solids (VS) and fixed solids (FS). TS is equal to the dry matter in the sediment, whereas volatile solids can be considered to present the organic fraction of the sediment.

Three subsamples of each sediment were dried overnight in 105°C and the dry weight compared to the wet weight gives the amount of TS in the sample. Next the amount of VS was determined by loss on ignition in 500°C. The amount in % of total solids (A.1), fixed solids (A.3) and volatile solids ((A.2)) were calculated with equations given below. There was not enough Silkeborg sediment for triplicate samples for test 4B. Therefore the DM determination was done on only one sample from wet sediment, which renders the Silkeborg result for 4B uncertain.

$$\%TS = \frac{(A-B)\times 100}{C-B} \tag{A.1}$$

$$\%VS = \frac{(A-D)\times 100}{A-B} \tag{A.2}$$

$$\%FS = \frac{(D-B)\times 100}{A-B} \tag{A.3}$$

Where

A	Weight of dried sediment + dish	[g]
B	Weight of dish	[g]
C	Weight of wet sample + dish	[g]
D	Weight of residue after ignition + dish	[g]

Table A.1: The amount in % of Total Solids (TS), Volatile Solids (VS) and the total Organic Matter (OM) of the sediments used in Tests 4A and 4B.

Solids	Test 4A		Test 4B	
fraction	Aarhus	Silkeborg	Aarhus	Silkeborg
TS [%]	63.8	75.4	50.8	39.8
VS [%]	2.7	2.1	4.2	2.8
OM/wet sediment [%]	1.72	1.58	2.13	1.11

A.2 Biocide Stock Solution

The samples were spiked with a stock solution mixture including all the three pesticides.The chemicals were produced by Sigma Aldrich (biocides) and Panreac (acetonitrile). The preparation of the 1000 ppm stock was prepared in the the following steps.

1. In a ventilation hood, a 25 mL volumetric flask was placed on a scale with accuracy of 0.1 mg.
2. 25 mg of each biocide powder was weighed to the volumetric flask.
3. With all biocides added, the flask was filled up with acetonitrile to form a 1000 ppm solution and stirred until dissolved.

Because of the very small amounts added into the same beaker, the weight of each biocide was not exactly 25 mg. The precise concentrations are presented in table A.2.

Biocide	M [mg]
Diuron	25.0
Terbutryn	25.3
Cybutryn	25.1

Table A.2: Weight of each biocide compound in prepared the stock solution.

A.3 Comparison of filter and centrifuge

A test was made to see the difference in results depending of whether the samples were centrifuged twice, or centrifuged and filtered. Picture A.1 shows that double centrifugation yields higher concentrations than the filter, because the filter removes finer fractions. The method of centrifuging + filtering was therefore switched to double centrifuging for the second round of experiments.

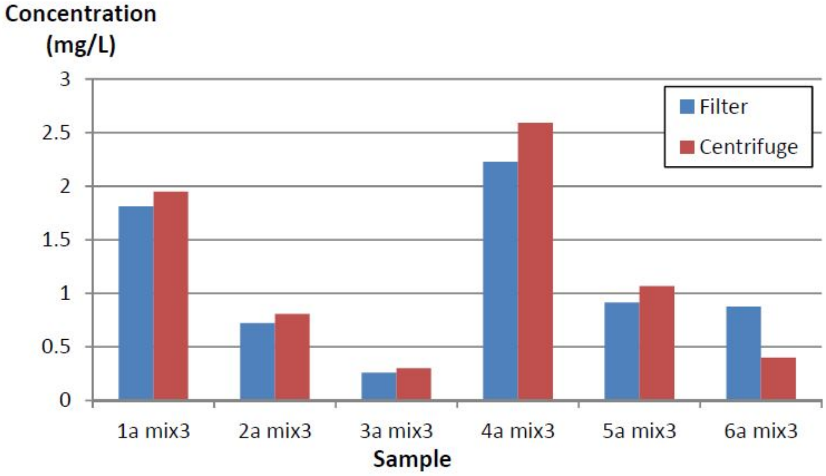

Figure A.1: Differences in concentration when pre-treating by double cetrifuging and filtering.

B Tables and Figures

B.1 Rates for Sorption and Degradation in Suspension

Table B.1: The sorption rates as concentration over time in Tests 4A and 4B. The missing result is caused by an invalid analysis result.

$\frac{[mg/L]}{[d]}$	Test 4A			Test 4B		
	Diuron	Terbutryn	Cybutryn	Diuron	Terbutryn	Cybutryn
Aarhus 5	1.3748	2.97	3.076	2.2174	3.7411	3.7893
Aarhus 2.5	0.8727	1.723	1.765	1.144	1.921	1.9454
Aarhus 1	0.3494	0.675	0.701	0.4781	0.7986	-
Silkeborg 5	1.1309	2.466	2.575	2.7561	4.1515	4.1467
Silkeborg 2.5	0.767	1.529	1.599	1.4176	2.1082	2.1161
Silkeborg 1	0.3041	0.627	0.657	0.539	0.088	0.0825

Table B.2: The degradation rates as concentration over time in Tests 4A and 4B.

$\frac{-[mg/L]}{[d]}$	Test 4A			Test 4B		
	Diuron	Terbutryn	Cybutryn	Diuron	Terbutryn	Cybutryn
Aarhus 5	0.038	0.037	0.039	0.0191	0.0088	0.0083
Aarhus 2.5	0.015	0.006	0.007	0.0072	0.0046	0.0038
Aarhus 1	0.001	0.005	0.005	0.0087	0.0034	0.0032
Silkeborg 5	0.0415	0.036	0.041	0.0153	0.0087	0.0089
Silkeborg 2.5	0.014	0.01	0.012	0.01	0.0082	0.0078
Silkeborg 1	0.01	0.007	0.008	0.0089	0.0044	0.0035

Table B.3: The fall in amount of biocide concentrations in % caused by degradation. The fall in concentration in test 4A happened during days 1-16 and in 4B between days 1-38. Values marked with * are calculated from day 28 instead of 38 and values marked with ** with values from day 3 instead of day 1 because of missing analysis results from the actual dates.

RR degradation	Test 4A			Test 4B		
%	Diuron	Terbutryn	Cybutryn	Diuron	Terbutryn	Cybutryn
Aarhus 5	14.2	25.3	27.7	26.3	34.7	35.3
Aarhus 2.5	12.3	11.6	14.6	21.3	30.4	30.3
Aarhus 1	21.0	23.7	24.3	48.9 *	60.2	61.2 **
Average	15.8	20.2	22.2	32.1	41.8	42.3
Silkeborg 5	14.2	21.4	24.4	27.5	44.3	48.2
Silkeborg 2.5	12.0	16.0	20.0	39.2	83.5	85.0
Silkeborg 1	19.8	20.7	25.5	45.0	80.6 *	87.7
Average	15.3	19.4	23.3	37.3	69.5	73.6

B.2 Rates for Sorption in Intact Sediment Cores

Table B.4: The fall in amount of biocide concentrations by sorption in the intact cores. The values are calculated as the difference between concentrations at hour 1 and hour 384 (day 16).

RR %	Diuron	Terbutryn	Cybutryn
Aarhus 1	77.69525001	89.61953719	90.2654031
Aarhus 2	78.37631128	92.17021898	92.94090678
Aarhus 3	74.82691194	90.25246819	91.04266877
Average	77.16244341	90.70026443	91.43171606
Silkeborg 1	72.41783329	88.57829475	89.24393735
Silkeborg 2	25.48322513	51.95906622	53.4304128
Silkeborg 3	67.91721375	84.69573	85.29904688
Average	63.65183373	80.2571079	81.0860121

B.3 HPLC Analysis Results

Figure B.1: Analysis results of suspension experiment 4A. The results are presented as [mg/L].

DIURON

DAY	Aarhus 5	Aarhus 2.5	Aarhus 1	Silke 5	Silke 2.5	Silke 1
0	5	2.5	1	5	2.5	1
1	3.625	1.627	0.651	3.869	1.733	0.696
3	3.424	1.558	0.596	3.684	1.664	0.654
6	3.191	1.478	0.570	3.369	1.586	0.591
10	3.106	1.423	0.514	3.322	1.605	0.570
14	3.112	1.428	0.514	3.320	1.526	0.558

TERBUTRYN

DAY	Aarhus 5	Aarhus 2.5	Aarhus 1	Silke 5	Silke 2.5	Silke 1
0	5	2.5	1	5	2.5	1
1	2.030	0.777	0.325	2.534	0.971	0.373
3	1.812	0.721	0.260	2.226	0.916	0.400
6	1.652	0.676	0.236	1.881	0.861	0.301
10	1.550	0.695	0.240	1.975	0.880	0.307
14	1.515	0.687	0.248	1.991	0.816	0.295

CYBUTRYN

DAY	Aarhus 5	Aarhus 2.5	Aarhus 1	Silke 5	Silke 2.5	Silke 1
0	5	2.5	1	5	2.5	1
1	1.924	0.735	0.299	2.426	0.901	0.343
3	1.711	0.668	0.244	2.122	0.858	0.384
6	1.527	0.625	0.231	1.720	0.772	0.260
10	1.421	0.624	0.221	1.788	0.821	0.290
14	1.392	0.628	0.226	1.834	0.721	0.255

Figure B.2: Analysis results of suspension experiment 4B. The results are presented as [mg/L].

DIURON

DAY	Aarhus 5	Aarhus 2.5	Aarhus 1	Silke 5	Silke 2.5	Silke 1
0	5	2.5	1	5	2.5	1
1	2.783	1.356	0.522	2.244	1.082	0.461
3	2.613	1.282	0.474	1.991	0.941	0.365
7	2.495	1.187	0.435	1.984	0.935	0.336
13	2.215	1.121	0.408	1.884	0.900	0.308
21	2.181	1.055	0.336	1.733	0.769	0.280
28	2.063	1.077	0.267	1.616	0.749	0.274
38	2.051	1.068		1.626	0.658	0.253

TERBUTRYN

DAY	Aarhus 5	Aarhus 2.5	Aarhus 1	Silke 5	Silke 2.5	Silke 1
0	5	2.5	1	5	2.5	1
1	1.259	0.579	0.201	0.849	0.392	0.192
3	1.105	0.517	0.193	0.661	0.296	0.104
7	1.081	0.512	0.174	0.797	0.376	0.127
13	1.041	0.536	0.147	0.860	0.417	0.089
21	1.178	0.419	0.120	0.729	0.249	0.066
28	0.922	0.415	0.097	0.541	0.177	0.037
38	0.823	0.403	0.080	0.473	0.065	

CYBUTRYN

DAY	Aarhus 5	Aarhus 2.5	Aarhus 1	Silke 5	Silke 2.5	Silke 1
0	5	2.5	1	5	2.5	1
1	1.211	0.555		0.853	0.384	0.185
3	1.059	0.495	0.187	0.650	0.287	0.103
7	0.986	0.445	0.168	0.735	0.325	0.118
13	0.929	0.481	0.139	0.779	0.372	0.082
21	1.075	0.410	0.120	0.674	0.229	0.066
28	0.880	0.408	0.100	0.527	0.171	0.041
38	0.783	0.387	0.073	0.442	0.058	0.023

Figure B.3: Analysis results of the core experiment test 5. The results are presented as [mg/L].

DIURON

DAY	AARHUS 1	AARHUS 2	AARHUS 3	SILKEBORG 1	SILKEBORG 2	SILKEBORG 3
0					2.500	
1	4.056	3.949	2.978	3.066	2.197	3.041
4	3.639	3.638	2.705	2.849	2.119	2.923
8	3.320	3.314	2.451	2.747	1.995	2.821
24	2.491	2.602	1.922	2.418	1.877	2.620
30	2.271	2.421	1.774	2.316	1.858	2.493
48	1.795	1.973	1.454	2.104	1.827	2.342
120	1.181	1.412	1.036	1.636	1.776	1.983
216	1.010	1.066	0.810	1.189	1.721	1.377
384	0.905	0.854	0.750	0.846	1.637	0.976

TERBUTRYN

DAY	AARHUS 1	AARHUS 2	AARHUS 3	SILKEBORG 1	SILKEBORG 2	SILKEBORG 3
0					2.500	
1	3.669	3.521	2.703	2.821	2.043	2.646
4	3.156	3.153	2.323	2.549	2.024	2.448
8	2.761	2.705	1.970	2.435	1.765	2.321
24	1.656	1.623	1.192	1.877	1.539	2.028
30	1.440	1.380	1.091	1.812	1.507	1.855
48	0.908	0.956	0.707	1.462	1.376	1.591
120	0.449	0.532	0.368	0.941	1.176	1.216
216	0.410	0.374	0.284	0.539	1.106	0.714
384	0.381	0.276	0.263	0.322	0.982	0.405

CYBUTRYN

DAY	AARHUS 1	AARHUS 2	AARHUS 3	SILKEBORG 1	SILKEBORG 2	SILKEBORG 3
0					2.500	
1	3.637	3.504	2.689	2.806	2.035	2.592
4	3.107	3.118	2.300	2.536	2.003	2.432
8	2.717	2.664	1.931	2.410	1.744	2.304
24	1.590	1.559	1.122	1.838	1.520	1.993
30	1.375	1.295	1.032	1.774	1.496	1.823
48	0.908	0.956	0.707	1.462	1.376	1.591
120	0.378	0.477	0.315	0.881	1.146	1.180
216	0.365	0.334	0.255	0.498	1.056	0.669
384	0.354	0.247	0.241	0.302	0.948	0.381