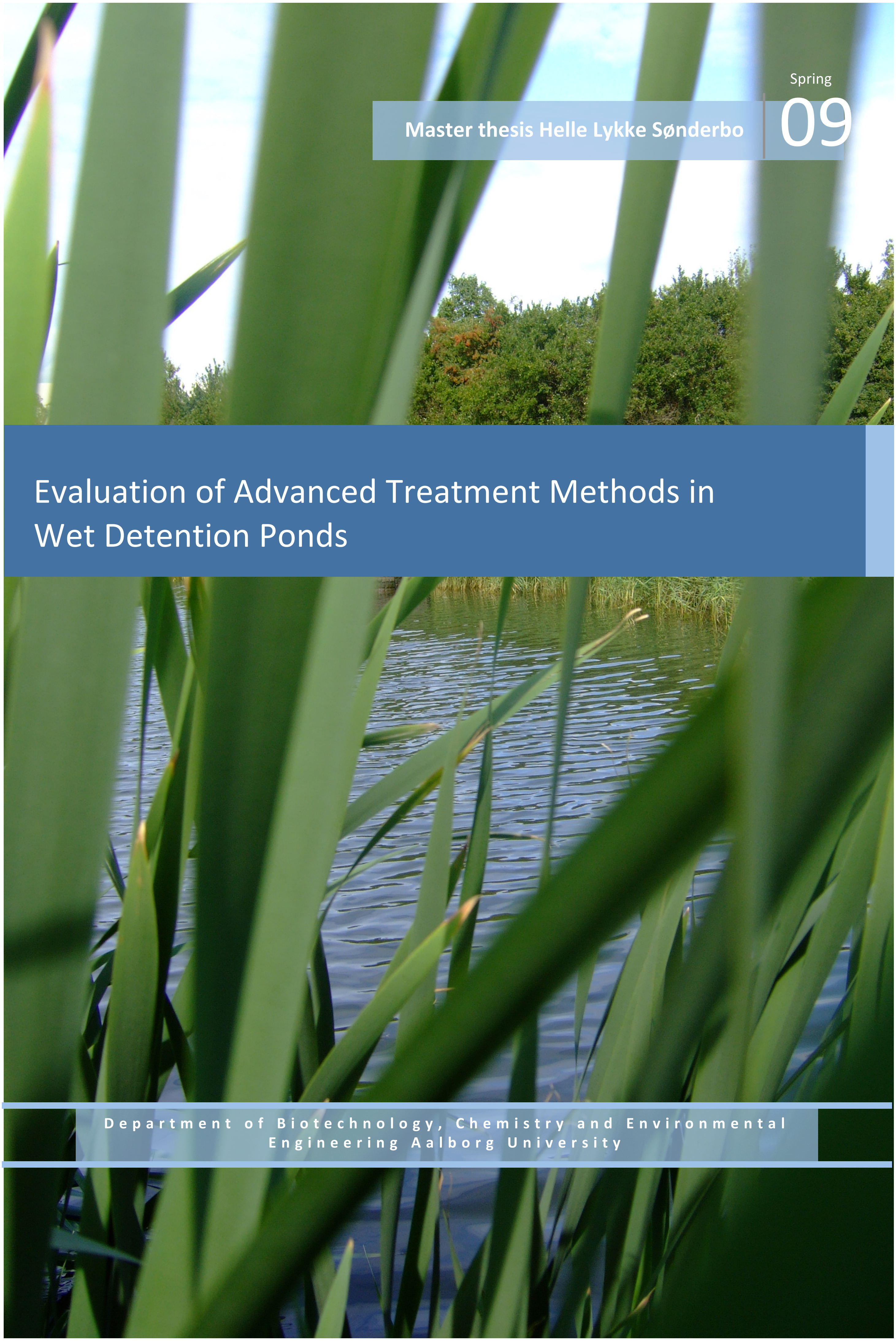

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Master thesis Helle Lykke Sønderbo

Evaluation of Advanced Treatment Methods in Wet Detention Ponds

Department of Biotechnology, Chemistry and Environmental Engineering Aalborg University

Title	Evaluation of Advanced Treatment Methods in Wet Detention Ponds
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Summary

Traditionally stormwater has been regarded as not polluted as most of the compounds are present in very low concentrations compared to e.g. wastewater. However in the recent years the rain related discharges have received increased attention and part of this increased attention is due to the enactment of the European Water Framework Directive, as the directive commits European Union member states to achieve good qualitative and quantitative ecological status of all water bodies by 2015. This will naturally bring increased focus on all discharges to the receiving waters also the rain related discharges from sewer systems.

Ongoing research deals with advanced technologies for treatment of stormwater, and the application of flocculants is one of them. If the implementation of the European Water Frame Directive entails that there will be placed demand on use of a technology that is the “best available” for the given purpose these “advanced methods” e.g. the use of flocculants can become very relevant.

This project evaluated the efficiency of the two flocculants iron chloride and aluminium chloride for removal of heavy metals from stormwater. The efficiency of the methods was evaluated by chemical Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis and by three different toxicological tests. The toxicological tests used three different test organisms; the bacteria *Vibrio fischeri*, the green algae *Selenastrum Capricornutum* and the daphnia *Daphnia magna*.

The results showed that it was possible to efficiently remove the heavy metals copper and zinc from the stormwater by addition of iron chloride or aluminium chloride, and the flocculants were evaluated to be equally efficient. The use of the two flocculants in combination was evaluated to be less effective and did not prove to enhance the removal further. Based on the results none of the flocculants can be recommended over the other. The removal of nickel was significantly lower for both flocculants. These findings were all equally supported by the Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis and by two of the toxicological test; the *S. capricornutum* green algae tests and the *D. magna* daphnia test.

This project also evaluated the effect of the concentration of the added flocculent by varying the concentration from 0 to 40 mg/L. Here it was found that the removal of copper and zinc were directly proportional with the concentration of added flocculants, and this was proven both by the chemical ICP analysis and by the toxicological tests with *D. magna* and *S. capricornutum*.

Additionally the effect of the precipitation period were evaluated and the results indicated that the longer the precipitation period the more efficient removal. However the precipitation experiments also revealed examples of equal removal regardless of the addition of flocculent or not, if just the precipitation period were long enough. These findings can be related to adsorption of the metals on to the glass container surfaces.

However irregularities were experienced with both the chemical analysis and the toxicological tests. Difficulties were experienced with the *V. fischeri* test and the *S. capricornutum* test and the use of flocculants is suspected to influence the test results in various ways resulting in the inconsistent results.

Generally is was found that the use of flocculants for removal of heavy metals from stormwater can not be rejected as a potential methods for implementation in wet detention ponds however more work is needed to document the efficiency and deal with the different problems experienced.

Resume

Traditionelt set er regnvand ikke blevet betragtet som værende forurenet, idet størstedelen af de forurenede stoffer er tilstede i meget lave koncentrationer sammenlignet med f.eks. spildevand. Regnbetingede udledninger har dog i de seneste år modtaget øget opmærksomhed, og en af årsagerne til denne forøgede opmærksomhed er vedtagelsen af EU's Vandrammedirektiv, idet direktivet forpligter EU medlemmerne til at opnå god kvalitativ og kvantitativ økologisk tilstand i alle vandmiljøer i 2015. Dette vil naturligt bringe øget fokus på alle udledninger til recipienterne også de regnbetingede udledninger fra afløbssystemer.

Igangværende forskning beskæftiger sig med avancerede renseteknologier til behandling af regnvand, og brugen af fældningsmidler er en af disse. Hvis implementeringen af EU's Vandrammedirektiv medfører, at der bliver udstedt krav om brug af en teknologi, som er den "bedst tilgængelige" så kan f.eks. brugen af fældningsmidler blive meget relevant.

Dette projekt evaluerede effektiviteten af de to fældningsmidler jernklorid og aluminiumklorid til fjernelse af tungmetaller fra regnvand. Effektiviteten af metoderne blev evalueret ved kemisk analyse Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and ved tre forskellige toksikologiske tests. De toksikologiske tests benyttede tre forskellige testorganismer; baterien *Vibrio fischeri*, den grønne alge *Selenastrum Capricornutum* and dafnien *Daphnia magna*.

Resultaterne viste, at det var muligt ved tilsætning af jernklorid eller aluminiumklorid effektivt at fjerne tungmetallerne kobber og zink fra regnvandet, og fældningsmidlerne blev vurderet til at være lige effektive. Brugen af de to fældningsmidler i kombination blev også undersøgt og blev vurderet til at være mindre effektiv og ikke forbedre fjernelsen yderligere. Baseret på resultaterne kan ingen af de to fældningsmidler anbefales i forhold til det andet. Fjernelsen af nikkel var signifikant lavere for begge fældningsmidler. Disse konklusioner kunne alle drages ud fra I analyserne med nductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) og ud fra to af de toksikologiske tests; *S. Capricornutum* algetesten og *D. magna* dafnietesten.

Dette projekt evaluerede også effekten af koncentrationen af tilsat fældningsmiddel ved at variere koncentrationen fra 0 til 40 mg /L. Her blev det fundet, at fjernelsen af kobber og zink var direkte proportional med koncentrationen af det tilsatte fældningsmiddel, og dette blev påvist både ved de kemiske ICP analyser og ved de toksikologiske tests med *D. magna* og *S. capricornutum*.

Endvidere blev effekten af bundfældningstiden undersøgt, og resultaterne indikerede at jo længere bundfældningstid jo mere effektiv fjernelse. Bundfældningsforsøgene afslørede dog også eksempler på lige stor fjernelse, uanset om der blev tilsat fældningsmiddel eller ej, bare bundfældningsperioden var lang nok. Disse resultater kan være relateret til adsorption af metallerne til glasoverfladerne i beholderne.

Uregelmæssigheder blev dog fundet ved både de kemiske analyser og ved de toksikologiske tests. Der blev også oplevet uregelmæssigheder ved *V. fischeri*- og *S. capricornutum*-testene, og brugen af fældningsmidler er mistænkt for at influere testresultaterne på forskellige måder og resultere i de uregelmæssige resultater.

Generelt blev det fundet, at brugen af fældningsmidler til fjernelse af tungmetaller ikke kan afvises at være en potentiel metode til implementering i våde regnvandsbassiner, men der kræves dog mere arbejde for at dokumentere effektiviteten og imødekomme de forskellige problemer, som blev oplevet.

Preface

This master thesis "Evaluation of Advanced Treatment Methods in Wet Detention Ponds" is conducted under the K-study Board of Biotechnology, Chemistry and Environmental Engineering at Aalborg University, Denmark. The project covers 30 ECTS points and has been conducted in the period from the 2nd of February 2009 to the 10th of June 2009.

The report is divided in eight chapters and figures and tables are assigned with continuous number throughout the report. Appendix are listed A1 to A8 and can be found in the end of this project. Additional data and graphs not showed in the report can be found on the attached DVD together with the literature that was available electronically.

Evaluation of Advanced Treatment Methods in Wet Detention Pods

Table of Contents

Appendix

1 Introduction

The future holds a lot of challenges for the existing and future sewer systems in Denmark both in regard to the expected increased frequency and intensity of heavy rain events, but also in regard to the increased focus on protection and improvement of the ecological water quality in the receiving waters (Winther *et al*. 2004; Djursing 2008).

Many aspects of these challenges are related to rain and the discharges of stormwater into the receiving waters. Stormwater runoff from urban and residential areas such as highways, parking lots and squares is handled both in combined sewer systems and in separate sewer systems. In the combined sewer systems, the stormwater is led, together with the wastewater, to the treatment plant and otherwise retained in rain reservoirs where the diluted stormwater is discharged by overflow structures to the receiving waters (Winther *et al*. 2004). In the separate sewer systems, the rainwater is conveyed in separate pipes to different structures such as dry basins, constructed wetlands, infiltration basins and wet basins or ponds and ultimately discharged into the receiving waters (Vollertsen *et al*. 2006). This report will focus on the rainwater discharged from separate sewer systems.

Discharge of rainwater often has some unwanted effects on the receiving waters; both physically due to the hydraulic load but also on the water quality. Traditionally, the discharged stormwater has been regarded as not polluted as most of the compounds are present in very low concentrations compared to e.g. wastewater (Hvitved-Jacobsen *et al*. 2004). However in the recent years the rain related discharges have received increased attention and part of this increased attention is due to the enactment of the European Water Framework Directive (Brinkmann 1985; Hvitved-Jacobsen *et al*. 2004). The directive commits European Union member states to achieve good qualitative and quantitative ecological status of all water bodies by 2015 and this will naturally bring increased focus on all discharges to the receiving waters also the rain related discharges from sewer systems (Hvitved-Jacobsen *et a*l. 2004).

As mentioned above, the mean concentrations of environmental harmful pollutants in stormwater from separate sewer systems are low, but if the discharged volumes are regarded, mass calculations show, that the yearly discharge of these compounds is noticeable (Dauro 2006; Spildevandskomiteen 2006). Additionally, both Danish and international studies show that stormwater from separate sewer systems can have toxic effects on the organisms in the receiving waters (Marselek *et al*. 1999A; Miljøstyrelsen 2001; Baun et *al*. 2005).

In the nearest future it must be expected that the directive must cause some kind of improved handling of the discharged stormwater from separate systems in order to achieve the established targets by 2015. However the amount of available data on environmental harmful pollutants in stormwater is limited and similar for the efficiency of the current treatment technologies (Hvidtved-Jacobsen *et al*. 2004).

Currently, the Danish environmental authorities have not made any conclusive statements on how to deal with this matter (Hvidtved-Jacobsen *et al*. 2004). One could not dissemble that specific demands on the discharges would be implemented in the wake of the Water Framework Directive, but it is more likely to expect that there will be placed demand on use of a technology that is the “best available” for the given purpose (Hvitved-Jacobsen *et al*. 2004; Vollertsen *et al*. 2006).

In Denmark today, the stormwater from the separate sewer system is primarily handled in wet and dry ponds or discharged directly to the receiving waters (Dauro 2006). However, the ponds are not designed with the purpose of implementing any particular treatment technology, besides removal of particulate matter. The primary design objective behind the ponds is to delay and even out the hydraulic load discharged into the receiving waters (Dauro 2006).

Besides the traditional functions of reducing the hydraulic load on the receiving waters and retaining particulate matter, the wet detention ponds also holds other potentials. If the design and the surroundings are right, the wet detention ponds can also serve as a "nature element" and thereby adding recreational value to an area. This is done by integration of the pond in the surroundings either as a natural born lake or as an artificial water element in the urban environment (Life Treasure 2007). The multiple potentials are some of the reasons why the use of wet detention ponds becomes more and more widespread.

To achieve the goals set by the Water Frame Directive it would be necessary to develop and implement actual technologies in the ponds that also reduce the load of dissolved environmental harmful pollutants and nutrients as well (Hvitved-Jacobsen *et al*. 2004). There are a number of existing technologies, developed for e.g. lake restoration and for wastewater and drinking water treatment, that holds potential as possible technologies for treatment of stormwater. Among these are e.g. the use of flocculants to enhance the removal of environmental harmful pollutants and nutrients. This technology is based on an enhancement of the sedimentation process by coagulation and flocculation of the dissolved and colloidal compounds in the stormwater, but it has not yet been used as a conventional technology for treatment of stormwater. Thus, the area requires further research before the technologies are applicable as usable options that could be implemented in the runoff management system in Denmark.

1.2 Objective

The main objective of this project is to evaluate the efficiency of flocculation methods for treatment of stormwater in wet detention ponds.

How effective are the use of flocculants as a technology to enhance the removal of environmental harmful pollutants in stormwater?

The efficiency of the methods is evaluated by chemical Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis and by three different toxicological tests. The focus of this project is on different aspects of the treatment; the type of flocculent, the concentration of the flocculent and the significance of the precipitation period. The toxicological testing is preformed with a test battery consisting of three different tests using bacteria, green algae and daphnia as test organisms. The efficiency of the flocculation methods is evaluated by comparing the results from the chemical analysis with the results from the toxicological tests.

2 Pollutants in Stormwater

Traditionally stormwater has not been regarded as polluted and discharge of stormwater has not been shown any particular attention compared to the treatment and discharge of wastewater from treatment plants (Brinkmann 1985; Miljøstyrelsen 2001; Spildevandskomiteen 2006). Stormwater handled in separate sewer systems is primarily run-off from covered areas such as roads, pavements, parking lots, squares etc., and the sources that contribute to the deterioration of the water can be divided in the following main groups (Miljøstyrelsen 1997).

Atmospheric - Load from atmospheric discharge of compounds and particles that deposits by either wet or dry deposition and originates from emissions from industrial areas or from the use of wood stoves in private houses.

Traffic - Load that is related directly to the traffic such as compounds from exhaust gasses, spill of oil, petrol and other liquids, material from tires, the road cover or from decomposition of components on the vehicles.

Function and Maintenance – Load from maintenance of roads, pavements, roadsides and private ground for instance by the use of road salt and pesticides.

Attrition and decomposition – Load from attrition and decomposition of materials in constructions such as trenches, roofs and other surfaces (Miljøstyrelsen 1997).

The variety of pollutants found in stormwater is to a certain limit the same as those found in wastewater, but with some obvious exceptions, as the water from domestic activities in households is not included. Another important difference between stormwater and wastewater is the great variability and inconsistency in the range of different pollutants and concentration levels found in stormwater. Both the type of pollutant and the concentrations vary significantly with time and are dependent of variables such as the weather, the time of year and the activities in the catchment area. Also the general distribution of roads, roofs, permeable and impermeable areas in the catchment area affects the characteristics of the stormwater from a particular catchment area (Brinkmann 1985; Hvitved-Jacobsen *et al*. 2004).

The amount of pollutants discharged from the separate sewer systems is predominately related to the pattern of rain events. The length of the dry period prior to rain events has also an influence on the concentrations found. The rain events after a long dry period has shown higher concentrations than rain events with shorter dry periods (Mijøstyrelsen 1997; Grout *et al*. 1999; Mosley and Peake 2001).

Besides the direct correlation with rain events, the concentrations in the stormwater can also be related to single events or more consistent but diffuse discharges (Spildevandskomiteen 2006). Single events of discharges could e.g. be pollutants from an industrial discharge or pollutants from washdown and disinfection of roofs in a residential area. Such single events and diffuse discharges over longer periods from unknown sources make it hard to make any conclusive statements on the typical pollutants and pollutants levels found in stormwater. The concentrations listed in the following section should therefore be not regarded as typical values, but more as examples of what have been reported in different studies.

2.0.1 Particulate versus dissolved compounds in stormwater

A common way to categorize the chemical content in water samples is in a “dissolved” and a “particulate” fraction. The prevalence of the pollutants in either the dissolved or the particulate fraction is important as it affects the treatability of the stormwater and also the bioavailability and toxicity of the pollutants.

Many of the pollutants in stormwater are found in the particulate fraction, meaning they are associated with the organic matter and the finer particles. This is predominantly true for the organic xenobiotic compounds such as total hydrocarbons, ∑PAH's and phthalates, as their chemical properties facilitates the association with organic material (Pitt *et al.* 1995; Miljøstyrelsen 1997; Miljøstyrelsen 2001).

For heavy metals the prevalence differs from metal to metal where some is predominately in the particulate fraction and others predominately in the dissolved fraction. It seems that metals as cobber and zinc are mainly found in the dissolved fraction, whereas metals such as lead, iron and cadmium are found associated with particles (Miljøstyrelsen 1997; Miljøstyrelsen 2001; Mosley and Peake 2001).

Most of the inorganic parameters have a tendency to be at dissolved form, most distinct for chloride (Miljøstryrelsen 1997). This kind of information about the prevalence of the compounds is important when is comes to finding the best technology for treatment of stormwater in the separate sewer system, due to the differences in removal of dissolved and particle-associated compounds, respectively.

2.0.2 Heavy metals

Heavy metals are a large group of metals that can cause of environmental pollution. They occur naturally in the ecosystem and some of them such as copper and manganese are essential as trace elements to organisms in low concentrations. Other heavy metals such as lead, cadmium and arsenic have no known beneficial effect on living organisms (Baird and Cann 2005; Kamo and Nagai 2008). Besides their naturally occurring sources, the metals also originate from a number of anthropogenic sources such as lead in petrol, industrial effluents and leaching of metal ions from the building materials and components on vehicles. The concentrations found in stormwater from separate sewer systems, which is described in the literature, varies from nothing to relatively large numbers dependant on the type of metal. The values listed in the table below are a collection of various measured concentrations from different Danish and foreign studies. The intervals listed are a clear example of the presence of extreme values, as a zinc concentration of 25.5 mg/L must be regarded as a single event and not a representative for the typical interval (Table 1).

Table 1 Selection of measured concentrations of heavy metals in stormwater from separate sewer systems (Spildevandskomiteen 2006).

Concentration in stormwater	Interval [μg/L]	Number of locations	Number of samples
Zinc	1.73-25500	112	868
Lead	<0.5-6400	122	1015
Copper	<0.5-1250	106	724
Cadmium	<0.05-2000	100	427
Nickel	<0.02-436	66	324

Investigations show that the heavy metals, as oppose to the NPO compounds, occur in the same order of magnitude in discharges from both separate and combined sewer systems (Miljøstyrelsen 1997; Miljøstyrelsen 2001).

Many studies deal with the correlation between traffic intensity in the catchment area and the concentration of heavy metals in the runoff. Other studies have also shown correlation between the concentrations of heavy metals in roadside snow and the distance to the road. The higher traffic load and the shorter distance to the highway the higher concentration of heavy metals (Table 2, Table 3 and Table 4)(Miljøstyrelsen 1997). The relatively high measured concentrations of lead listed in the tables are probably due to the fact that the studies were conducted before unleaded petrol was introduced.

Table 2 Concentrations of five heavy metals in road runoff from two roads with medium and high traffic load (Miljøstyrelsen 1997).

	Zinc [μg/L]	Lead [μg/L]	Copper [μg/L]	Cadmium [μg/L]	Nickel [μg/L]
Medium traffic load	204	22	17.2	0.68	9.9
High traffic load	342	202.9	109	2.0	35.5

Table 3 Concentrations of three heavy metals in wet detention ponds in Sweden compared to traffic load in the catchment areas. * Residential area that has the largest area with zinc and copper and a higher population density (Miljøstyrelsen 1997).

Traffic load [cars · km/day]	Zinc [μg/L]	Lead [μg/L]	Copper [μg/L]
4170	643	477	323
3130	436	232	254
120*	272	234	33
340	269	71	26

Table 4 Concentration of five heavy metals in snow as a function of distance to a highway in Norway (Miljøstyrelsen 1997).

Distance from highway [m]	Zinc [μg/L]	Lead [μg/L]	Copper [μg/L]	Cadmium [μg/L]	Nickel [μg/L]
5	338	500	117	2.4	49
13	63	109	37	0.7	14
19	75	53	43	0.5	19
50	90	154	0.1	0.7	14
53	50	44	20	0.7	19
150	50	80	0.1	0.3	13
300	50	19	0.1	0.3	64

2.0.3 Polyaromatic hydrocarbons (PAH's)

Polyaromatic hydrocarbons or PAH's is a collective term for chemical compounds that consists of only carbon and hydrogen atoms and contains aromatic rings. The sources of PAH's are various and they occur in oil, coal, and tar deposits, and are produced as by-products of fuel burning both from fossil fuels or biomass fuels. PAH's are also used in several products such as binding and proofing agents and especially in creosot, a product that contains a number of polyaromatic compounds (Miljøstyrelsen 2007).

In the wake of the European Water Framework Directive the European Union has enacted a new directive concerning environmental water quality standards. Part of this directive, is a list of priority substances that encompass 33 substances. Twenty of these substances are labelled as priority hazardous substances among these twenty are a number of PAH's (Europaparlementet 2008).

Studies of polyaromatic hydrocarbons in stormwater often measure the sum of PAH's, but compounds as flouranthene, phenanthrene and anthracene are mentioned more often than others. Table 5 shows a summary of measured concentrations of PAH's in stormwater from separate sewer systems in Denmark. Five of the six PAH's are listed as hazardous priority substances on the list from the European Union (Miljøstyrelsen 1997; Miljøstyrelsen 2007).

Table 5 Observed mean values of different PAH's in stormwater from separate sewer systems in Denmark and from run-off from highways in Germany with different traffic load (Miljøstyrelsen 1997; Miljøstyrelsen 2007).

	Stormwater from separate sewers [μg/L]	Run-off from highways [μg/L]
Benzo(a)pyrene	0.14	0.2-5.2
Benzo(b,j,k)fluoranthene	0.39	-
Benzo(g,h,i)perylene	0.25	0.03-7.7
Indeno(1,2,4-cd)pyrene	0.11	-
Anthracene	0.088	-
Fluoranthene	0.66	0.3-13.9

As for heavy metals, studies have found a correlation between the traffic intensity and the concentration of PAH's in the run-off. Similarly a Norwegian study shows that there is a correlation between the distance from highways and the concentration of PAH's measured in snow (Miljøstyrelsen 1997).

2.0.4 Pesticides

Pesticides is a large group of toxic compounds used for control of unwanted weed, insects, fungi and other organisms that are considered harmful or unwanted e.g. in cultivating, in private gardens and public parks, at roadsides and pavements. Due to these various applications the pesticides end up in stormwater.

The studies on pesticides in stormwater are limited, and as for most of the other compounds in stormwater, the measured concentrations are very variable and seem to be dependant of the land-use of the catchment and of the ongoing activities in the catchment in the sampling period (Pitt *et al*. 1996; Schiff and Sutula 2004).

Schiff and Sutula (2004) studied three catchment types; agricultural, residential and open land. The study showed that the run-off from catchments, which were dominated by agricultural land use, had the highest concentrations of the insecticides (Schiff and Sutula 2004). Another American study investigated the insecticide chlordane in run-off from different areas and found that runoff from auto service area contained 0.8 μg/L, from roofs 1.6 μg/L and from parking areas contained 1.0 μg/L (Pitt *et al*. 1995).

In connection with establishment of a system for collection of rainwater in Denmark, the water was analysed for a number of pesticides and seven different pesticides were found above the detection limit at 0.01 μg/L. Four of the seven pesticides were prohibited in Denmark at the time of the study in 2003, however they were present in the stormwater, which illustrates that the presence of pollutants in stormwater is hard to predict (Table 6).

Table 6 Concentrations of seven different pesticides found in a system for collection of stormwater in Denmark (Miljøstyrelsen 2003). 1) (Rosbjerg and Frederica 2000), 2) (Miljøstyrelsen 2007), 3) (Miljøministeriet 2009), 4) (Miljøstyrelsen 2009).

Pesticide	n	Mean concentration [μg/L]	Prohibited in Denmark since
4,6-Dinitro-o-creso (DNOC)	8	0.530 (0.131–1.18)	1986¹⁾
Atrazine	6	0.60 (0.14–0.108)	1994²⁾
Desethylatrazine	4	0.56 (0.16–0.86)	1994²⁾ Metabolite of atrazine.
Isoproturone	3	0.42 (0.40–0.47)	Prohibited⁴⁾
Pendimethaline	6	0.86 (0.4–0.152)	Not prohibited⁴⁾
Terbuthylazine	2	0.74 (0.10–0.138)	2009³⁾
Desethylter- buthylazine	1	0.50	Not prohibited⁴⁾

2.0.5 NPO compounds

NPO compounds is a collective term for total nitrogen, total phosphorus and organic material that results in oxygen consumption in the receiving waters measured by Chemical Oxygen Demand (COD). In wastewater the group of compounds make up the greatest part of the compounds, but in stormwater the concentrations of these compounds are substantially lower (Table 7) (Miljøstyrelsen 2001).

Table 7 Concentration of NPO compounds in water discharged from combined and separate sewer systems respectively (Miljøstyrelsen 2001).

	Combined sewer systems	Separate sewer systems
COD [mg/L]	160	50
Total nitrogen [mg/L]	10	2
Total phosphorus [mg/L]	2-3	0.5
Suspended solids [mg/L]	150-200	30-100

3 Treatment of Stormwater in Wet Detention Ponds

Run-off collected by the separate sewer system is discharged to the receiving waters or infiltrated to the ground water though a number of different ways; mainly through ponds or through direct discharge to the receiving waters or by local percolation through a fascine to the groundwater (Dauro, 2006).

The ponds can have different designs and can be both wet and dry; termed wet detentions ponds or dry detentions ponds. The main objective of ponds is to reduce the hydraulic load by delay and smoothing of the water flow by storage of the stormwater. The reduction of the water flow protects the receiving waters from erosion and generally helps to reduces the disturbance of the organisms in the receiving water (Dauro 2006).

The wet detention ponds are designed in such way that there is a permanent water surface that rises during rain events and decreases during the dry periods. Furthermore the ponds are designed in such way that the water level should not rise to more than 1.5 meters during rain events. During dry periods the basin should be relatively shallow approximately 0.8-1.5 meters deep (Figure 1). For dry ponds there is no permanent water table and the volume is only flooded during and in a short period after rain events (Dauro 2006).

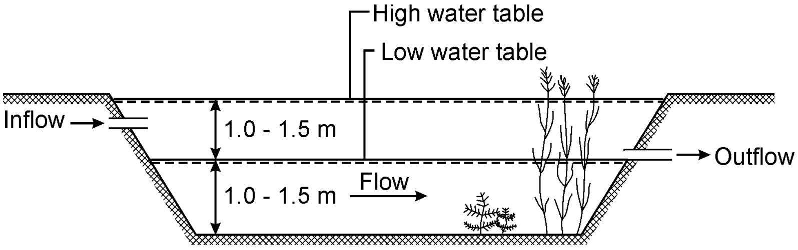

Figure 1 Principal sketch of the design of a wet detention pond (Life Treasure 2007).

Sedimentation is the most important process for removal of a great part of the compounds. Most wet detention ponds are designed with the inlet in one end of the pond and the outlet in the opposite end. This should secure a steady flow and prevent stagnant areas, but it should also secure that the retention time is long enough to facilitate that sedimentation of the particles will occur naturally.

Besides this sedimentation there is no actual treatment technology applied to the ponds, but two other processes remove unwanted compounds from the stormwater; uptake of compounds by plants and sorption of compounds to surfaces such as the plants and the bottom in the pond (Vollertsen *et al.* 2006).

The relatively low water level in the wet detention ponds should secure that the re-aeration is sufficient to avoid anaerobic conditions on/near the bottom of the pond. Such depletion of oxygen in the lower part of the water body can facilitate release of bound phosphorous, iron and hydrogen sulphide from the sediment (Vollertsen *et al.* 2006). This release of iron and phosphorous will counteract the original purpose of the wet water detention ponds and raise the possibility of discharge of these compounds to the receiving waters.

As mentioned in the previous section, the concentrations of pollutants in stormwater are often low and a great part of the polluting compounds are associated with particles. This association to particles is what utilizes the removal of the compounds in the wet detention ponds. As described by the gravitation law and Stokes law, particles with a certain density will precipitate with a certain settling velocity. This means that if the hydraulic retention time in the pond is longer than the time it takes for the individual particulate compounds to settle, the compound will settle in the sediment of the pond and thereby not be discharged to the receiving water body (Winther *et al.* 2004).

Table 8 show an example of the annual treatment efficiency of a Norwegian wet detention pond. The removal of compounds in the pond is solely based on sedimentation (Vollertsen *et al.* 2007). The actual treatment efficiency of the traditional wet detention ponds vary dependant of the type of compounds. The treatment efficiencies are relatively high for the suspended solids and the organic compounds such as oil, fat and PAH's. The treatment efficiencies for the nutrients and for the heavy metals are the lowest and thus treatment performance towards these compounds could be improved as sedimentation as a sole process does not seem to be sufficient.

Table 8 Annual treatment efficiency of a Norwegian wet detention pond (Vollertsen *et al.* 2007).

	Inlet [kg/year]	Outlet [kg/year]	Overall treatment efficiency [%]	Treatment efficiency excl. snowmelt [%]
Suspended solids	3790	582	85	93
Total phosphorous	8.81	3.59	59	84
Bioavailable phosphorous	5.31	2.00	62	88
Total nitrogen	20.4	14.4	29	37
Oil and fat	67.9	12.0	82	89
Total PAH	0.0242	0.0035	86	92
Lead	0.234	0.056	76	83
Cadmium	0.00285	0.00115	60	70
Copper	1.17	0.50	58	66
Zinc	3.74	1.07	71	81

It can however be very difficult to determine which particles that will have the sufficient settling velocity to settle during the flow through a certain pond. As in the Danish study by Miljøstyrelsen (1997) most other studies separate the compounds found in water samples in a "dissolved" and in a "particulate" fraction using a arbitrary pore size value of a filter – typically 0.45 μm (Stanley *et al.* 2003; Grout *et al.* 1999). It is a common assumption that most of the particulate fraction will precipitate if the retention time is long enough, whereas the dissolved fractions will not, as the gravitational force is not significant enough due to their small size.

However it is not always that simple. Studies have showed that the settleability of specific pollutants differs widely and that the settleability also differs from site to site within the same class of pollutants (Whipple and Hunter 1981). These variations in settleability of pollutants should be seen in relation to the studies that emphasise that the particle size distribution is important in determination of the settleability and efficiency of the removal process (Whipple and Hunter 1981; Mann and Hammerschmid 1989; Vaze and Chiew 2004). The differences in settleability of the same type of compound and from site to site is likely to be associated with differences in particles size distributions in the respective runoff events.

In the traditional wet detention ponds, the settleability is of great importance in relation to the removal efficiency (Allen *et al.* 1997; Vaze and Chiew 2004). Vaze and Chiew (2004) concludes that treatment facilities for stormwater must be able to remove particles down to a diameter of 11 μm and they found that > 85 % of the total amount of nutrients were associated with particles smaller than 300 μm (Vaze and Chiew 2004).

Many studies examine the relationship between particle size and the distribution of the associated of heavy metals (Grout *et al.* 1999; Miljøstyrelsen 2000; German and Svensson 2002). These studies show an increasing metal concentration with decreasing particle size and the smaller particles were more susceptible to sourcing and transport of pollutants than the larger particles (Miljøstyrelsen 2000; German and Svensson 2002; Stanley *et al.* 2003). The term colloids are also used in relation to fractions in water samples and colloids are found to account for 29-85 % of the total content of heavy metals in water samples depending of the type of metal (Harrison and Wilson 1985; Grout *et al.* 1999).

The term colloids encounters for small particles that roughly have a radius in the interval from 1 nm to 1 μm and the gravitational effects are insignificant in relation to their movement. Brownian motion governs their movements and they interact by intermolecular forces and aggregate into larger particles by coagulation/flocculation processes (Keiding, unpub.; Shaw 1997)

The many terms for the particle sizes as either dissolved, particulate, small, large, colloids and so on can be confusing even for scientists. Furthermore it is hard to relate these classifications to the ability of the compounds to settle and thereby their behaviour in wet water detention ponds.

Another alternative classification has been proposed, that might be more relatable in practical purposes in relation to evaluation and development of new methods for treatment of urban stormwater. Three classes are proposed; "dissolved", "colloidal", and "gravitoidal". Colloids are defined as "any constituent that provides a molecular milieu into and onto which chemical can escape from the aqueous solution and whose environmental fate is predominantly affected by coagulation-breakup mechanisms, as opposed to removal by settling." Gravitoids are defined as "particles that are significantly affected by gravitational settling". Dissolved substances are defined as "particles that provide no internal environment for a contaminant to partition into, and their properties are essentially molecular in nature (e.g., ionized functional groups can be treated as point charges, rather than in terms of surface potentials and electric double layer theory)" (Stanley *et al.* 2003).

Figure 2 shows a schematic representation of the particle size distribution in stormwater characterized by four fractions; dissolved, colloid, gravitoidal and sedimentary fraction. The small horizontal arrows represent the processes that facilitate the transition from one pool to another e.g. the coagulation process of the compounds from the gravitodal fraction to the colloid fraction.

The large arrow pointing towards right illustrates, that the larger density and stability of the particles, the more efficient will the sedimentation process in the wet water detention ponds become, and this will also mean a more efficient removal of the pollutants associated with the particles. This means that the removal of the smaller entities is less effective. However the removal of these smaller entities is not less important as they exhibit a larger mobility and bioavailability and thereby increasing the unwanted effects in the receiving waters (Stanley *et al.* 2003). These relationships between size, mobility, settleability entail that sedimentation as a sole process is not able to remove a great fraction of the pollutants, and as the focus on the rain related discharges increases, this calls for new ways to treat and handle the discharge of stormwater.

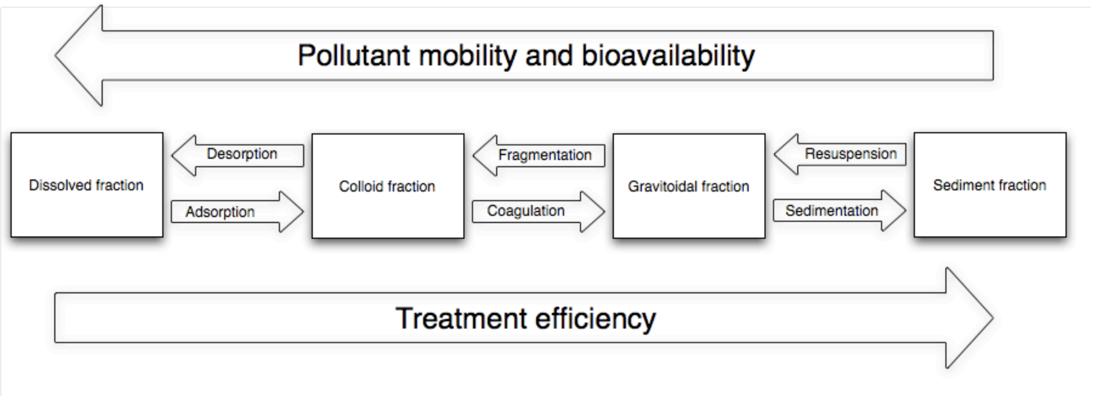

Figure 2 Schematic representation of the effect of particle size in stormwater on the treatment efficiency, the pollutant mobility and the bioavailability (Based on Stanley *et al.* 2003).

3.1 New technologies

In order to optimize the retaining and removal of compounds facilitated by wet detention ponds new technical facilities are developed and technologies from other areas are adapted to the ponds. These initiatives can under a collective term be called “advanced methods” for treatment of stormwater. If the implementation of the European Water Frame Directive entails that there will be placed demand on use of a technology that is the “best available” for the given purpose these “advanced methods” can become very relevant.

An example of some of the activities in this field is the Life Treasure Project (Treatment and Re-use of urban stormwater runoff by innovative technologies for removal of pollutants). The Life Treasure project is co-financed by the European Union and carried out by a consortium of three municipalities, two Danish universities and the Danish Environmental Protection Agency (Life Treasure 2009). The purpose of the Life Treasure project is to implement and demonstrate technologies that can efficiently reduce diffuse urban pollutant loads onto receiving waters. Focus is on the removal of phosphorous and on toxic substances such as heavy metals and organic micropollutants (Life Treasure 2009).

As part of the project, three wet detention ponds for treating stormwater runoff have been constructed. The ponds are constructed with three different technologies that aim to optimize the treatment process to include more than just the particulate fractions, but also remove small particles and colloidal and soluble bound pollutants. Besides the three advanced technologies in the ponds, each pond facilitates sedimentation and filtration through sand filters and also benefits from plant uptake of dissolved pollutants and sorption of pollutants to surfaces in the pond. The facilities are located in Silkeborg, Århus and Odense, Denmark (Life Treasure 2009).

The facility in Århus is based on sorption to iron-enriched bottom sediments and the facility in Silkeborg is based on the principle of enhancement of the precipitation by addition of aluminium flocculent. The facility in Odense is based on sorption of the pollutants to a fixed media filter. The filter media technology also holds great potential as a future technology for removal of pollutants from stormwater, but the technology will not be described further in this thesis.

3.1.1 Sorption to iron-enriched bottom soil

The extended treatment technology in the wet detention pond located in Århus is based on the addition of iron chloride and the chemical characteristics of the iron species. This technology is used for treatment of wastewater but has potential for treatment of stormwater run-off (Life Treasure 2007).

Figure 3 show a principle sketch of the process that removes pollutants from the stormwater in the pond in Århus. An iron chloride solution has been dispersed into the pond by a perforated hose and a pump on the pond side. Approximately 63 g Fe(III) has been added pr. m^3 pond volume (Kemira 2009). This addition of iron chloride results in formation of iron hydroxides that precipitates and thereby making the bottom enriched with iron products, which enhances the sorption ability of the bottom sediments (Vollertsen *et al*. 2009).

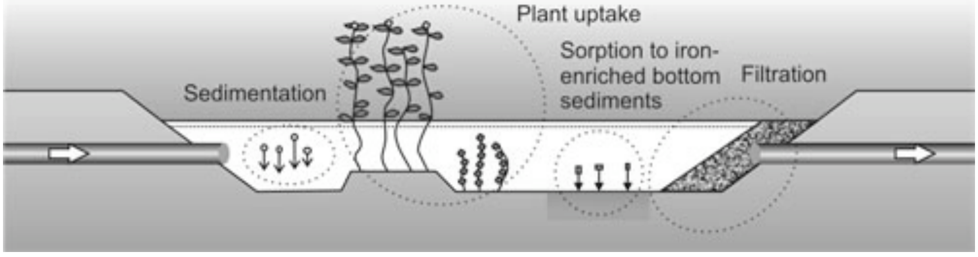

Figure 3 Principal sketch of the pond in Århus where addition of iron chloride is tested (PH Consult 2007).

The chemistry of iron species is very complex and is dependent of mainly the redox potential and pH. What is most important in the use of iron chloride in wet water detention ponds is the ability to form different species of iron hydroxide and its ability to form precipitates of iron salts.

When the redox potential is high e.g. under aerobic conditions and the pH is between 7-10 iron(III)hydroxide ($Fe(OH)_3$) is relatively stable and has a low solubility (Figure 4). This is favourable for the removal of compounds from the water as iron(III)hydroxide provides sorption sites for a number of compounds and particles due to very complex interactions between the molecules and colloid particles in the water (Gregory 2005; Life Treasure 2007). Stormwater in ponds is in general slightly alkaline and the pH varies between 7.5 and 9 (Vollertsen *et al*. 2009). As Figure 4 shows $Fe(OH)_3$ is the most dominating species under neutral pH conditions however the other species of iron (oxy)hydroxides can also provide sorption sites for pollutants. These molecules complexes of iron hydroxides will form larger flocs by coagulation and flocculation if the conditions are favourable and thereby enhance the removal of colloid and dissolved compounds (Gregory 2005; Life Treasure 2007).

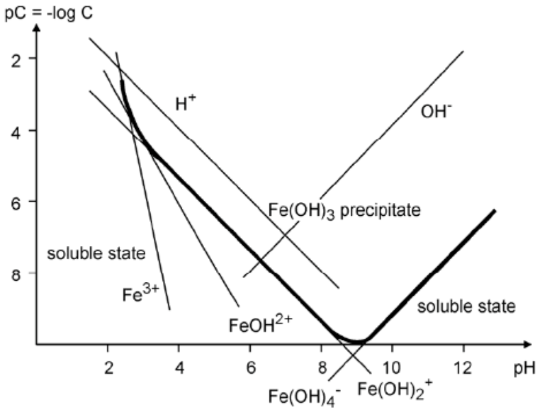

Figure 4 Solubility diagram of iron hydroxides as a function of pH and concentration in an aqueous solution at high (aerobic) redox potential (Life Treasure 2007).

Besides the iron(oxy)hydroxides, precipitates can also be formed from ion bindings with ferri and ferro ions. In this matter a high redox potential e.g. by aerobic conditions will also be favourable. Aerobic conditions will keep iron as ferri-iron (Fe(III) - oxidized form) that binds phosphorous easily whereas ferro-iron (Fe(II) - reduced form) does not bind phosphorous as the complex become unstable (Bondo Christensen *et al*. 2004).

3.3.2 Coagulation and flocculation by aluminium addition

Another technology for enhancement of the removal of pollutants is tested in the wet water detention pond in Silkeborg. The method is based on addition of an aluminium salt and its ability to form different species of aluminium hydroxide that enhances the precipitation due to coagulation and flocculation of the hydroxides. Figure 5 shows a principal sketch of the process in the pond. The aluminium is added as natrium aluminate ($AlNaO_2$) proportional with the flow at the inlet to the pond; approximately 5 g Al/m^3. Addition of aluminium salts is a method also used for treatment of drinking and wastewater and also for restoration of eutrophic lakes (Life Treasure 2007; PH Consult 2007).

As for iron-salts aluminium salts form a wide spectrum of aluminium hydroxides when dissolved in water. Similarly, phosphorus and other dissolved particles can sorp to the hydroxides and create flocs by coagulation and flocculation process that subsequently settle in the pond sediment (PH Consult 2007; Life Treasure 2007).

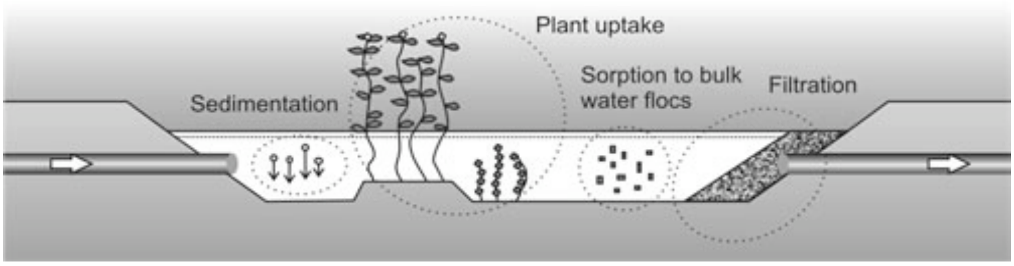

Figure 5 Principal sketch of the pond is Silkeborg where addition of an aluminium salt is tested (PH Consult 2007).

The different species is very dependent of the pH value (Figure 6). At pH values between 6 and 8 the predominating species of aluminium hydroxides is $Al(OH)_3$ and it has a very low solubility in water. An advantages of $Al(OH)_3$ over $Fe(OH)_3$ is that the sorption potential of $Al(OH)_3$ is inert to the changes in redox potential which means that it will not dissolve during anoxic periods and release the bound compounds (Life Treasure, 2007). However at high pH values above 8.5 will the aluminium hydroxide $Al(OH)_3$ become unstable and dissolve thereby release the bound pollutants again. Two of the aluminium compounds $Al(OH)^{4-}$ and $Al(H_2O)_6^{3+}$ are toxic and they form when the pH is above 8.5 and below 4.5 respectively (Life Treasure 2007).

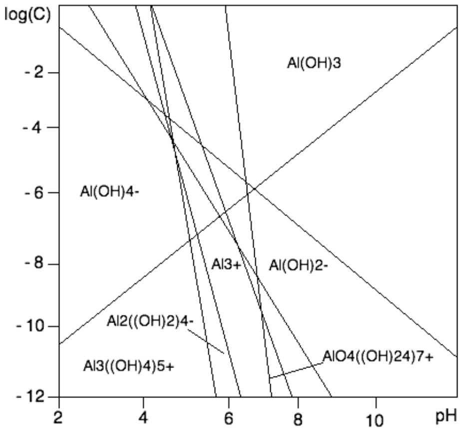

Figure 6 Diagram of the aluminium hydroxide species as a function of pH and concentration (Gregory 2005).

4 Toxicity of Stormwater

Many studies show that stormwater can be toxic in relation to the organisms in the receiving waters (Marsalek *et al*. 1999A; Marsalek *et al*. 1999B; Miljøstyrelsen 2001; Baun *et al*. 2005), and the risk that discharges of stormwater will have toxic effects in the water environment has received increased attention as the unwanted effects of environmental harmful pollutants have become more recognised (Miljøstyrelsen 2001).

Toxicity is in general divided into "acute" and "chronic" toxicity; where the acute effects will set in during hours to days, and the chronic effect will show over weeks to months to years (Walker *et al*. 2006). Both kinds of toxicity are relevant when dealing with discharges of stormwater. Chronic effects can develop due to the consistent discharge of pollutants in predominantly low concentrations as some of the pollutants can accumulate in the sediment or in the food chain and thereby reach concentrations that will have chronic effects on the aquatic organisms (Miljøstyrelsen 2001). The acute toxic effects will occur noticeably faster e.g. during and after rain events where there in the shorter periods will be discharged high concentrations of pollutants to the receiving waters (Marsalek *et al*., 1999A; Miljøstyrelsen 2001). The general toxicity of stormwater has been reported to vary significantly during individual rain events and also between events from the same catchment and this could be explained by the likewise variations in event mean concentrations of pollutants in the stormwater (Marsalek *et al*. 1999A).

It has been reported that the toxicity of stormwater runoff from highways is more toxic than runoff from residential areas (Marsalek *et al*. 1999A), however this correlation is not always reported as others have found that there was no significant correlation between toxicity and type of catchment (Miljøstyrelsen 2001). This non-significant correlation between toxicity and type of catchment area was reported even though the runoff from highways had higher concentrations of pollutants than the runoff from the residential areas (Miljøstyrelsen 2001).

Another interesting aspect of stormwater toxicity is weather the toxicity is mainly due to the content of pollutants associated with particles or due to the dissolved and colloid fractions. Stormwater that have had a period for precipitation has markedly reduced concentrations of a number of chemicals, especially those that due their physical and chemical characteristics has affinity for particulate materials in the water. However the toxicity of the water was not likewise reduced due to the precipitation, and this must be a result of that the toxicity is to some extent related to the dissolved and colloid fraction of the stormwater (Miljøstyrelsen 2001). These findings would enhance the importance of optimizing the current treatment facilities for discharge of stormwater runoff in relation to a more effective removal of the dissolved and colloid fraction.

4.1 Testing stormwater toxicity

In general, toxicity testing is not a part of the traditional water quality test program in the western countries and the monitoring of the environmental state of the aquatic environment is most often based on measurements of a number of physical and chemical parameters (Baun 1998). The newest water quality criteria from the European Union due to the Water Framework Directive are also based on primarily physical and chemical parameters. However the biological aspects are included for lakes and inland water by chlorophyll *a* measurements and the Danish fauna index is also a part of the water quality program (Bondo 2005; Bruus 2009).

The sole use of chemical analysis has some disadvantages that biological toxicity tests can approach. The chemical analysis gives a value for the concentration of one or more compounds in a water sample, but they do not provide any answers in relation to the actual effect in the aquatic environment (Keddy *et al*. 1995; Baun 1998). As environmental water samples are complex and contain a variety of pollutants in different concentrations, a chemical analysis will not integrate the composition of the water sample in evaluation of the results. This is possible in biological toxicological testing as the tests provide information on the total effects of a given combination of compounds in the actual water sample. The biological tests appear to give a more direct measure of the environmental effects than do chemical analysis (Keddy *et al*. 1995; Baun 1998).

There are many different ways in which toxicity can be measured, but the basic principle is to quantify the effect in a organism related to a certain dose or concentration of a toxic compound (Baun 1998; Walker *et al*. 2006). The dose or concentration of a pollutant that will cause a predefined toxic response at 50 % level is used as a standard of reference between different kinds of toxicity tests. This dose or concentration is termed lethal dose/lethal concentration and is abbreviated LD_{50} or LC_{50}. The term Effect Dose or Effect Concentration is also used (ED_{50}/EC_{50}) if the predefined response is not death. Other reference measures are NOED or NOEC that describe the No Observed Effect Dose or Concentration (Walker *et al*. 2006).

A very important characteristic of biological toxicity testing is, that the toxicity is species-specific meaning that the degree of toxicity of one compound/one sample differs from species to species of test organism (Walker *et al*., 2006). This entail that the use of one sole species as test organism will give a limited picture of the effects of the compound, as the test only asses the effect on that particular species. To avoid this, it is best practice to use several test species in evaluation of toxicity – often named a test battery or a bioassay (Blaylock *et al*. 1985; Dutka 1988; Keddy *et al*. 1995; Baun 1998; Marsalek *et al*. 1999A; Miljøstyrelsen 2001).

The range of species will often be chosen in relation to what media is tested and the ecological relevance for that media. For water samples the natural choice will be any form of aquatic species such as bacteria, algae, zooplankton, crustacean, fish and in rare circumstances birds (Walker *et al*. 2006). Another step to increase the ecological relevance is to include species from different trophic levels, as this will give a broader view of the effects in the natural ecosystem (Blaylock *et al*. 1985; Dutka 1988; Keddy *et al*. 1995; Baun 1998; Marsalek *et al*. 1999A; Miljøstyrelsen 2001).

To illustrate the importance of using a range of different organisms the table below show, how the LC_{50} value for cobber varies form species to species. Towards cobber the crustacean appears to be the most sensitive, however the values vary over a wide spectre and different studies state different values for the same organism. It seems like that the larger species, as fish, is more resistant than the smaller species, and this illustrates that the toxicity is species-specific and why it is best practice to use species from different trophic levels.

Table 9 Lethal concentration (LC_{50}) values for different test organisms of copper $^{1)}$ (Smith and Heath 1979), $^{2)}$ (Flemming and Trevors 1989), $^{3)}$ (Blaylock *et al.* 1985), $^{4)}$ (Villavicencio *et al.* 2005), $^{5)}$ (Tsiridis *et al.* 2006), $^{6)}$ (Utgikar *et al.* 2004).

Test organism	LC_{50} values for cobber [μg/L]
Trout – fish	100-1000 $^{1)}$
Bluegill – fish	2000-4000 $^{1)}$
Bluegill – fish	6000 $^{2)}$
Bluegill – fish	2200 $^{3)}$
Daphnia magna - crustacean	3.7-66.2 $^{4)}$
Selenastrum capricornutum - algae	400 $^{3)}$
Chlorella vulgaris - algae	200 $^{3)}$
Vibrio fischeri - bacteria	250 $^{5)}$
Vibrio fischeri - bacteria	500 ± 100 $^{6)}$

Another compound that is likely to be found in runoff from squares and covered areas is glyphosate, which is the active compound in the herbicide Roundup®. Roundup® is one of the most used herbicides worldwide and it proven to be very effective as weed control (Hendricks and Rhodes 1992; Alberdi *et al*. 1996; Busse *et al*. 2001; Amoros *et al*. 2007).

With the knowledge of that glyphosate is widely used and also highly water-soluble its toxic effect in the ecological system is of great interest. Moreover glyphosate is also among the compounds found in stormwater discharged to Harrestup Å in Denmark (Alberdi *et al.* 1996; Eriksson *et al.* 2007; Amoros *et al.* 2007).

A handful of studies evaluate the toxic effects of glyphosate in relation to different organisms (Hendricks and Rhodes 1992; Alberdi *et al.* 1996; Busse *et al.* 2001; Amoros *et al.* 2007; Hernando *et al.* 2007). The tabled LC_{50} values for glyphosate are thousand folds higher than the concentrations listed above for the toxicity of copper. This means that the natural occurring cobber has a larger acute effect than the artificial herbicide glyphosate in relation to the selected test organisms (Table 10).

Table 10 Lethal concentration (LC_{50}) values for glyphosate for different test organisms.

Test organism	LC_{50} [mg/L]	
Vibrio fischeri	36.4	(Amoros *et al.* 2007)
Vibrio fischeri	44.2	(Hernando *et al.* 2007)
Daphnia spinulata	66.18	(Alberdi *et al.* 1996)
Daphnia magna	61.72	(Alberdi *et al.* 1996)

If the two compounds, copper and glyphosate, are classified by the toxicity categories from European Union legislation the herbicide glyphosate is classified as "harmful" whereas cobber is classified as "very toxic" (Table 11).

Table 11 EU classifications of compounds based on their EC value (Hernando *et al.* 2007).

EC_{50} [mg/L]	Classification
< 1	Very toxic
1-10	Toxic
10-100	Harmful

This comparison between an herbicide and a heavy metal show, that it can be difficult to predict and evaluate what effects different kinds of compounds will have in the natural environment.

In reality there is not just one pollutant, such as glyphosate or cobber in stormwater, but a complex composition of several different compounds of different origin. This will naturally create the possibility of combination effects or so-called "cocktail effects" between the compounds when they are mixed in the aquatic environment. Chemical analysis does not take such combination effects into account (Baun 1998; Walker *et al.* 2006). However as the biological toxicity tests not aim towards a specific compound, but the effect of the combination of the present compounds, the toxicity tests will provide a more tangible picture of what will happen in the environment due to the different discharges (Baun 1998).

A total chemical analysis of a water sample would be almost impossible, mostly because the chemical analysis require knowing the identity of the compounds to be quantified, and this far from possible in practice (Baun 1998). Another advantage in using biological toxicity tests is that they only account for the compounds that actual are bioavailable, and this enhances the validity of the results even more (Baun 1998). The amount of organic matter in the water is for instance proven to reduce the toxicity and also the general speciation of the compounds will affect the toxicity (Glover *et al.* 2005; Brooks *et al.* 2007) Cobber for instance can occur in different species, where some are more toxic than others (Allen and Hansen 1996). Collectively biological tests provide different and presumable more ecological relevant information on the effects of polluted stormwater in the receiving waters than the chemical tests.

5 Methods

5.1 Laboratory tests for reduction of toxic compounds from stormwater

The main purpose of the experiments was to evaluate the efficiency of two flocculants for treatment of stormwater. The efficiency of the flocculants was tested by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) elemental analysis for selected elements, and by a test battery of three biological tests.

Experiments were conducted with stormwater collected during spring 2009 from a wet detention pond located in Northern Jutland near Hjallerup City to obtain an alkalinity as in stormwater. The pH values were in the range form 7.25 to 7.83. The catchment area of the pond is unknown, but a highway is located near by. The samples were stored in dark at 5 °C in 10 L containers for maximum 14 days prior to the experiments.

Prior to analysis the stormwater was filtered through a 0.45 μm filter. This had multiple purposes. One of the selected toxicity tests required this filtration and to standardise the three tests, it was chosen to filter the water prior to all analysis, as a part of the toxicity in the samples could be associated with the particles larger than 0.45 μm (Figure 7) (1).

To obtain toxic solutions the stormwater was spiked with different compounds; the heavy metal cobber or the quaternary ammonium compound benzalkonium chloride (Figure 7) (2). The copper solution was prepared from a stock solution of cobber(II)sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$) diluted in demineralised water. Cobber was chosen as a representative for the heavy metals as cobber was found to be one of the heavy metals that predominately exists in the dissolved and colloid fraction (Sansalone and Buchberger 1997; Mosley and Peake 2001; Morquecho 2005). Furthermore cobber was the heavy metals found in the highest concentrations (up to 490 μg/L) in one of the newest Danish ingestions of stormwater runoff from the Danish Environmental Protection Agency (Miljøstyrelsen 2001).

The benzalkonium chloride solution was prepared from a 10 %'s benzalkonium chloride solution, which is used for disinfection and algae removal of roofs and garden furniture and also as an herbicide in aquaculture (Bartolome and Sanchez-Fortun 2005). It is the active compound in the commercial product Rodalon®. The stock solution of benzalkonium chloride was likewise prepared by demineralised water.

Additional experiments were also conducted with stormwater spiked with a mixture of copper, nickel and zinc in the following concentrations; 500 μg Cu/L, 100 μg Ni/L and 500 μg Zn/L. The solution was prepared from a stock solution of cobber(II)sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$), nickel sulfate hexahydrate ($NiSo_4 \bullet 6H_2O$) and zinc sulfate heptahydrate ($ZnSO_4 \bullet 7H_2O$) diluted in demineralised water.

The basic setup of the experiments is illustrated at Figure 7 and described in Appendix A5. Before the samples were spiked with the toxic compound the natural toxicity of the stormwater was tested in either of the toxicity tests. See Appendix A6. After the samples were filtered and spiked with the toxic solutions, samples were withdrawn for the toxicity analysis by the test battery and for the heavy metals analysis by the ICP analysis (Figure 7)(3).

After thoroughly mixing of the samples the flocculent solutions were added (Figure 7) (4). The flocculants tested are iron chloride and aluminium chloride, as these are among the most effective flocculants and holds potential for treatment of stormwater (Life Treasure 2007; PH Consult 2007). The iron stock solution were prepared from iron(III) chloride hexahydrate ($FeCl_3 \bullet 6H_2O$) and diluted with filtered stormwater. Similarly the aluminium stock solution were prepared from aluminium(III) chloride hexahydrate ($AlCl_3 \bullet 6H_2O$).

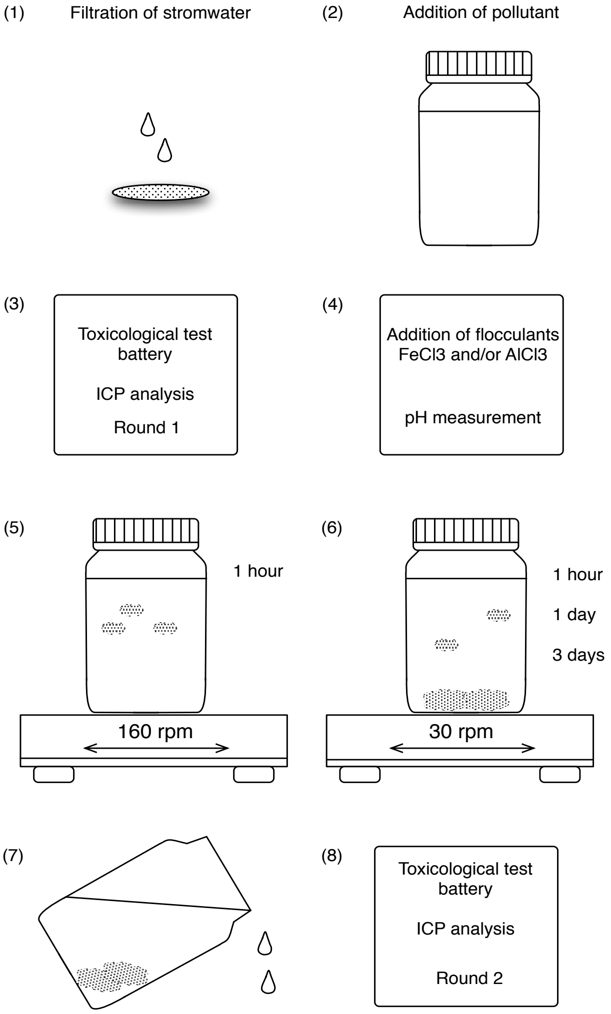

Figure 7 Sketch of general setup and procedure.

The water samples were then added flocculent solution in a concentration series and the pH was measured. Afterwards the sample containers were placed on a vibrating table at 160 rpm (Figure 7) (5). This was done to enhance the interactions between the molecules and ions in the solution, as the forces that influence the coagulation process require relatively short distance between the molecules to have en effect (Keiding unpub.). After one hour the rotation were turned to 30 rpm to provide a gently stirring, that would enhance further coagulation and flocculation but not break up the existing flocs. At 30 rpm the sample are left for precipitation for either 1 hour, 1 day or 3 days (Figure 7) (6). This variation in settling time should clarify the effect of the precipitation time. Three days were chosen, as this is a realistic hydraulic retention time for a Danish wet water pond (Wium-Andersen *et al.* 2009), whereas the 1-hour settling time would represent the retention time during a heavy rain event. After the precipitation period the supernatant was withdrawn and tested for ICP analysis and for toxic effects by three different toxicity tests (Figure 7) (7) (8).

The two flocculants, $FeCl_3$ and $AlCl_3$, were tested both individually and in combination. The most beneficial addition of flocculants is dependant on various parameters such as alkalinity, redox potential, concentration of the unwanted pollutants for removal and also the hydraulic retention time (Life Treasure 2007). Theoretically this is very complex and calculation of the most appropriate addition of flocculent is not applicable in practice. To give an estimate of the range of the most appropriate concentration the flocculants were added to the stormwater in the following concentration series; 0, 5, 10, 20 and 40 mg/L. Furthermore the use of such concentration series should also clarify the upper boundary in relation to any toxic effects of the flocculants. A detailed description of the procedure can be found in Appendix A5.

5.2 Chemical analysis

Heavy metal concentration of the stormwater was analysed prior and after the treatment with flocculants using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (iCAP 6300 from Thermo Scientific). The ICP analysis is based on the decomposition of the sample into atoms, which are exited in argon plasma and the quantity of each element is measured by emission of ultraviolet or visible radiation by the gaseous atoms (Harris 2003).

Prior to analysis; the samples were diluted in ultra pure water and acidified to 0.2 % with 65 % nitric acid (HNO_3). The nitric acid was not of highest analytical quality and could thus contain trace impurities of aluminium, copper, iron, nickel and zinc (Baker 2009). As the detection limits of the ICP are very low, it is not sufficient to prepare to solutions from demineralised water as especially iron could be released from the water pipes. Thus ultra pure water was used to prepare and dilute all samples and solutions.

The samples are drawn into a nebulizer, where fine aerosols are created. Second a stream of argon gas is passed through a heated tube, where the solvent evaporates, and carries the aerosols to a plasma flame of argon. The dry aerosols, the analyte, are atomized and exited in the plasma flame and the emission signal is detected (Harris 2003).

The iCAP 6300 is capable of multi-element analysis and every sample was analysed for the content of copper, nickel, zinc and the flocculants iron and aluminium, respectively. Each element were measured at certain wavelengths and these were chosen in such way that the highest signal intensity and the lowest interference were achieved. For details on the ICP analysis and test procedure see Appendix A1 and A5. The detection limits of the instruments are very low down to less than 1 μg/L if the instrument is optimal adjusted (Table 12).

Table 12 Intervals for the detection limits of the Inductively Coupled Plasma-Optical Emission Spectrophotometer iCAP 6300 when the instrument are optimally adjusted (Thermo Scientific 2009).

	Aluminium and Zinc	Iron, Copper and Nickel
Detection limit interval [μg/L]	0.1-1	0.01-1

The actual detection limits obtained during the analysis performed in relation to this project is much lower than the limits listed above. The actual detection limits were around 30 μg/L for aluminium and zinc, around 6 μg/L for iron and less than 6 μg/L for copper based on the standard curves. The standard curves produced for each of the five elements can be found on the attached DVD.

5.3 Toxicological analysis

The toxicity of stormwater samples were investigated by a battery of tests using *Vibrio fischeri* (bacteria), *Selenastrum capricornutum* (algae) and *Daphnia magna* (crustaceans) as test organisms. The same selection of test organisms has also been used in a number of other Danish studies (Baun 1998; Miljøstryelsen 2001; Wium-Andersen *et al*. 2009).

The toxicity tests were chosen on the basis the following criteria; the tests should be validated and comply with a standard; an existing protocol should be available; the tests should use organisms on different trophic levels; and the test organisms should be from an aquatic habitat to raise the relevance of the attained results. *Vibrio fischeri* is a marine bacterium and will therefore not be found naturally in wet detention ponds, but both *Daphnia magna* and *Selenastrum capricornutum* are indigenous to freshwater.

All three tests are designed to measure acute toxic effects, but with different endpoints. The choice of acute tests was made to attain a more time effective evaluation process of the samples as oppose to tests to measure chronic effects as they mostly run for longer periods.

5.3.1 Bacterial Luminescence Toxicity Test

The test is based on inhibition of the metabolic activity in the marine bacterium *Vibrio fisheri*. The inhibition of the metabolic activity is direct proportional to the light emission of bioluminescence. The light emission by *Vibrio fischeri* is due to the oxidation of flavin mononucleotide ($FMNH_2$) to the oxidized form (FMN) as this produces a blue-green light of 490 nm (See equation below) (Parvez *et al*. 2006).

$$FMN\,H_2 + O_2\ R\text{-}CHO \rightarrow FMN + H_2O + R\text{-}COOH\ \left(hv490\,nm\right)$$

Serial dilutions of the toxic sample were made in 96-well microtitplates and *Vibrio fischeri* culture was added. Prior to analysis the samples were adjusted to a salinity of 2 % as *Vibrio fisheri* is a marine bacterium. The luminescence of the samples was measured after a contact time at 0, 15 and 30 minutes at a Perkin Multilabel Reader. Eight replicates were made for each sample. Decrease in luminescence relative to a control gives an expression for the toxic effect and LC-values can be estimated. Due to the low contact time the test is relatively rapid compared to other toxicity tests and *Vibrio fischeri* is regarded as the most sensitive compared to other bacterial toxicity tests (Parvez *et al*. 2006). The test was carried out as a modified version of the international standard DS/EN ISO 11348-1 (ISO 1999). See Appendix A4 for details.

5.3.2 Algae Growth Rate Toxicity Test

The test is based on inhibition of the growth of the freshwater algae *Selenastrum capricornutum*. The growth of the algae is measured as the change in cell density by optical density (OD) measurements at a wavelength of 670 nm, as the absorbance maxima of the plant pigment chlorophyll a, is found to be at 662 nm (Gross 1991).

Appropriate volumes of algae nutrient media and algae stock were added to the toxic samples corresponding to a cell density of 10^4 cells/mL. The absorbance at 670 nm was then measured at a Shimadzu UVmini-1240 spectrophotometer after a contact time of 24, 48 and 72 hours. Triplicates were made for each sample. The decrease in absorbance relative to a control gives an expression for the toxic effect, as an inhibition in growth rate. When testing for toxicity of heavy metals, EDTA had been omitted from the algae nutrient media, as this forms complex bonds with the metals and thereby reducing the toxicity (American Public Health Association 2005). In this study the toxicity test kit Algaltoxkit F™, MicroBioTests Inc. was applied, but this procedure corresponds to the procedure described in the international standard DS/EN ISO 8692 (ISO 2005). See Appendix A2 for details.

5.3.3 Daphnia Immobility Toxicity Test

The test is based on immobility of the crustacean *Daphnia magna*. The ability of the daphnia to swim is affected by toxic compounds and is therefore used as an expression of the toxic effect. Daphnia magna is easier to handle than other species of daphnia because of its larger size and the studies of the toxicity of cobber have shown that this species exhibits the same degree of sensitivity as other daphnia species (Winner and Farrell 1976). The daphnia were exposed to toxic samples and immobile daphnia were counted after 24 and 48 hours of exposure. Five daphnia were incubated in small wells and triplicates were made for each sample. For each test, a control was made to ensure that the immobilization was caused by the toxic effect and not by any physical stresses. In this study the toxicity test kit Daphtoxkit F™, MicroBioTests Inc., was applied, based on the international standard DS/EN ISO 6341 (ISO 1997). See Appendix A3 for details.

6 Results on Removal of Pollutants by Addition of Flocculants

6.1 Evaluation by heavy metal analysis

The removal of pollutants from the stormwater was evaluated by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis to determine the concentrations of selected heavy metals in the stormwater. The analysis primarily focused on copper and the two metals in the flocculants; aluminium and iron. Some stormwater samples were also spiked with the metals nickel and zinc, and in these cases the ICP analysis also included these metals. The date where the individual tests were performed is written in parenthesis in the caption under each figure.

Iron flocculent

The efficiency of iron chloride ($FeCl_3$) as a flocculent for removal of heavy metals from stormwater was tested by addition of iron chloride in a series of concentrations; 0, 5, 10, 20 and 40 mg $FeCl_3$/L. Besides this, the effect of the length of the precipitation period was also tested by varying the period that the stormwater was left to precipitate before the decantation. The tested lengths were; 1 hour, 1 day and 3 days. Where the precipitation period is not specified it is 1 hour.

The efficiency of iron chloride as a flocculent for removal of heavy metals proved to be directly proportional with the addition of flocculent as the general picture showed that the concentration of cobber in the decanted stormwater decreased as the concentration of added iron chloride increased (Figure 8 and Figure 11). The same tendency was seen with the removal of zinc, when the stormwater was spiked with this, but the tendency was not observed for nickel (Figure 9).

All samples were spiked with copper, zinc and/or nickel. Copper and zinc were added to obtain a concentration of 500 μg/L and nickel to obtain a concentration of 100 μg/L. In most experiments a water sample were withdrawn after the addition of metal and before the addition of flocculants, and this concentration is termed "start". The concentrations of metal in the start samples were found to be significantly lower than the added amount at 500 μg copper or zinc pr. litre and 100 μg nickel pr. litre (Figure 8 and Figure 9). The reason for this is not known, but it could be do to adsorption of the metals to the surfaces in the glass bottles.

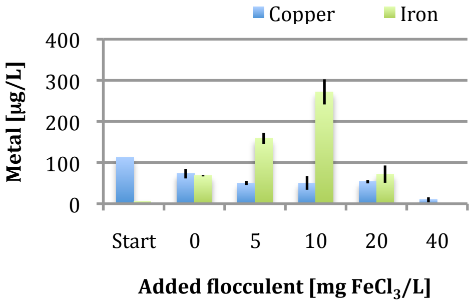

Figure 8 Concentrations of copper and iron in stormwater spiked with 500 μg Cu/L and treated with different concentrations of iron chloride. The precipitation period is 1 hour (18.03.09).

Besides the addition of different concentrations, the significance of the length of the precipitation period was also tested. The tests with iron chloride as flocculent showed that, the longer the precipitation period, the more efficient removal of copper and zinc (Figure 9, Figure 10 and Figure 11). A precipitation period of 3 days resulted in up to 100 % removal of copper and zinc and this was regardless of the added flocculent concentration, but the results also showed that the 3 days precipitation period also resulted in a up to 100 % removal without the addition of iron flocculent (Figure 10). The removal of nickel was not as effective and the removal did not seem to be dependant of the flocculent concentration added (Figure 9 and Figure 10).

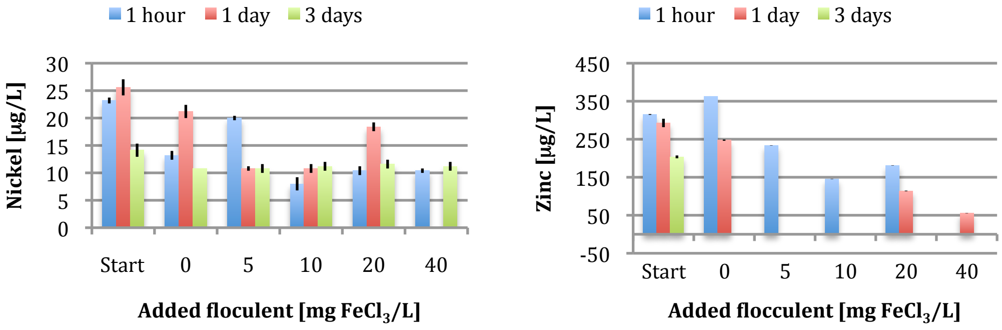

Figure 9 Concentration of nickel and zinc in stormwater treated with iron chloride flocculent in different concentrations. The "start" concentration represents the concentration of nickel and zinc measured in the stormwater after the addition of nickel and zinc, but before the addition of flocculent (07.05.09).

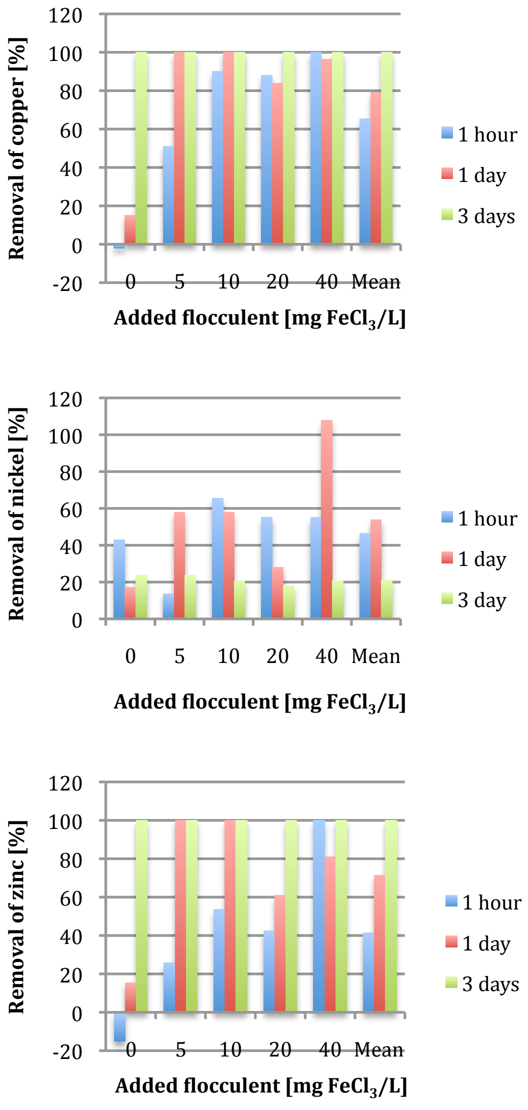

Figure 10 Percentage removal of copper, nickel and zinc from stormwater treated with iron flocculent in different concentrations (07.05.09).

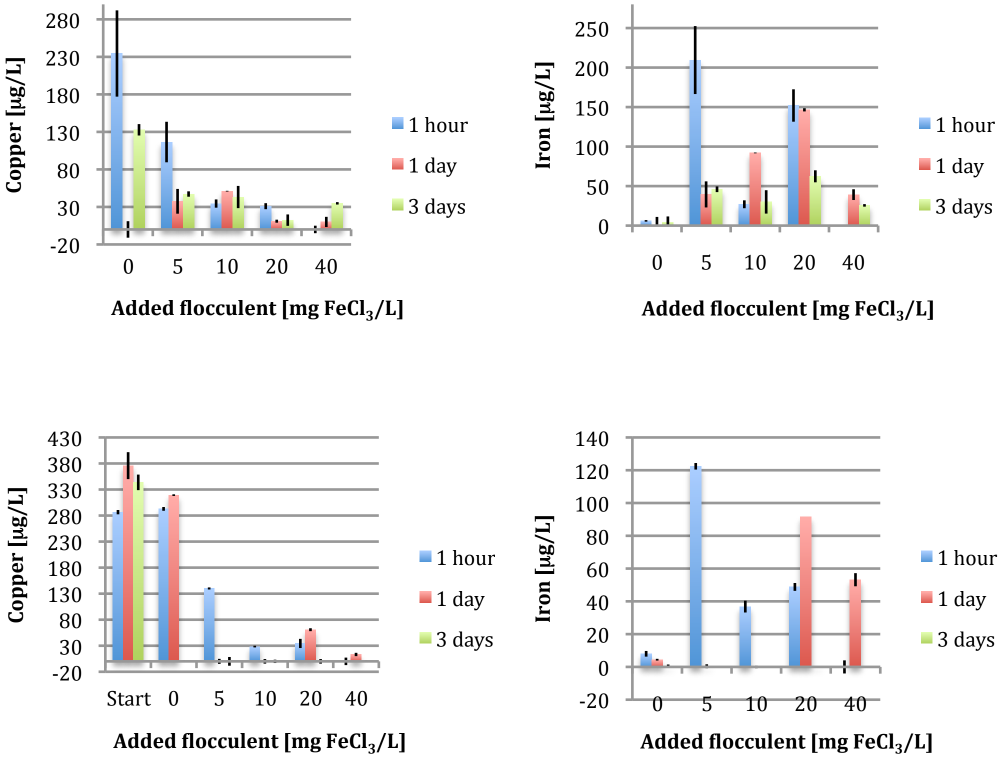

Figure 11 Concentrations of copper (left) and residual iron (right) in stormwater spiked with 500 µg Cu/L and treated with different concentrations of iron chloride. The stormwater was left to settle for 1 hour, 1 day and 3 days respectively (Top - 05.04.09) (Bottom - 07.05.09).

The addition of iron chloride as flocculent also resulted in increased iron concentrations in the different samples, up to 270 µg/L. However the concentrations of iron left after the precipitation and decantation did not seem to be related to the flocculent concentrations added originally (Figure 8 and Figure 11).

Aluminium flocculent

Like for iron chloride, aluminium chloride was also tested in a series of concentrations and precipitations periods. The removal of copper by addition of aluminium flocculent showed the same tendency as the removal by the iron flocculent; that the removal was directly proportional to the addition of flocculent (Figure 12 and Figure 13). In addition to copper, aluminium chloride was also tested in relation to stormwater spiked with both nickel and zinc. Only a single test was performed with these metals. As for the iron flocculent, the aluminium chloride did not result in an effective removal of nickel from the stormwater, and the removal of zinc was independent of the concentration of added flocculent (Figure 13). The removal of zinc was as effective as the removal of copper and for both metals up to 100 % was removed from the stormwater after a precipitation period of 1 day and 3 days (Figure 15).

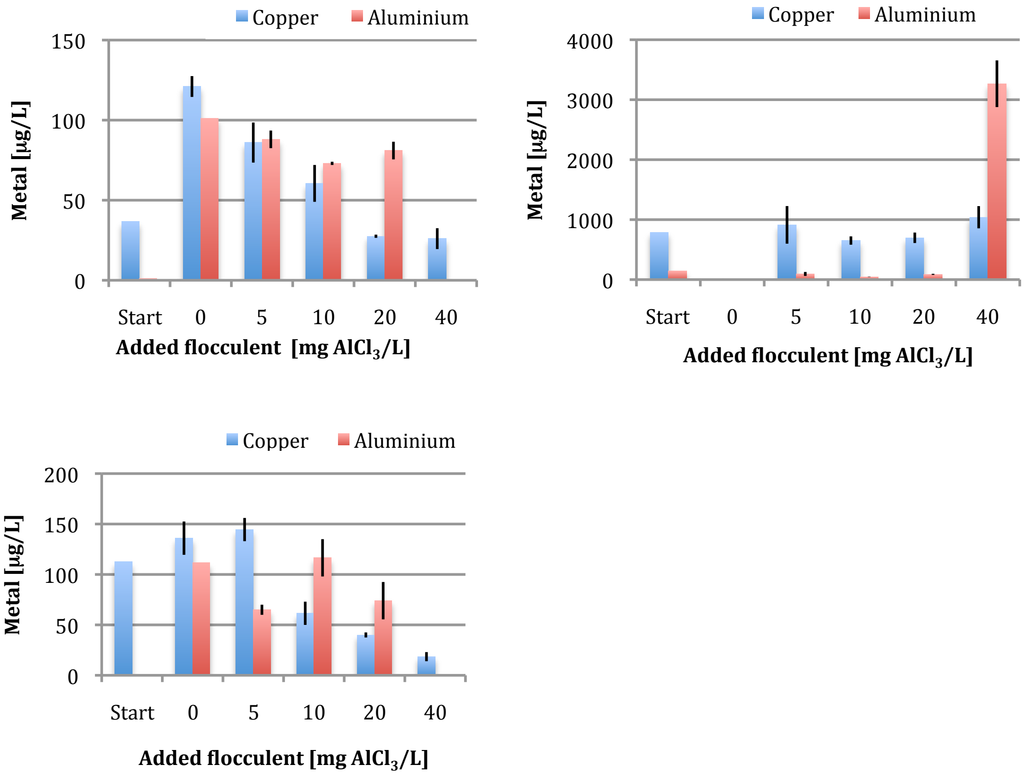

Figure 12 Concentration of copper and aluminium in stormwater spiked with 500 µg Cu/L and treated with different concentrations of aluminium chloride. The stormwater was left to precipitate for 1 hour (Top left - 29.03.09) (Top right - 25.03.09) (Bottom right – 18.03.09).

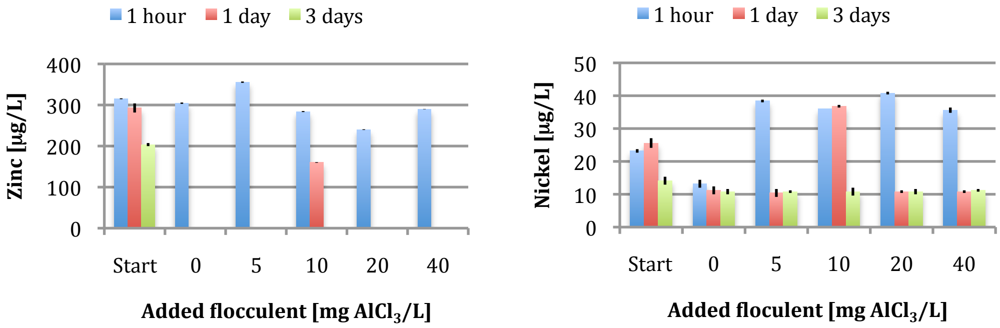

Figure 13 Concentration of nickel and zinc in stormwater spiked with 100 µg Ni/L and 500 µg Zn/L. The stormwater was treated with aluminium chloride in different concentrations and was left to precipitate for 1 hour, 1 day and 3 days respectively after the addition of flocculent (07.05.09).

Most of the tests preformed with aluminium chloride also showed that the addition of aluminium resulted in an increase in the stormwater aluminium concentration after the precipitation and decantation. The aluminium concentration found in the treated stormwater varied from 0-3200 µg/L (Figure 12 and Figure 14).

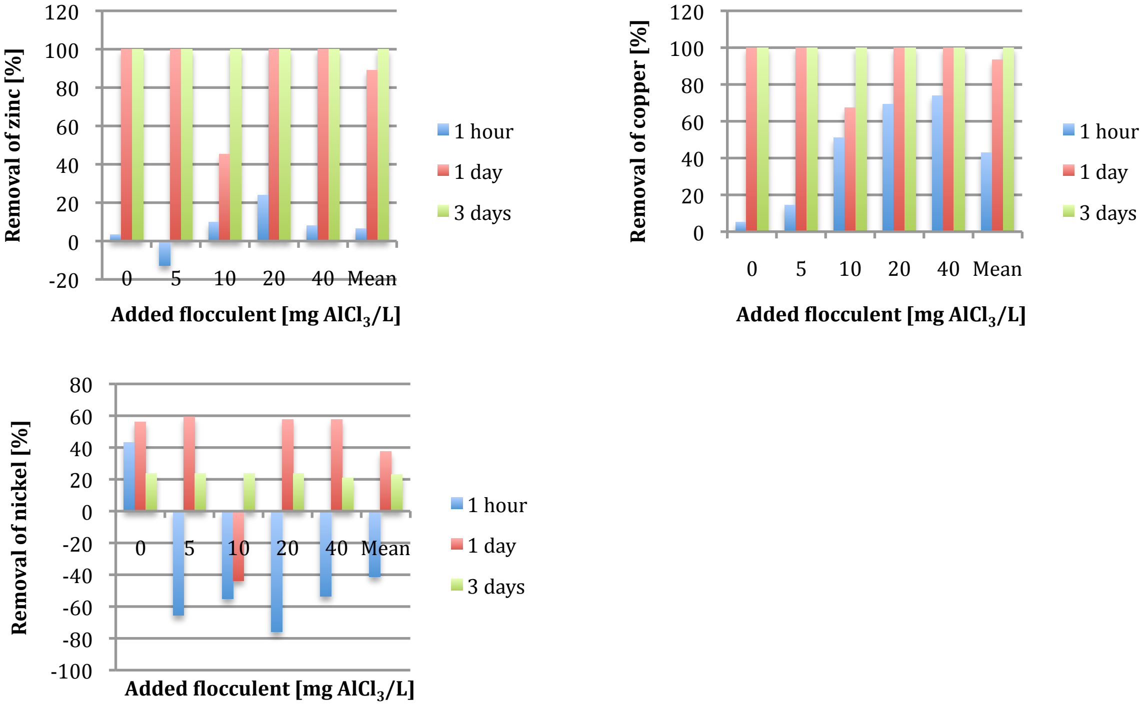

Figure 15 Percentage removal of copper, nickel and zinc from stormwater treated with aluminium chloride and left to precipitate for 1 hour, 1 day and 3 days respectively (05.07.09).

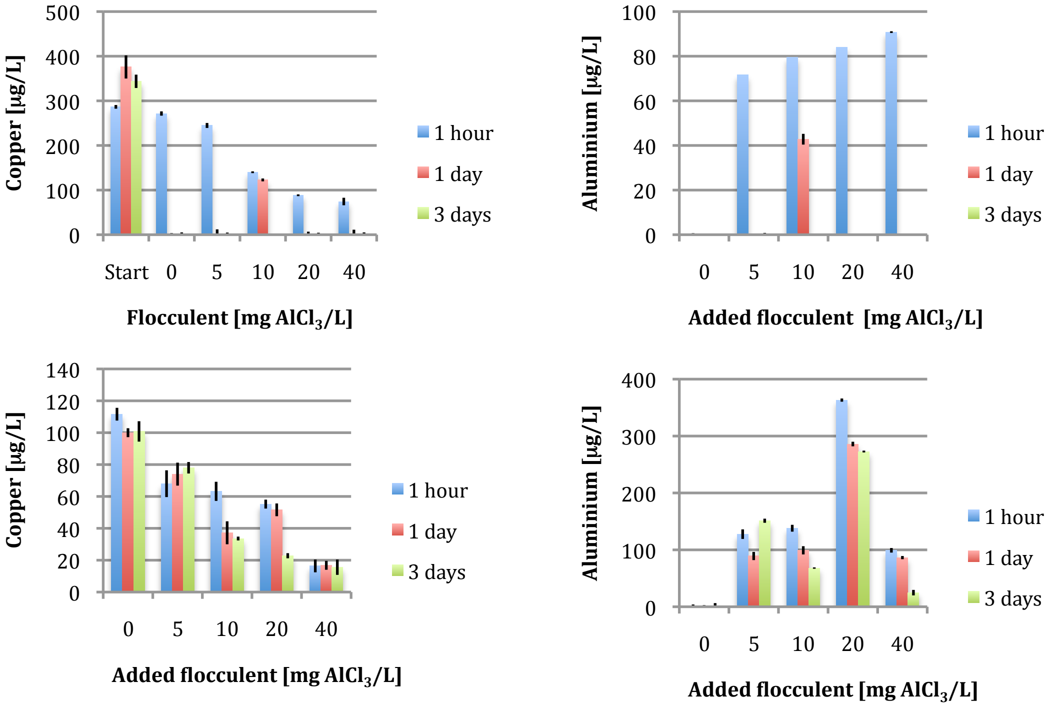

Figure 14 Concentration of copper and aluminium in stormwater spiked with 500 g Cu/L and treated with different concentrations of aluminium chloride. The stormwater has been left to settle after the addition of aluminium flocculent for 1 hour, 1 day and 3 days respectively (Top - 05.04.09) (Bottom – 07.05.09).

Iron and Aluminium flocculants in combination

The flocculants iron and aluminium chloride were also tested in combination where the concentrations series consisted of 50 % aluminium chloride and 50 % iron chloride based on weight. The concentration series also went from 0 mg/L to 40 mg/L as the series for addition of single flocculants. The tests with the combination of flocculants showed the same direct proportionality between the removal and the concentration of added flocculent, as the addition of flocculants alone, however the tendency was not as clear compared to the use of a single type of flocculent (Figure 16 and Figure 17). The experiments also showed that the removal of metals also was more effective with a 1 hour and 1 day precipitation period compared to a 3-day period (Figure 18).

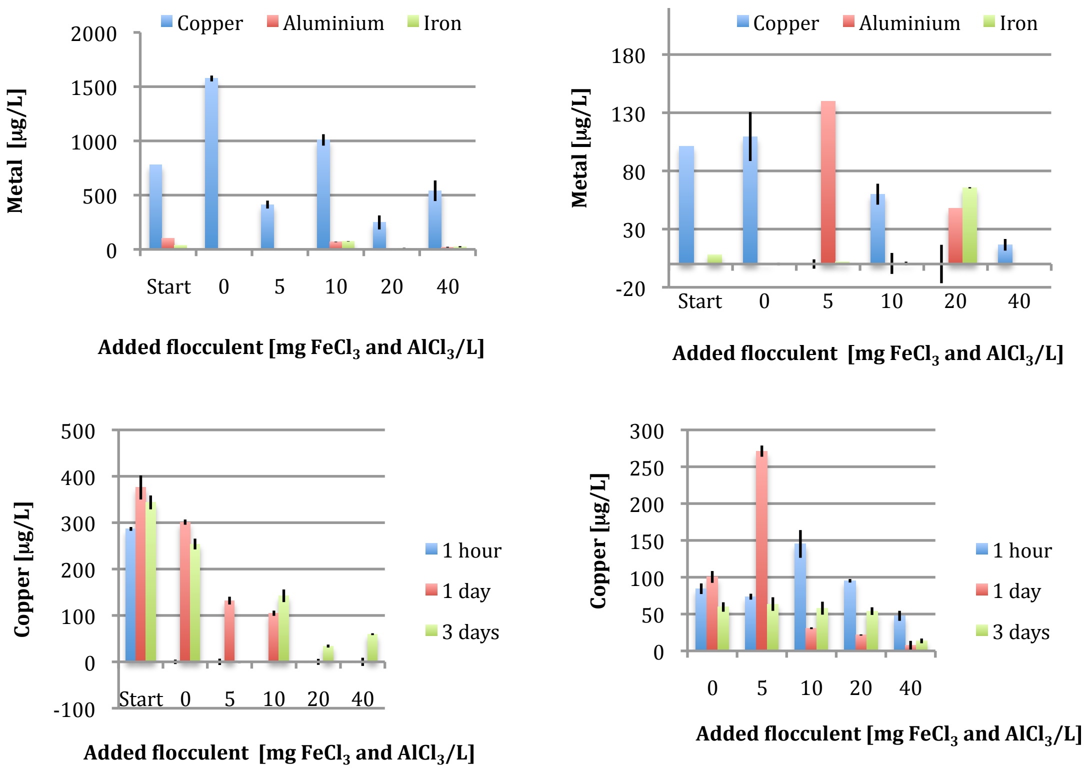

Figure 16 Concentration of copper, aluminium and iron in stormwater spiked with 500 µg Cu/L and treated with a combination of iron and aluminium chloride in different concentrations (Upper left – 25.03.09) (Upper right – 29.03.09) (Lower right 05.04.09) (Lower left 05.07.09).

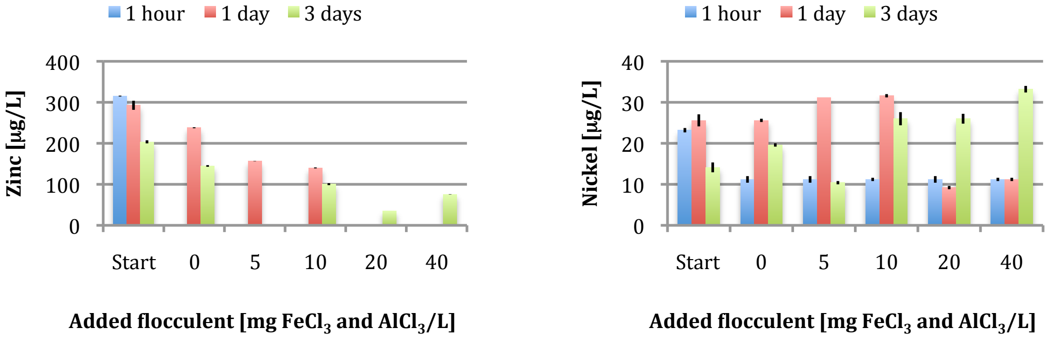

Figure 17 Concentration of zinc and nickel in stormwater spiked with 500 µg Cu/L and treated with a combination of iron and aluminium chloride in different concentrations. The stormwater was left to precipitate for 1 hour, 1 day and 3 days respectively (07.05.09).

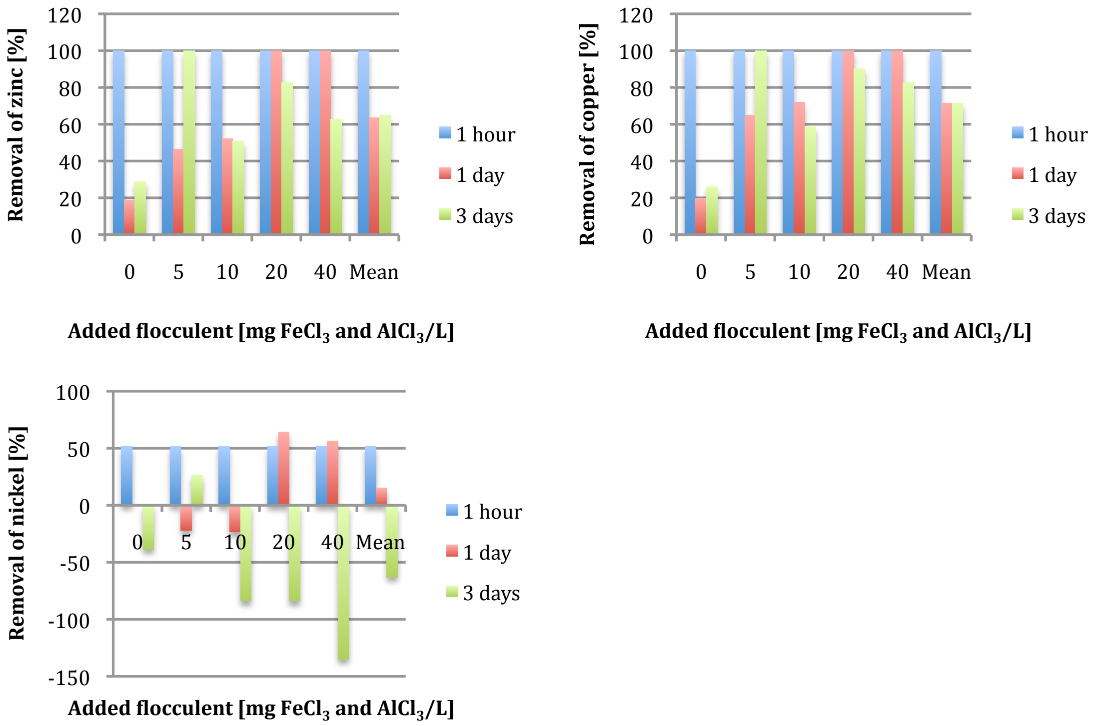

Figure 18 Removal of copper, zinc and nickel from stormwater treated with iron and aluminium chloride in combination (07.05.09).

As showed at Figure 19 substantial parts of the added iron and aluminium flocculent are left in the stormwater after the precipitation and decantation up to 800 µg Fe and Al/L. Surprisingly the stormwater with the shortest precipitation period has the lowest content of flocculent left. Looking at the partitioning between iron and aluminium it seems that more aluminium is left in the stormwater (Figure 20).

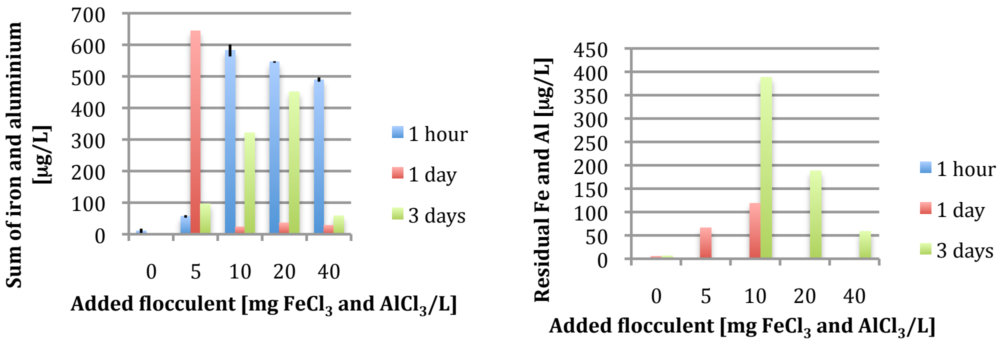

Figure 19 Concentration of aluminium and iron left in the stormwater after precipitation and decantation. The stormwater was left to precipitate for 1 hour, 1 day and 3 days, respectively (Left 05.04.09) (Right 07.05.09).

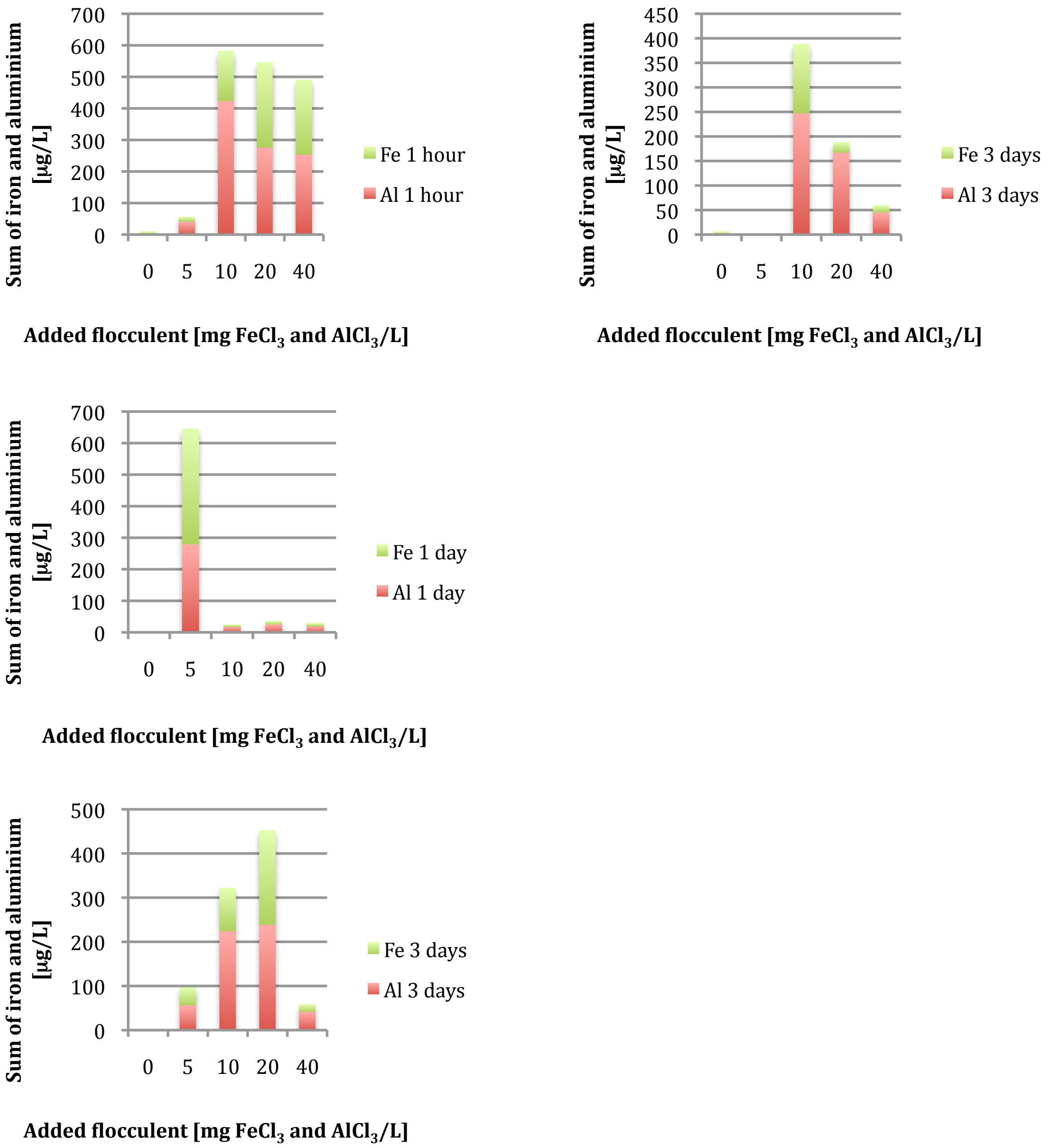

Figure 20 Residual aluminium and iron in stormwater after decantation and 1 hour, 1 day and 3 days of precipitation (Left column 05.04.09) (Right column 07.05.09).

6.2 Evaluation by biological toxicity test

In addition to the chemical analysis, three toxicity tests using three different test organisms evaluated the addition of iron or/and aluminium chloride as flocculants. The test organisms were bacteria (*V. fischeri*), green algae (*S. capricornutum*) and daphnia (*D. magna*). The efficiency of the flocculants was evaluated both in regard to stormwater spiked with the heavy metals copper, zinc and nickel and to the disinfection agent benzalkonium chloride. Where it is possible the results from the *D. manga* test are displayed in red and the results from the *S. capricornutum* test are displayed in light blue, and where the precipitation period is not mentioned the precipitation period is 1 hour.

Iron flocculent

The iron flocculent was evaluated by the *S. capricornutum* algae toxicity test in relation to stormwater spiked with copper (500 µg/L), but the test organism proved to be very sensitive and the performance of the test were complicated by difficulties with the growth of the algae. See more details on this in Appendix A8. The results from the toxicity tests with *S. capricornutum* showed a more mixed picture than the chemical analysis. One test showed that the addition of iron chloride decreased the toxicity of the stormwater, however another test showed that the flocculent was not able to remove the toxicity of the stormwater (Figure 21).

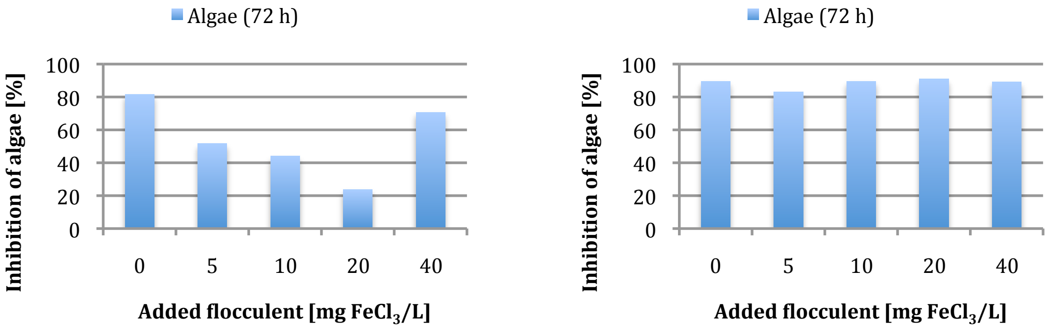

Figure 21 Toxic responses from *S. Capricornutum* to stormwater spiked with 500 µg Cu/L and treated with iron chloride flocculent in five different concentrations (Right 13.03.09 - left 18.03.09). The date of the performance of the individual tests is written in parenthesis.

Besides the *S. Capricornutum* tests, the efficiency of the iron flocculent was also tested by the *D. magna* daphnia test where the stormwater was spiked with 500 µg copper. These tests showed that the addition of iron chloride was able to reduce the toxicity of the stormwater to a level were no toxicity could be detected. The reduction in toxicity proved in most experiments to be directly proportional with the concentration of added flocculent (Figure 22).

However, two other tests, that were identical to the above-mentioned tests, were also conducted with the daphnia. In one of these tests (09.03.09) did all the daphnia survive 48 hours in the stormwater spiked with 500 µg copper/L and treated with 0, 5, 10, 20 and 40 mg $FeCl_3$/L, respectively. In another identical test all the daphnia were dead after only 24 hours (26.03.09) (Figure 23). Except from these two tests, the daphnia test was experienced to be more reproducible than the algae test and easier to perform.

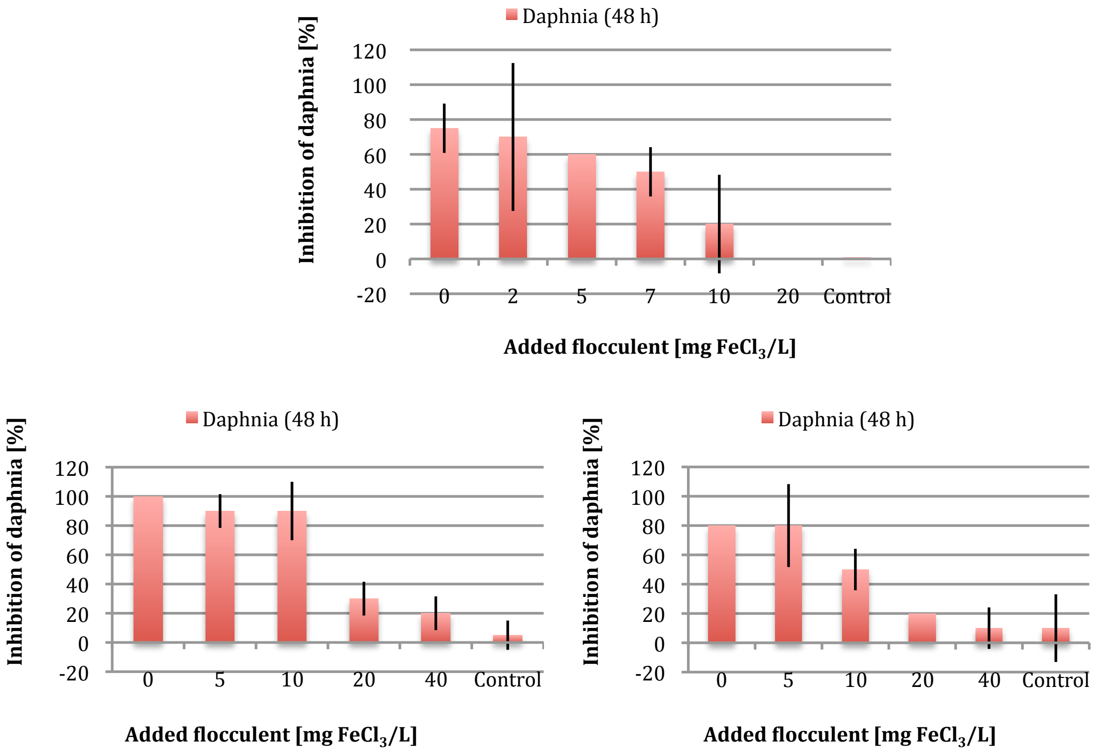

Figure 22 Toxic response from *D. magma* in relation to stormwater spiked with 500 µg Cu/L and treated with different concentrations of iron flocculent (Top 02.03.09) (Bottom left 12.03.09) (Bottom right 18.03.09).

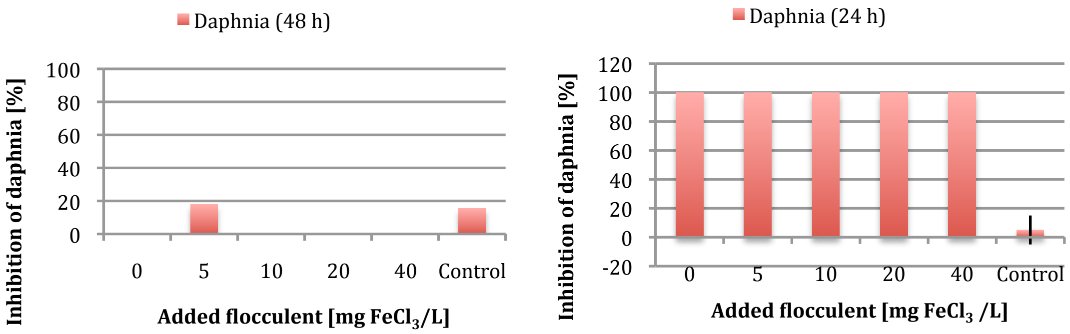

Figure 23 Inhibition of *D. magna* from stormwater spiked with copper (500 g Cu/L) and treated with iron chloride in different concentrations (Left 09.03.09) (Right 26.03.09).

The effect of the length of the time period available for the flocculation, coagulation and precipitation was also tested with the daphnia. This was done by varying the length of period from the addition of the flocculent and to the stormwater, was decanted. The time period was varied from 1 hour to 1 day and to 3 days respectively. The toxic response from the daphnia test indicated, that it had no impact weather the period was hours or days (Figure 24).

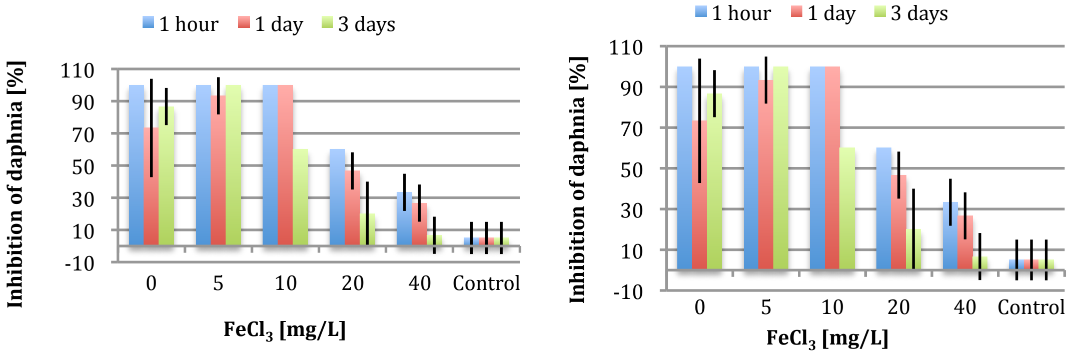

Figure 24 Toxic response from *D. magna* in relation to stormwater spiked with 500 µg Cu/L and treated with iron chloride in different concentrations were the precipitation period is varied from 1 hour to 1 day to 3 days (05.04.09) (07.05.09).

Additional tests were preformed with the bacteria *V. fischeri* to test the toxic response of this organism towards the spiked stormwater. The experiments with stormwater spiked with copper showed that the test organism was insensitive to the spiked copper concentration of 500 µg Cu/L, so no further experiments were conducted with *V. fischeri* to test the flocculants in relation to removal of copper.

The *V. fischeri* bacteria proved to be more sensitive towards the disinfection agent benzalkonium chloride. The ISO standard procedure (ISO 11348-1) that describes the *V. fischeri* test proscribes a total concentration of 10^7 cells/mL for a valid test, but to obtain any measurable change in the light emission from the bacteria it was necessary to dilute the bacteria culture by a factor 10^3. The experiments with diluted culture and benzalkonium chloride showed that the benzalkonium chloride induced an inhibition of the emission signal of 50 %, but this was regardless of the concentration of benzalkonium chloride (Figure 25). Similarly the addition of iron flocculent did not lower the toxicity of the stormwater in relation to the *V. fischeri* bacteria and this was similarly regardless of the flocculent concentration, which was varied from 5 to 40 mg/L (Figure 25). The same pattern was measured for the aluminium chloride flocculent and aluminium and iron chloride in combination). The remaining dose-response curves and data from this experiment also showed no decrease in the toxicity of the stormwater and can be viewed on the attached CD.

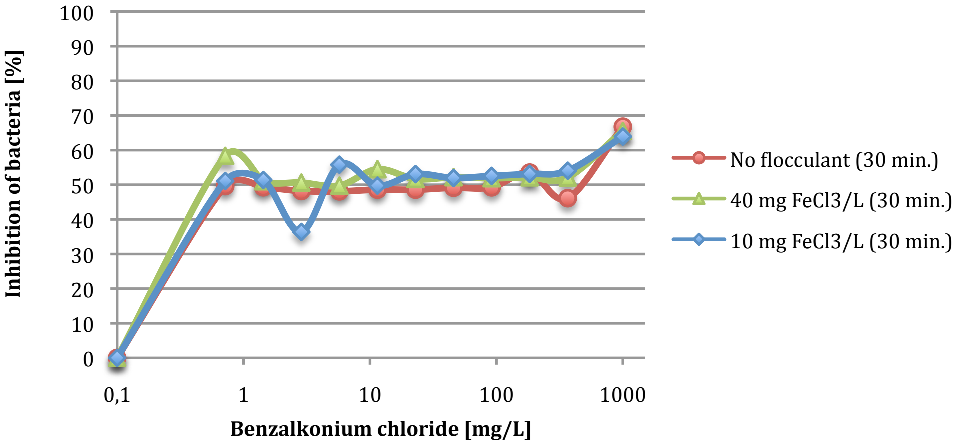

Figure 25 Dose-response curve for *V. fischeri* for stormwater spiked with benzalkonium chloride and treated with iron flocculent (17.04.09).

Aluminium flocculent

Similarly, the efficiency of aluminium chloride was also tested on stormwater spiked with copper. The flocculent concentration was also added in different concentrations in the following series; 0, 5, 10, 20 and 40 mg $AlCl_3$/L. As for the iron flocculent, the efficiency of the flocculent was directly proportional with the concentration of flocculent, however the tendency was weaker and not significant. This was found both by the algae and the daphnia tests (Figure 26).

As for the iron flocculent, the tests with aluminium flocculent also resulted in some irregular results where 100 % to 80 % of the daphnia were dead after 48 hours in stormwater spiked with 500 µg Cu/L and treatment with aluminium chloride in different concentrations (25.03.09)(29.03.09) (Figure 26). As for iron chloride, aluminium chloride was also tested in relation to different precipitation periods. The results showed great variability in the toxic responses and there are no clear effects of the varying precipitation periods (Figure 27).

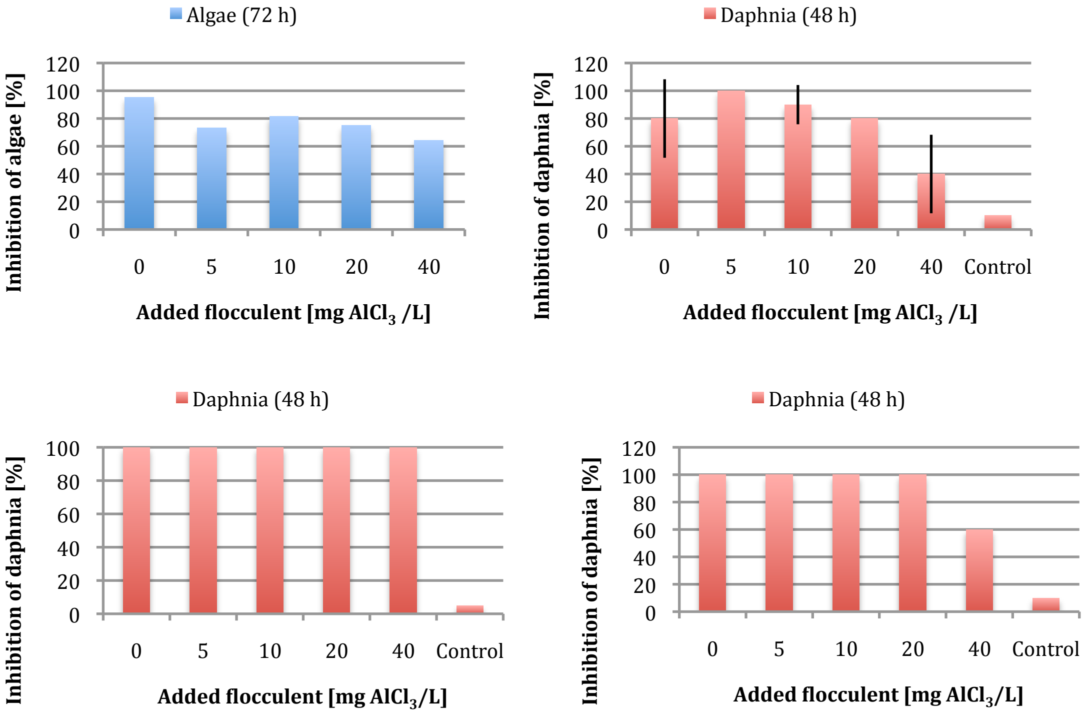

Figure 26 Toxic response of *S. capricornutum* and *D. magna* in relation to stormwater spiked with 500 g Cu/L and treated with aluminium chloride in varying concentrations (Top left - 25.03.09) (Top right 29.03.09) (Bottom left 29.03.09 Bottom left 25.03.09).

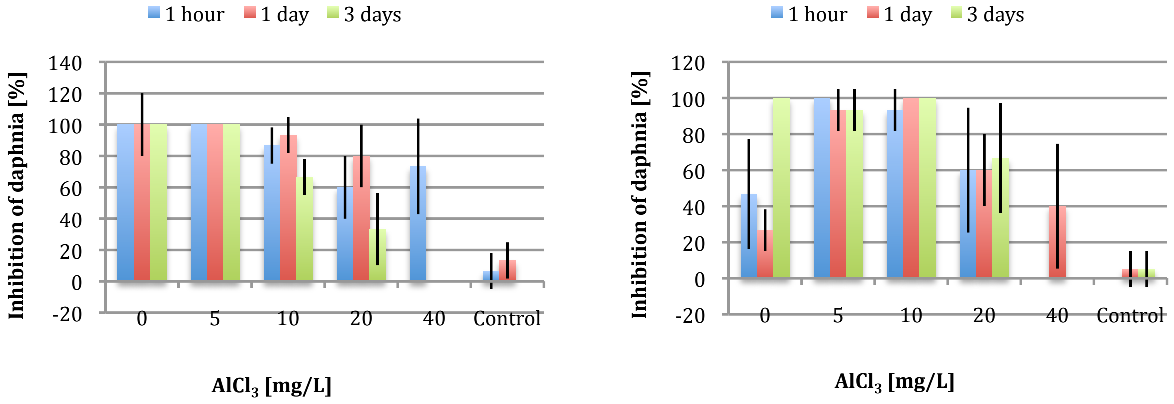

Figure 27 Toxic response of *D. magna* in relation to stormwater spiked with 500 µg Cu/L and treated with aluminium chloride in different concentrations were the precipitation period is varied from 1 hour to 1 day to 3 days (Left - 08.04.09) (Right - 07.05.09).

Iron and Aluminium flocculants in combination

A few tests were also conducted where the flocculants iron and aluminium chloride were used in combination. One test showed a decrease in the toxic response from *D. magma* proportional to the flocculent concentration, whereas another test showed a toxic response of 100 % regardless of the flocculent concentration (Figure 28).

The effect of the precipitation period was also tested for the two flocculants in combination, but these tests showed that there was no significant difference in the toxic response of the stormwater with different precipitation periods (Figure 29).

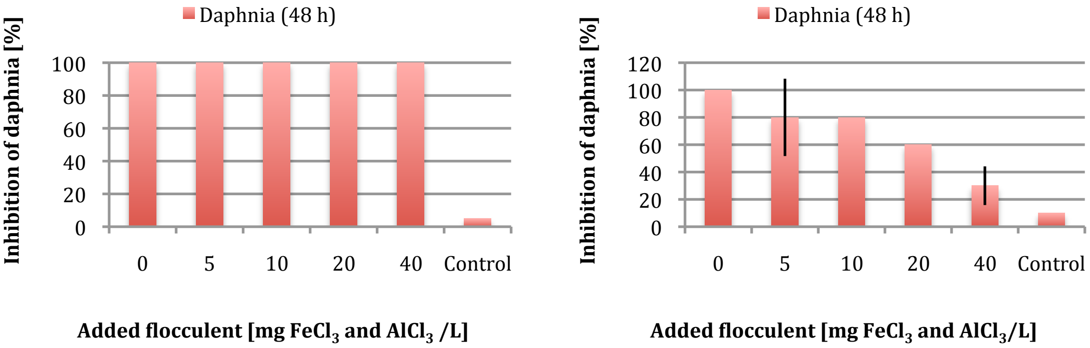

Figure 28 Toxic response from *D. magna* in relation to stormwater spiked with 500 g Cu/L and treated with the flocculants aluminium and iron chloride in combination (Right 25.03.09) (Left 29.03.09).

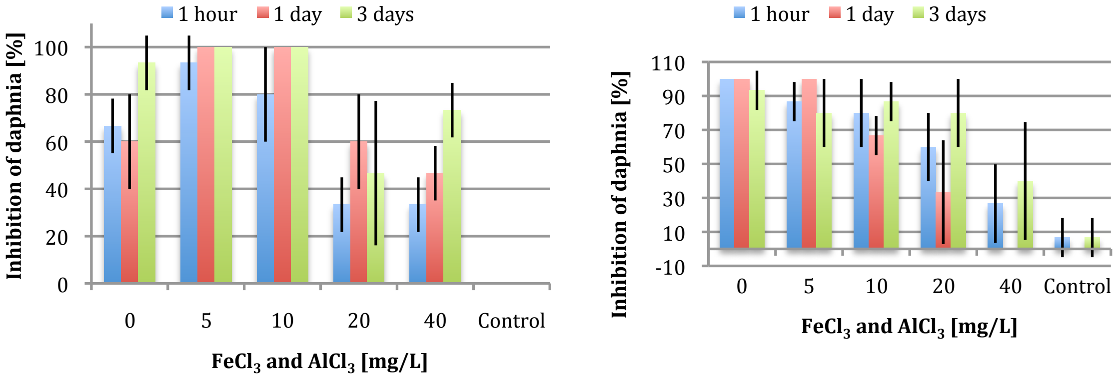

Figure 29 Toxic response of *D. magna* in relation to stormwater spiked with 500 µg Cu/L and treated with aluminium and iron chloride in combination in different concentrations were the precipitation time is varied from 1 hour to 1 day to 3 days (08.04.09) (07.05.09).

6.2.1 Sensitivity of toxicological tests

The sensitivity of the three biological toxicological tests can be described in different ways. Below is the sensitivity of the tests described by a comparison of the LC_{50} values for each of the three test organisms towards cobber and benzalkonium chloride, respectively. The algae test with *S. capricornutum* proved to be the most sensitive of the three tests followed by the daphnia test with *D. magna* and the bacteria test with *V. fischeri.* This order was valid for both copper and benzalkonium chloride. Appendix A7 shows the dose-response curves associated with listed the LC_{50} values.

Table 13 LC_{50} values of copper for the three test organisms *V. fischeri*, *S. capricornutum* and *D. magna*. It was not possible to attain any usable dose-response curves for the algae test thus it was not possible to determine any LC_{50} values towards copper for the algae.

LC_{50} [µg/L] *V. fischeri* – bacteria	LC_{50} [µg/L] *S. capricornutum* - algae	LC_{50} [µg/L] *D. magma* – crustacean
1100	n.d.	170
1600	n.d.	

Table 14 LC_{50} values of benzalkonium chloride to the three test organisms *V. fischeri*, *S. capricornutum* and *D. magna*.

LC_{50} [µg/L] *V. fischeri* – bacteria	LC_{50} [µg/L] *S. capricornutum* - algae	LC_{50} [µg/L] *D. magna* – crustacean
400	90	180

6.2.2 Summary

In general, both iron chloride and aluminium chloride proved to be able to remove copper and zinc from stormwater. This was documented by both the chemical analysis, which measured the concentration of the compounds and also by the biological toxicity tests. The removal of metals proved to be directly proportional to the concentration of added flocculent. However some tests both chemical and toxicological did not show this tendency and in some test the flocculants were not able to remove the toxicity and metals.

The experiments also showed that the addition of iron chloride and aluminium chloride resulted in elevated levels of iron and aluminium in the stormwater after the precipitation and decantation of the stormwater.

Additionally the effect of the precipitation period were evaluated and the results indicated that the longer the precipitation period the more efficient removal as the precipitation period of 3 days resulted in the most efficient removal. However the precipitation experiments also revealed examples of equal removal regardless of the addition of flocculent or not, if just the precipitation period were long enough. The toxicological tests did not show any significant effect in the toxic responses weather the precipitation period were 1 hour, 1 day or 3 days.

7 Discussion

7.1 Evaluation of flocculants

The results from the flocculation experiments showed that both flocculants were able to remove copper and zinc from stormwater. The removal was documented by both the chemical analysis and the toxicological analysis, and generally the removal of the metals was directly proportional with the added concentration of flocculent.

These findings were not surprising as the use of iron and aluminium flocculants in other contexts such as lake restoration, drinking and wastewater treatment is widespread and have proven to be effective (Boers *et al*. 1992; Quaak *et al.*1993; Jaeger 1994; Deppe and Benndorf 2002; Karlby and Sørensen 2002; Burton and Stensel 2003; Winther *et al*. 2004). Limited studies have been preformed on treatment of stormwater with flocculants and it has also been concluded that the toxicity of stormwater and pollutants content can be reduced by treatment with aluminium flocculent (Heinzmann 1994; Wood *et al*. 2005; Wium-Andersen *et al*. 2009).

In lake restoration projects, the target compounds are primarily nutrients and phosphorous in particular, whereas the spectre is wider for wastewater, where the target compounds are suspended solids, BOD, nutrients, organic micro pollutants and heavy metals (Wetzel 1983; Burton and Stensel 2003). In these connections, the flocculants are added in excess compared to the target pollutants and this strategy would also be reasonable in relation to treatment of stormwater.

In this project, both iron chloride and aluminium chloride were added in concentrations ranging from 5 to 40 mg/L, and in comparison the stormwater was spiked with copper to a concentration of 0.5 mg/L. Based on the stoichiometry, this means that 3-27 times more iron or aluminium flocculent was added than copper. An actual dosage is hard to give recommendations on, as this would depend on e.g. the alkalinity of the water and the actual concentrations of metals/target compounds in the pond, which varies.

The dosage of the flocculent can be applied in different ways. In lakes the flocculants are added either to the sediments or by dispersion in the water phase and on treatment plants the dosage is often preformed flow proportional into the process tanks (Wetzel 1983; Karlby and Sørensen 2002; Burton and Stensel 2003). All applications are possible in wet detention ponds dependant on the location. In the Life Treasure project two application forms are tested as aluminium is dosed flow proportional and iron is dosed by dispersion in the water phase and left to precipitate thereby augmenting the sediment concentration of iron (Life Treasure 2009).

Removal efficiency

The percentile removal of the metals was found to be lowest for nickel (-41-54 %) whereas the percentiles for copper and zinc were significantly higher. Generally the removal of copper was more effective (43–100 %) than the removal of zinc (6-100 %). The removal also proved to be more effective the longer the precipitation period were (Table 15).

Compared to the performance of wet detention ponds with no advanced treatment, the removal is clearly enhanced by the addition of flocculants if only the percentiles for a 3 days precipitation period are regarded (Table 15, Table 16 and Table 17). The retention times in the listed wet detention ponds are not known, but are assumed to be days. The pond investigated by Vollertsen *et al*. 2007 was designed with a hydraulic retention time of 3 days with a return period of 3-4 pr. year.

The high removal percentiles for the treated stormwater should however be evaluated as laboratory rates, whereas the listed data for normal wet detention ponds are full-scale numbers. The experiments conduced in the laboratory with the flocculants are performed with addition of copper sulfate that fast dissociate into copper ions and under very controlled conditions. The small volume of the blue cap bottles and the high concentrations of both target compounds and flocculent enhances the contact between the two. The situation is much more complex in an actual retention pond where other compounds are present, were the circulation is not as consistent and steady and where the metals are part of a dynamic pool that changes due to various processes.

Table 15 Mean percentile removal of copper, nickel and zinc from stormwater by addition of iron and aluminium flocculants (04.-0.7.05.09).

Flocculent and precipitation	Removal – Cu [%]	Removal Ni – [%]	Removal –Zn [%]
Al – 1 hour	43	- 41	6
Al – 1 day	93	38	89
Al – 3 days	100	23	100
Fe – 1 hour	65	47	41
Fe – 1 day	79	54	72
Fe – 3 days	100	21	100

Table 16 Treatment performance of a stormwater detention pond with no advanced treatment applied. The pond was designed with a hydraulic retention time of 3 days with a return period of 3-4 pr. year (Vollertsen *et al*. 2007).

	Removal – Cu [%]	Removal –Zn [%]	Removal –Pb [%]	Removal – Cd [%]
Overall	58	71	76	60
Only summer	66	81	83	70

Table 17 Performance of 25 wet detention ponds with no advanced treatment applied in relation to heavy metals based on data from 1999 to 2008 (International Stormwater Best Management Practices (BMP) Database 2008).

	Removal - total [%}	Removal – dissolved [%]
Cadmium	45	42
Copper	29	40
Chromium	76	63
Lead	27	52
Zinc	-	31

Residual flocculent in treated stormwater

The chemical analysis showed that both iron and aluminium were left in the stormwater after precipitation and decantation. This could be due the ineffective flocculation, coagulation or precipitation, but it is more likely to be due to the decantation. The experiments were conducted in 100 mL blue cap bottles with a total height of 10 cm, and this small height of the bottle makes the decantation difficult.

On average there were 113 μg Al/L in the stormwater after precipitation and decantation whereas there were 44 μg Fe/L in the stormwater. The aluminium flocs were harder to identify visually, due to their white colour, whereas the iron flocs were easily identified. This difference in the visibility of the iron flocs made the decantation easier, and this could have resulted in a more effective decantation for stormwater treated with iron.

This surplus of iron and aluminium in the stormwater is problematic as aluminium can be toxic to living organisms. The toxicity varies greatly with pH and other parameters, and under different circumstances aluminium is not toxic (Gostomski 1990; Gensemer and Playle 1999). Most studies report aluminium to be toxic under acid conditions, and above pH 8.5 and below 4.5 there is a risk of formation of toxic forms of aluminium hydroxides (Baker and Schofield 1982; Havas 1985; Gostomski 1990; Gensemer and Playle 1999; Life Treasure 2007). In actual application in wet detention ponds, the pH can rise above 8.5 during intense photosynthesis and the toxic $Al(OH)_4^-$ could be formed.

The observed pH in the stormwater analysed in this project was in the range from 7.25 to 7.83. According to theory, the aluminium ion will become hydrated and the dominating aluminium hydroxide form will be $Al(OH)_3$. Generally this form of aluminium hydroxide is characterised as insoluble and not regarded as toxic (Life Treasure 2007). Some studies specifically deal with the toxicity of aluminium towards *D. magna* and *S. capricornutum* and they found that aluminium is toxic to the organisms in concern (Gensemer and Playle 1999; Guida *et al*. 2004). At aluminium concentration of 790 μg/L the inhibition of the daphnia was 100 % and at aluminium concentration of 500 μg/L the inhibition of the algae was 72 % (Guida *et al*. 2004).

Another disadvantages in relation to the use of aluminium flocculants is that $Al(OH)_3$ precipitates on contact with gills of fish thereby preventing the normal oxygen intake from the water, thus suffocating the fish (Rosseland *et al*. 1990; Poleo 1995; Baird and Cann 2005).

As described above, the toxicity of aluminium and its different hydroxides is complex and highly dependant of pH. The observed pH values in this project do not indicate that toxic forms of aluminium hydroxide have been formed.

When evaluating the surplus aluminium concentration it is important to remember that the measured aluminium values are not a measure of the dissolved aluminium, but the total content. In the tested stormwater, the aluminium is probably primarily present as aluminium hydroxide forms especially $Al(OH)_3$, that is insoluble at pH around 6 to 8 (PH Consult 2007).

The surplus iron in the stormwater may not constitute a problem. In the observed pH range in this project, the dominating iron hydroxide would be $Fe(OH)_3$ and this hydroxide is stable, has a low solubility and is generally not considered toxic. One study also investigated the toxicity of a iron-based flocculent; polymeric ferric sulfate, and no toxic response were found (Hendrich *et al*. 2001). This would serve as an argument for a preference for ferric-based flocculants rather than aluminium based flocculants.

Test procedures

Besides the difficulties with decantation, irregularities were also experienced with the measured concentrations of added metals. The concentrations of metal in the “start” measurements of the stormwater all displayed significantly lower concentrations than the actual amounts that were added. The explanation for this is unknown, however adsorption of the metals to the surfaces in the glass bottles is a possible explanation for the some of the loss. The glass bottles are made of borosilicate glass and others have found that such glass can adsorb 20 % of the added zinc ions and 15 % of nickel ions during contact periods of up to 30 days (Struempler 1973).

Another part of the explanation for irregularities in the measured metals concentrations is the fact that the Inductively Coupled Plasma-Optical Emission Spectrophotometer had just been set up and these samples were the first to be analyzed at the instrument. Such instrument normally needs some adjustments before the analysis are run optimal and give the most correct results.

7.2 Chemical analysis versus toxicological analysis

Correlation between chemical and toxicological results

To evaluate the results from the chemical analysis and the toxicological tests, the concentrations of metals could be correlated with the percentile inhibition of the test organisms. The correlations analysis show that the inhibition of the daphnia is strongest correlated with the total concentration of copper, nickel and zinc (R = 0.33) (Table 18). The data is however on different scales as the metals concentrations are on an infinite scale (0-∞ μg/L), whereas the inhibition of the daphnia is on a finite scale (0-100 %). If the concentrations of copper, nickel and zinc are adjusted to a finite scale the correlation coefficient (R) is 0.35 (Table 18).

The weakest correlation of the toxicity is found in relation to the iron concentration, and this supports that iron in these concentration can be categorized as non-toxic. The correlation with the aluminium concentration is also week (Table 18). The week correlation of the toxicity with both flocculants could be because the flocculants are present in flocs and forms that are relatively insoluble and thus does not inhibit the test organisms.

Table 18 Correlation between concentration and inhibition of daphnia. * The metal concentrations were adjusted to a finite scale for at better comparison with the inhibition data. n = 117 for Fe, Al and Cu, n = 43 for Zn and Ni.

Correlation (R)							
Cu, Ni and Zn	Cu, Ni and Zn *	Copper	Aluminium	Iron	Nickel	Zinc	All metals
0.33	0.35	0.33	0.10	0.08	0.20	0.24	0.14

Another and more simple analysis shows correlation between toxicity and metal concentration more clearly. In this analysis the inhibition is divided into five intervals making the estimate more rough (Table 19). The analysis shows a clear connection between the toxicity and the concentration of copper and zinc in the stormwater, as the toxicity being direct proportional to the mean concentration of these metals in the stormwater (Table 19). However the relationship is not observed for nickel. The large standard deviations confirm the variability that was observed in the toxicological tests in general.

Table 19 Percentile inhibition of daphnia compared to the mean concentration of copper, nickel and zinc in the stormwater based on 117 and 43 samples. σ denotes the standard deviation.

Inhibition [%]	Cu [μg/L] n = 117	σ	Ni [μg/L] n = 43	σ	Zn [μg/L] n = 43	σ
76-100	176	290	18	10	93	130
51-75	54	70	19	13	120	116
31-50	33	34	14	10	67	81
11-30	21	34	11	0	0	0
0-10	20	13	0	0	0	0

Based on the observed inhibition and the measured concentrations of copper, nickel and zinc it is possible to estimate the LC_{50} values for the three metals. This gives rise to the LC_{50} values listed below. These values can be compared to the LC_{50} values determined by the dose-response tests were the LC_{50} values not are based on measured metal concentrations but on the calculated addition by a dilutions series (Table 20). The order of toxicity of the three metals to *D. magna* is not alike in the two calculations, however nickel is stated as the most toxic in both series. Others have reported copper to be the most toxic for *D. magna* followed by zinc and then nickel to be the least toxic (Khangarat and Ray 1987; Khangarat and Ray 1989). These different results illustrate some of the irregularities related to toxicological testing.

Table 20 Two rows of LC_{50} values of copper, nickel and zinc to *D. magna* based on estimates from the flocculent tests or based on the actual dose-response tests.

	LC_{50} [μg/L] – Average estimated from flocculent tests	LC_{50} [μg/L] – Individual dose-response test
Cu	44	170
Ni	17	33
Zn	94	170

Looking in details on the individual tests, there are some good examples of how the chemical tests supports the toxicological results. Two flocculent tests with aluminium flocculent showed that 60 to 100 % of the daphnia were dead after 24 hours of exposure regardless of the flocculent concentration (Figure 26). However the chemical analysis from these two tests revealed that substantial amounts of copper were present in the stormwater after the treatment with aluminium chloride, and this could explain why the daphnia did not survive (Figure 12). The concentrations and appurtenant toxic response from the two experiments are summarized in Table 21. As shown the copper concentrations from the 25.03.09 are up to twice as high as the amount of copper that presumable were added, thus the measured concentrations must be due to mistakes during the process.

Table 21 Inhibition and concentration of copper in two tests conducted with aluminium chloride.

Added flocculent [mg/L]	Inhibition [%] 25.03.09	Cu [µg/L] 25.03.09	Inhibition [%] 29.03.09	Cu [µg/L] 29.03.09
0	100	0	100	121
5	100	913	100	86
10	100	651,5	100	60,5
20	100	697,5	100	27,5
40	100	1040,5	60	26

Another test confirms the relationship between the toxic response and the sum of concentrations of copper, zinc and nickel. The correlation coefficient (R) between the metal concentration and the inhibition in this test is 0.69 (18.03.09) (Figure 30 left). Reversely another test exhibits a low correlation coefficient of 0.23 between the toxicity and the metal concentration (06.05.09) (Figure 30 right).

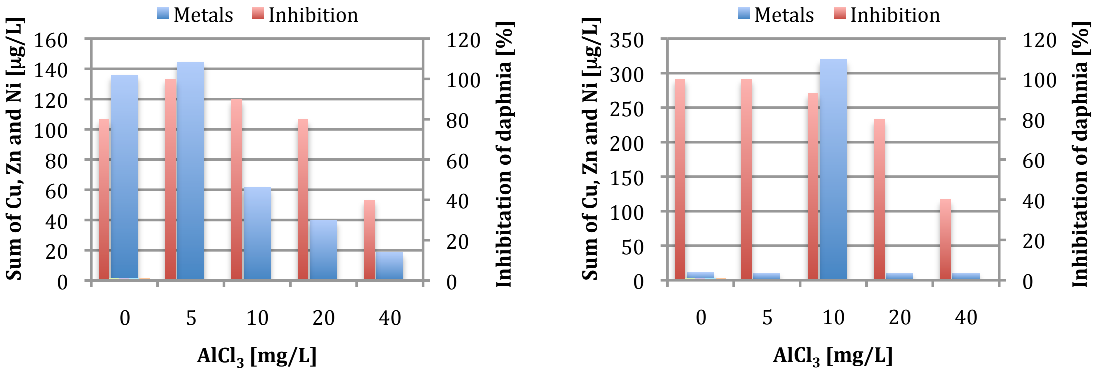

Figure 30 Concentration of copper, nickel and zinc found by the chemical analysis displayed with the corresponding toxicity found by the toxicological tests (Left - 18.03.09) (Right 06.05.09).

Generally the evaluation of the chemical versus the toxicological analysis is a little mixed as some observations and analysis show that there is a good correlation between the two, whereas other show that the correlation is low. One must remember that both types of analysis, chemical and biological, have its limitations and advantages.

One of the largest disadvantages of the chemical analysis must be the lack of ability to distinguish between which compounds that are bioavailable and which are not. In natural samples this is often of great importance, as e.g. the content of organic matter can reduce the harmful effect of a compound (Di Toro *et al.* 2001; Glover *et al.* 2005). Some chemical analysis can however determine the amount of metal in different fractions e.g. the fraction bound to organic matter. These are sequential extraction methods such as "Tessier" extraction, however the procedure is exhaustive (Tessier *et al.* 1979).

Also the effect of the speciation of the compounds will not be taken into account. A good example is aluminium, where some aluminium molecules such as $Al(H_2O)_6^{3+}$ are toxic whereas the $Al(OH)_3$ is generally not, and in both cases the chemical test will find the same result (Life Treasure 2007). Another disadvantage is that additive effects of the complete content of an environmental sample cannot be evaluated by chemical tests, whereas this is possible with the toxicological tests, and the question of cocktail and additive effects is often a big issue in relation to the presence of environmental pollutants in the aquatic environment.

Metal speciation and concentration

The metals tested in this report are added to the stormwater in relatively high, but not unrealistic concentrations, and more ever the metals are added as dissolved ions, enhancing the bioavailability compared to the content in natural samples. However, in relation to handling of stormwater such extreme values can be expected, as the concentration range is known to be very wide. One of the purposes of advanced treatment of stormwater should namely be to avoid the discharge of pollutants that occur as single events with high concentrations such as illegal discharges or accidents. The relatively high concentration of metals added to the stormwater was also a compromise in order to find a concentration range that would cover all three toxicity test organisms; *V. fischeri, S. capricornutum* and *D. magna*.

Sensitivity of toxicological tests

Three different toxicological tests were used in this project to evaluate the effect of treatment of stormwater in relation to the toxicity of the stormwater. The three tests were chosen so three different trophic levels were represented and to be able to target stormwater concentrations over a wide range. This proved to be a good strategy as the tests displayed different sensitivity towards the spiked stormwater.

Generally the algae test with *S. capricornutum* were found to be the most sensitive of the three tests, followed by the daphnia test with *D. manga* and then the bacteria test with *V. fischeri* as the least sensitive test (Figure 31). This order of sensitivity is based on the LC_{50} values described in Appendix A7 and is in relation to copper and benzalkonium chloride. Others have also reported this same order of sensitivity in relation to other compounds (Baun 1998; Marsalek *et al*. 1999A; Marsalek *et al*. 1999B; Wium-Andersen *et al.* 2009).

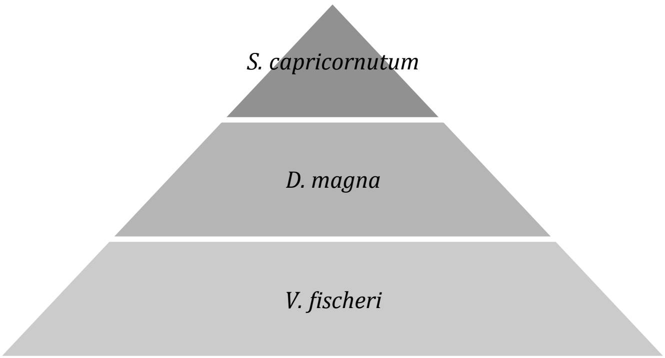

Figure 31 Sensitivity of test organisms towards copper and benzalkonium chloride.

Practical application of toxicological tests

The experiences achieved from working with the *S. capricornutum*, the *V. fischeri* and the *D. manga* tests in this project show that the application of toxicological tests can be difficult. This was e.g. illustrated by the growth problems experienced with *S. capricornutum*. See Appendix A8. Many of the implications with the toxicity tests could possible be related to the addition of flocculants in high concentrations and the rather inaccurate and rough decantation procedure, as substantial iron and aluminium were left in the stormwater. The residual iron and aluminium in the stormwater could remove nutrients and other compounds that are essential to e.g. the algae growth and thereby influence the outcome of the toxicity tests.

Difficulties were also experienced with the culturing of the *V. fischeri* culture. The culture should be propagated 24 hours prior to the performance of test, and grown to a biomass of 10^6 cells/μL. The propagation of the *V. fischeri* culture were not always successful and in general it takes carefulness to work with bacterial cultures. See Appendix A4. The hatching of the daphnia was less sensitive and no problems were experienced in this project, but it should be preformed 3 days prior to analysis of the water sample, which makes the tests time-consuming. All tests demanded either 1-3 days of preparation and 30 minutes to 3 days of subsequent processing with reading the results. These rather laborious procedures do not speak in favour of the use of toxicological tests.

However the advantages of a direct measure of the biological effects and the possibility to evaluate the total environmental sample speak in favour of a more widespread use of such tests as a supplement to chemical analysis. The use of toxicological tests are not currently a part of the traditional water quality programs in Denmark and other western countries, but it could be a possibility to implement them to some extent in the consequence of many changes that are expected due to the European Water Framework Directive. A battery of toxicological tests could be enforced as a collective European tool like the Danish Stream Fauna Index (DSFI) is today in Denmark as a supplement to the chemical analysis (Bruus 2009).

8 Conclusion

The objective of this project was to test the use of two flocculants, aluminium and iron chloride, and their ability to remove heavy metals from stormwater. The use of these two flocculants is already widespread in lake restoration and in clarification of drinking water and treatment of wastewater, but has only, as far a known, be used in relation to stormwater on scientific basis (Boers *et al*. 1992; Quaak *et al*.1993; Heinzmann 1994; Jaeger 1994; Deppe and Benndorf 2002; Karlby and Sørensen 2002; Burton and Stensel 2003; Winther *et al*. 2004; Wood *et al*. 2005; Wium-Andersen *et al*. 2009).

The laboratory experiments set up in this project found that removal of copper and zinc from stormwater by the use of iron and aluminium chloride is possible. This was documented by both chemical analysis, ICP-OES analysis and also by toxicological tests using the green algae *Selenastrum capricornutum* and the daphnia *Daphnia magna*. It was not possible to document the removal of copper and zinc by the toxicity test that uses the bacteria *Vibrio fischeri* as test organism.

The results from both the chemical and the toxicological analysis also showed that the removal efficiency were directly proportional with the concentration of added flocculent both with regard to aluminium chloride and iron chloride. The addition of flocculants was in concentrations ranging from 5 to 40 mg/L based on weight and the addition of 40 mg flocculent/L gave the best removal. Weather this dosage would be the best solution in an actual detention pond is hard to say, however the findings gives an indication of the concentration range.

Based on the results, none of the flocculants could be recommended over the other. Looking only at the findings from the toxicological tests least irregularities were experienced with the addition of iron chloride, however this is suspected to be due to the separation process by decantation. The use of a combination of the two flocculants did not result in an improved removal as could have been expected due to the theory of the solubility of the hydroxides formed by the flocculants.

The removal efficiency of the flocculants were also tested in the situation where the length of the precipitation period was varied from 1 hour to 1 day and to 3 days respectively. The chemical analysis showed clearly that there was an improved removal if the stormwater were left to precipitate for 3 days. When the water samples were analysed with the toxicological tests it was not possible to detect the connection between precipitation period and removal.

The removal of both copper and zinc were up to 100 % when the precipitation period were 3 days, however the removal for stormwater not treated with flocculants, were similarly up to 100 % for the two metals, when the precipitation period were 3 days. These findings question the use of flocculants as an efficient method, however a lot of the irregularities displayed in the results are assumed to be due to a rather rough procedure for decantation, adsorption of metals to the glass container surfaces and also due to the influence of the flocculants on the test organisms.

The findings is however useable as preliminary results, that indicate that the use of flocculants is a applicable method for treatment of stormwater, however the efficiency is not well documented and needs further research to be categorized as a effective and applicable treatment method. E.g. the effect of the pH is another aspects that need to be addressed and it would be interesting to perform similar experiments on stormwater not spiked with metals, to see the effect in low concentration levels.

The use of toxicity tests in relation to treatment of the stormwater showed several interesting things. First of all the use of toxicity tests were more difficult than first assumed, and the toxic response of the test organisms can be influenced by many parameters, that not always can be mapped. The toxic responses can however rank and classify stormwater samples in rough categories in relation to their toxicity without knowledge of the actual content of the sample, and when the samples were divided in rough intervals the correlation between the measured concentration of metals and the measured inhibition of the test organisms were good.

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Appendix

A1 - Procedure for Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) element analysis

Principle

The principle of Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) element analysis is based on detection of emission signals from individual elements. The analyte, are atomized in a plasma flame and the emission signal is detected (Harris 2003).

Materials

Finn pipettes

Fume hood

Greiner tubes

Test tubes and racks for test tubes

iCAP 6300 Inductively Coupled Plasma-Optical Emission Spectrometer form Thermo Scientific

Solutions

65 % nitric acid of analytic quality (HNO_3).

(However in this project the nitric acid was not of highest analytic quality and could contain impurities of different elements such as iron, aluminium and others).

0.2 % nitric acid solution

MilliQ water

Appropriate element standards; Fe, Al, Cu, Ni and Zn in this analysis.

Procedure and preparations

1. Turn on the iCAP 6300, the cooler, the autosampler and the argon gas tap that is connected to the iCAP (Figure 32).

Figure 32 Inductively Coupled Plasma-Optical Emission Spectrometer – iCAP6300

2. Control that the exhaust devise is on.
3. Prepare a 0.2 % HNO_3 solution from the 65 % HNO_3 and MilliQ water in the fume hood. Use gloves and glasses. The solution is for acidifying of the standard curve and for cleansing water.
4. Make the appropriate element standards diluted with the 0.2 % HNO_3 solution.
5. Create a "Method" in the iCAP software "Analyst". It is in the method the elements for analysis is selected.
6. For aluminium, iron, nickel, zinc and copper could a choice of elements look like listed in the table below.

Element	Aluminium	Iron	Nickel	Zinc	Copper
Wavelength [nm]	167.679	259.837	231.604	213.856	324.754
Signal [relative intensity]	1825000	600000	600000	3000000	5000000

7. Run the "Method" to test the standard curve. The standard curve can be measured either manually or by creating a new sequence in "Analyst" to be run be the autosampler.
8. Check for each element if there is any interference from other elements. This can be done in the "Element parameters" under "Subarray".
9. If any large interference is present change the wavelength of that particular element.
10. After the standard curve series have been analyzed use "Analyst" to adjust the standard curve by removing points or changing the weight of the individual data points.
11. If the wavelength of one or more elements is changed or if the standard curve is not satisfying repeat the procedure. When the standard curve is satisfying the samples can be analyzed.
12. Prior to analysis each sample has to be acidified, and if wished the samples can also be further diluted. The samples should have a final concentration of 0.2 % HNO_3 like the standard curves.
13. The acidification and dilution can e.g. be done by using 1 mL of a 2 % nitric acid, 2 mL sample and 7 mL milliQ water.
14. Place the acidified samples in the autosampler and create a new sequence in "Analyst" using the method with the right standard curve.
15. Check that the "interlocks" are ready and that the tubes are correctly connected. The "interlocks" are e.g. the purge gas flow, the plasma gas flow, the exhaust and the temperature of the optics. If these things are not ready it is not possible to turn on the ICP.
16. Run the sequence in "Analyst".

Data treatment

1. Withdraw the results from "Analyst" in txt.-file.
2. As the file contains a lot of additional information, which is not needed in the data treatment a small program can with benefit be created to extract the necessary data.
3. Import the modified txt.file to Excel or a similar data treatment program. The concentrations of the individual elements are given in ppm = mg/L.
4. Calculate the actual concentration in the samples.
5. Present the results in graphs e.g. showing the concentration in the stormwater before and after treatment with iron chloride.

A2 - Procedure for Algae Growth Rate Inhibition Toxicity Test

Modified version of DS/EN ISO 8692 (2005): Water quality – Freshwater algal growth inhibition test with unicellular green algae.

Principle

The toxicity of a sample is determined as the inhibition of the growth rate of the planktonic green algae *Selenastrum capricornutum*. Inhibition is measured as acute toxicity after a contact time of 24, 48 and 72 hours at 20 °C.

Materials

Vials with beads of *Selenastrum capricornutum* and matrix dissolving medium provided by MicroBioTests.

10 cm length cuvets and holdings trays provided by MicroBioTests.

UV-VIS-mini 1240 Shimadzu spectrophotometer

pH meter

Temperature controlled room and lamp at 6000 lux

Vacuum pump and 0.45 μm filters

Solutions, substances and media

Algal culturing medium (A1-A4)

1. The algal culturing medium is prepared by four stock solutions A1 to A4.
2. A1: 1.5 g NH_4Cl/L, 1.2 g $MgCl_2*6H_2O$/L, 1.8 g $CaCl_2 * 2H_2O$/L, 1.5 g $MgSO_4 * 7H_2O$/L, 0.16 g KH_2PO_4/L and dilute to 1 L with de-ionized water.
3. A2: 64 mg $FeCl3 * 6H2O$/L, 100 mg $Na_2EDTA * 2H_2O$/L and dilute to 1 L with de-ionized water.
4. A3: 185 H_3BO_3/L, 415 mg $MnCl_2 * 4H_2O$/L, 3 mg $ZnCl_2$/L, $CoCl_2 * 6H_2O$/L, 0.01 mg $CuCl_2 * 2H_2O$/L and 7 mg $NaMoO_4 * H_2O$/L and dilute to 1 L with de-ionized water.
5. A4: 50 g $NaHCO3$/L and dilute to 1 L with de-ionized water.
6. Sterilize the stock solutions by membrane filtration (mean pore diameter 0.2 μm) or by autoclaving at 120 °C for 15 minutes.
7. Mix 10 mL of nutrient solution A and 1 mL and nutrient solution B, C and D and add de-ionized water to a total volume of 1 L.
8. If necessary adjust the pH to 8.0 ± 0.2 by adding NaOH or HCl solution.
9. NB. If testing the toxicity of metal EDTA MUST be omitted from stock solution A2 (American Public Health Association 2005).

Water sample (B)

1. Filtered stormwater
2. Toxic reagent in solution e.g. copper or N-alkyl dimethyl-benzyl-ammonium chloride

Store water samples at 2-4 °C, and equilibrate the samples to app. 20-22 °C before the test starts.

Procedure and preparations

De-immobilization of the algae

1. Turn on the spectrophotometer and adjust the wavelength to 670 nm.

2. Pour the liquid from the vial with algal beads and transfer 5 mL of matrix dissolving medium. Cap the vial and shake it vigorously until the algae beads are dissolved.
3. Centrifuge the vial for 10 minutes at 3000 rpm.
4. Pour out the supernatant and replace it by 10 mL de-ionized water to eliminate all traces of the matrix-dissolving medium.
5. Cap the vial and shake it until the algae re-suspended. Centrifuge again for 10 minutes at 3000 rpm.
6. Decant the supernatant and re-suspend the algae in 10 mL algal culturing medium.
7. Pour the algal suspension into a 25 mL volumetric flask and add algal culturing medium to the 25 mL mark.
8. Fill a cuvet with 25 mL algal stock solution and a calibration cuvet with 25 mL algal culturing medium and measure the OD. Calculate the cell number by the prefabricated standard curve.
9. To avoid to high cell number at the end of the experiment the start concentration of algae in the samples should be app. 10^4 cells/mL. Therefore the stock solution should be diluted to app. 10^6 cells/mL. Determine the dilution factor (D) by dividing the cells number of the stock dilution (N_1) with 10^6 cells/mL (N_2) and multiply with the volume (25 mL).

$$D = \frac{N_1}{N_2} 25\ mL$$

10. Dilute the algal stock solution with the calculated volume of algal culturing medium and transfer 25 mL to a cuvet and measure the OD again.
11. Check that the new OD corresponds with the desired 10^6 cells/mL.

Toxicant dilutions

1. Take six 200 mL volumetric flasks and label them C0 to C5.
2. The experiment is done in triplicates.
3. Fill all the flasks except flask C1 with 100 mL algal culturing medium.
4. Transfer 200 mL water sample to flask C1.
5. Add 2 mL of nutrient stock solution A to flask C1 and 0,2 mL of nutrient stock solutions B, C and D to flask C1.
6. Transfer 100 mL from flask C1 to flask C2. Stopper flask C2 and shake it to mix.
7. Now transfer 100 mL from flask C2 to flask C3. Stopper flask C3 and shake it to mix. Repeat the same transfer procedure until flask C5. Remember to remove 100 mL from flask C5 as last step.
8. Add 1 mL of the algal solution to each flask.
9. Transfer 25 mL from each flask to a labeled cuvets and measure the OD of each cuvet.

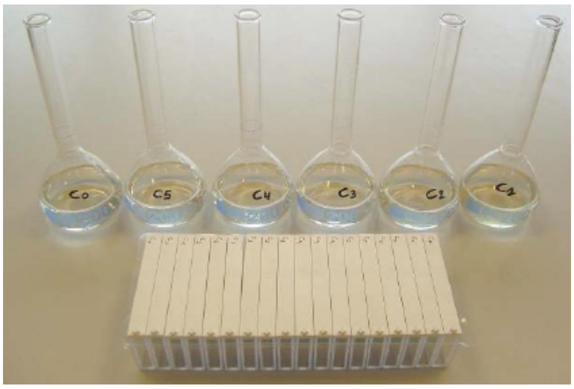


Figure 33 Dilution series of toxic sample in flasks from C0 to C5 and 10 cm long cuvets (MicroBioTests).

10. Place the cuvets in the holding trays. Make sure that they turn the same way, as the lids should left slightly open by the provided plastic strip that can be put just under the lids of the cuvets the keep the open.
11. Incubate the cuvets at 21 to 25 °C under app. 6000 lux for 72 hours.
12. To ensure that the algal get randomly amount of light the cuvets must placed randomly in the tray.
13. Measure the OD of the cuvets after 24, 48 and 72 hours and remember to place the cuvets in a random way after each measurement round.

Additional remarks

The cuvets must always be placed in the spectrophotometer in the same direction, namely with the imprinted arrows pointing to the left.

Due to settling of the algae it is important to shake the cuvets prior to each OD measurements additionally to obtain representative measurements it is important to read the OD after a fixed amount of seconds after placement of the cuvet in the spectrophotometer e.g. 3 seconds.

Data treatment

Calculate the toxicity response e.g. as LC_{50} values according to the procedure in DS/EN ISO 8692 (2005).

A3 - Procedure for *Daphnia magna* Immobility Toxicity Test

Modified version of DS/EN ISO 6341 (1997): Determination of the inhibition of the mobility of *Daphnia magna* Straus – Acute Toxicity test.

Principle

The toxicity of a sample is determined as the inhibition of the mobility of the planktonic crustacean *Daphnia magna*. Inhibition is measured as acute toxicity after a contact time of 24 and 48 hours in dark at 20 °C.

Materials

Vials with ephippia of *Daphnia magna* provided by MicroBioTests.

Petri dish 5 or 10 cm in diameter

Multiwell test plates with lids provided by MicroBioTests.

Vials with Spirulina powder provided by MicroBioTests.

Micropipette and microsieve

pH meter

Temperature controlled room and lamp at 6000 lux

Vacuum pump and 0.45 μm filters

Solutions, substances and media

Media for hatching and incubation of *Daphnia magna* – standard freshwater (A1-A4)

1. A1: 11,76 g $CaCl_2 * 2H_2O$ and dilute to 1 L with de-ionized water.
2. A2: 4,93 g $MgSo_4 * 7H_2O$ and dilute to 1 L with de-ionized water.
3. A3: 2,59 g $NaHCO_3$ and dilute to 1 L with de-ionized water.
4. A4: 0,23 g KCl and dilute to 1 L with de-ionized water.
5. Mix 25 mL of each of the four solutions and add de-ionized water to a total volume of 1 L.
6. If necessary adjust the pH to 7,8 ± 0.2 by adding NaOH or HCl solution.

Water sample (B)

1. Filtered stormwater
2. Toxic reagent in solution e.g. copper or N-alkyl dimethyl-benzyl-ammonium chloride
3. Store water samples at 2-4 °C, and equilibrate the samples to app. 20-22 °C before the test starts.

Procedure and preparations

Hatching of Daphnia magna

1. Pour the ephippia into the microsieve and rinse thoroughly with tap water to eliminate all traces of the storage medium.
2. Transfer the ephippia to a petri dish with pre-aerated standard freshwater (A) and incubate for 72 h at 20-22 °C under illumination of 6000 lux.

3. As the standard freshwater does not contain any food the daphnia needs to be pre-fed two hours before the test starts. This provides them with an energy reserve and prevents that they die form starvation during the test.
4. Pour the Spirulina powder into the hatching petri dish and swirl gently to distribute the food evenly.

Toxicant dilutions

1. Take five 100 mL volumetric flasks and label them C1 to C5
2. Fill C1 with 100 mL non-diluted water sample.
3. Fill 50 mL standard freshwater into flasks C2 to C5.
4. Transfer 50 mL from flask C1 to flask C2. Stopper flask C2 and shake it to mix.
5. Now transfer 50 mL from flask C2 to flask C3. Stopper flask C3 and shake it to mix. Repeat the same transfer procedure until flask C5. Remember to remove 50 mL from flask C5 as last step.

Filling the test plate

1. The test plate contains 25 wells (Table 22). Fill the five wells in row 0 with 10 mL standard freshwater (A). Transfer 10 mL from flask C1 to each well in row 1.
2. Transfer 10 mL from flask C2 to each well in row 2. Repeat this procedure for flasks C3 to C3 and rows 3 to 5.
3. The wells in column 0 are rinsing wells and are for daphnia that is not included the test.
4. Remove the hatching petri dish from incubation and transfer minimum 20 daphnia to the rinsing wells. The transfer can be done with a micropipette. Because of the small size of the daphnia is can be necessary to carry out the transfer under a microscope or by means of a light table or similar equipment.
5. Now transfer exactly 5 daphnia from the rinsing well in row 0 to the remaining wells in row 0 meaning well A0, B0, C0 and D0. Repeat this for row 1 to 5 and rinse the pipette thoroughly between each row.
6. Control that each well (except the rinsing wells) contains exactly 5 actively swimming daphnia.
7. Incubate the test plate in dark at 20-22 °C.
8. Read the test plates after 24 and 48 hours respectively. Count the number of immobile daphnia in each well. An immobile daphnia is defined, as a daphnia that is not able to swim after gentle agitation of the liquid and movement of the antennae is not valid as a swimming daphnia.

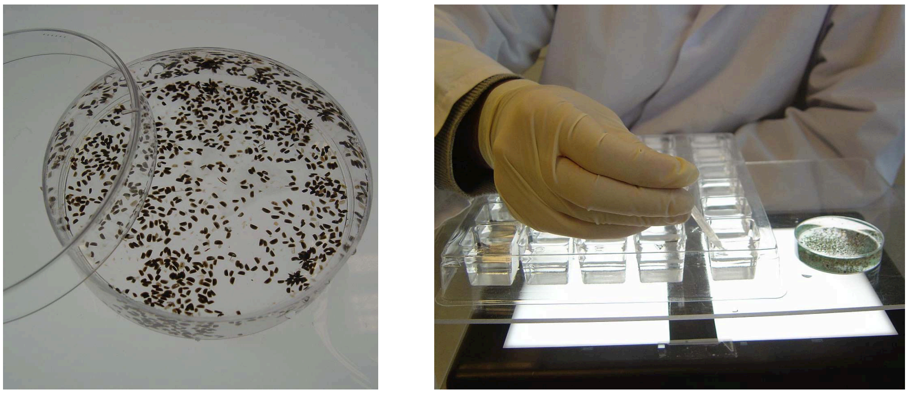


Figure 34 Petri dish with ephippia and daphnia (left) and display of how to transfer daphnia to the test plate (right) (MicroBioTests).

The test is regarded as valid if the number of immobile daphnia in the controls not exceeds 10 % and the dissolved oxygen concentration at the end of the test is greater than or equal to 2 mg/L.

	A	B	C	D
Rinse	FW	FW	FW	FW
Rinse	D0	D0	D0	D0
Rinse	D2	D2	D2	D2
Rinse	D4	D4	D4	D4
Rinse	D8	D8	D8	D8
Rinse	D16	D16	D16	D16

Table 22 Example of how to fill a test plate.

Freshwater = ☐ and water sample = ▨

Data treatment

Calculate the toxicity response e.g. as LC_{50} values. See ISO DS/EN ISO 6341 (1997).

A4 - Procedure for Bacterial Bioluminescence Toxicity Test

Modified version of ISO 11348-1 (2007): Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test).

Principle

The toxicity of a sample is determined as the inhibition of the light emission of the bioluminescent bacterium *Vibrio fischeri* DSM 7151 (*Vibrio fischeri* DSM 7151). Inhibition is measured as acute toxicity after a contact time of 15 min and 30 min at 20 °C.

Materials

Multilabel Reader at 20 °C

White 24- or 96-well Microplates

Pipettes and sterile tips

V. fischeri DSM 7151

Shaker at 20 °C

pH meter

Freezer

Solutions, substances and media

Medium for Propagation of *Vibrio fischeri* (VF Broth and VF Agar) (A)

Distilled water

10 g/L Brain-Hart Infusion Broth.

0.5 g/L Yeast extract

0.5 g/L $(NH_4)_2SO_4$

25 g/L NaCl

0.2 g/L $MgSO_2 \times 7H_2O$

50 ml/L of 1 M Phosfate Buffer Stock (pH 7) (or 6.1 g $NaH_2PO_4 \times H_2O$ + 2.8 g $K_2HPO_4 \times 3H_2O$)

3 ml/L glycerol

[15 g/L agar –*for agar plates*]

Sterilize in autoclave

Medium for Toxicity Testing (VF Tox Medium) (B)

Distilled water

8 g/L D(+)-Glucose monohydrate

20 g/L NaCl

2.035 g MgCl2*6H2O

0.3 g KCL

11.9 g/L HEPES (N-2-Hydroxyethyl)piperazine-N-(2-ethanesulfonic acid)

Adjust to pH 7 with 1 M NaOH or HCl

Sterilize in autoclave

Medium for freezing of Vibrio fischeri (VF Freeze Medium) (C)

Distilled water

66 g/L D(+)-Glucose monohydrate

4 g/L NaCl

2 g/L L-Histidin

0.5 g/L Bovine serum albumin (BSA)

Adjust to pH 7 with 1 M NaOH or HCl

Sterilize in autoclave

Alternatively use the VF Medium with 40% glycerol.

NaCl medium for dilutions (D)

Distilled water

20 g/L NaCl

Sterilize in autoclave

Reference substances (E)

0. 22 g/L $ZnSO_4 \times 7H_2O$ + 20 g/L NaCl or

0.009 g/L 3,5-Dichlorphenol ($C_6H_4OCl_2$) + 20 g/L NaCl or

0.023 g/L $K_2Cr_2O_7$ (potassium dichromate) + 20 g/L NaCl

Procedure and preparations

Propagation of Vibrio fischeri

1. Thaw frozen *V. fischeri* (A).
2. Inoculate VF Broth with frozen V. fischeri (A). Alternatively inoculate VF Broth with luminescent *V. fischeri* colonies from VF agar plates (A).
3. Incubate for 24-72 h days at 20 °C on a shaker at 180 rpm.
4. Transfer *V. fischeri* culture to fresh VF Broth (A) (1:100) e.g. 0.5 ml culture to 50 ml broth in 250 ml Blue Cap bottles.
5. Incubate for 20-24 h days at 22 °C on a shaker at 180 rpm. Use the culture for toxicity test (see below). Alternatively, freeze *V. fischeri* cultures according to ISO 11348-1 (2007) using the VF Freeze Medium (C).

Storage and preparation of samples

1. Store water samples at 2-4 °C. Non aqueous extracts may be frozen.
2. Equilibrate the samples to 22 °C.
3. Adjust test samples to 20 per mille salt (20 g/L) by adding pure NaCl.

Toxicity Test Procedure for fresh *V. fischeri* cultures

1. Make serial dilutions of the toxic sample (**TS**) in the Microplate (see outline below). All dilutions should be carried out using sterile 2% NaCl. The final volume in 96-well plates should be 100 µl test sample and 100 µl diluted *V. fischeri* culture. Suggestions for dilution series see Table 23.

Water Samples (WS) One column is used for undiluted test samples (100µl sample+100 µl culture = D2) and 10 columns are used for serial dilutions of the test sample in 100 µl 2% NaCl (D4-D1024).

Negative controls (NC). Include cells without added toxic compounds (100 µl NaCl + 100 µl culture).

Positive controls (PC). Include positive controls with a known toxic compound such as $ZnSO_4$ x $7H_2O$ (LC_{50} = 26 mg Zn/l) or $K_2Cr_2O_7$ (LC_{50} = 3.5 mg Cr/l. The positive controls may be included in the same microplate or by preparing a separate microplate with serial dilutions of a stock solution of the positive control, e.g., a stock solution of 2.2 g/l of $ZnSO_4$ x $7H_2O$.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	D2048	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2
B	NC	D2048	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2
C	NC	D2048	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2
D	NC	D2048	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2
E	NC	D2048	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2
F	NC	D2048	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2
G	NC	D2048	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2
H	NC	D2048	D1024	D512	D256	D128	D64	D32	D16	D8	D4	D2

Table 23 Suggestions for dilution series in the microplates.

NC = ☐ and WS = ▨

2. Collect fresh *V. fischeri* grown for 20-24 h at 22 °C in VF Broth as described above.
3. Dilute the culture 1:10 with VF Tox Medium (B). A test with a 96-well microplate requires approximately 10 ml diluted culture.
4. The luminescence for diluted cultures should be $>10^6$ RLU per 100 µl diluted culture as measured on the Perkin Elmer Multilabel Reader (appr. 10^7 cells per 100 µl).
5. 9. Add 100 µl diluted *V. fischeri* cultures to all wells.
6. 10. Measure luminescence in all wells at t=0 min, t=15 min, and t=30 min using a counting time of 0.1 sec/well by the Perkin Elmer Multilabel Reader.

Data treatment

Calculate the toxicity response e.g. as LC_{50} values. See ISO 11348-1 (2007).

A5 - Laboratory procedure for "Removal of toxicity by addition of aluminium and iron flocculants"

Scope

To improve sedimentation of both the dissolved and particulate compounds in stormwater a flocculants such as iron(III) chloride and aluminium will be added to the stormwater in different concentrations and combinations. The test will evaluate the effect of the addition of different concentrations and combinations flocculants by applying ICP-analysis and battery of toxicity test before and after addition of the flocculants. The test will also evaluated the effect of the precipitation period as the stormwater treated with flocculent will be left to precipitate for different time intervals.

Materials and equipment

Vacuum pump and 0,45 μm filters

100 mL "blue cap" glass bottles (or other volume)

Scale

Vibrating table

pH meter

Solutions, substances and media

Stormwater should be stored in darkness at 5 °C prior to testing

Stormwater with toxic reagent e.g. copper

Flocculent solution (e.g. iron(III) chloride hexahydrate, $FeCl_3 * 6H_2O$ or aluminium chloride hexahydrate, $AlCl_3 * 6H_2O$) diluted with stormwater with toxic reagent.

Variables

Three parameters will be varied in this experiment.

A: Type of flocculent:

Iron chloride, aluminium chloride and Iron chloride and aluminium chloride in combination.

B: Concentration of flocculent:

0, 5, 10, 20 and 40 mg flocculent/L

C: Precipitation period:

3 days, 1 day and 1 hour.

Procedure and setup

1. See a sketch of the general procedure at Figure 35.
2. Filter the stormwater (A) through a 0,45 μm filter to remove particles and naturally occurring algal.
3. Prepare the test water consisting of stormwater and toxic reagent in the wanted concentration (B).
4. Extract the volumes for the first round of toxicity tests from the test water and for the measurements of the composition of compounds by ICP analysis.
5. Set up and label the glass bottles with the different additions of flocculent.

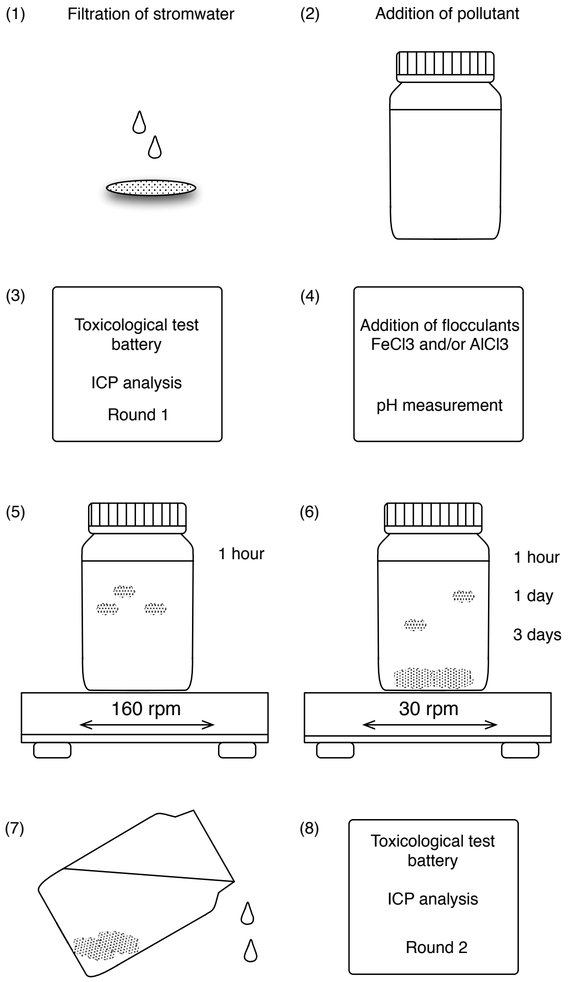


Figure 35 General procedure and setup.

6. Prepare two solutions with e.g. 40 mg flocculent/L from the toxic stormwater (C).
7. Add volumes of the solution with flocculent and the solution with toxic stormwater to the blue cap bottles to make up the different concentrations of $FeCl_3$ and $AlCl_3$. See Table 24.
8. Add the remaining volume of the toxic stormwater to make the total volume of 100 mL.

Table 24 Volumes of toxic stormwater and flocculent solution to make the different concentrations of a single flocculent. The volumes are based on a flocculent solution with 40 mg flocculent pr. L. If two flocculants are tested in combination the volumes in row three should be divided by two for each flocculants.

mL toxic stormwater	0 mg flocculent/L	5 mg flocculent /L	10 mg flocculent /L	20 mg flocculent /L	40 mg flocculent /L
No flocculent	100	87,5	75	50	0
Flocculent added ($AlCl_3$ or $FeCl_3$)	0	12,5	25	50	100

9. Place the glass bottles on the vibrating table and start the table at 160 rpm.
10. Leave the samples for continuously shaking for 1 hour.

11. Adjust the vibrating table to 30 rpm and leave the samples to precipitate for 3 days, 1 day and 1 hour respectively.
12. Leave the samples for settling for 2 hour and decant the supernatants for the second round of toxicity tests and for the measurements of the composition of compounds by ICP analysis.

Additional remarks

Samples ready for toxicity tests should be kept in darkness and at 5 °C and the tests should be preformed within 4 hours after the end of the experiment.

Data treatment

Calculate the toxicity response from the toxicity tests battery from each of the samples before and after the addition of flocculent.

Calculate the removal of compounds from the ICP-analysis results due to the treatment with flocculants and compare with the change in toxicity after the treatment.

A6 - Analysis of stormwater not spiked with heavy metals

Stormwater from a retention pond in North Jutland south of Hjallerup City was collected during the spring 2009. The stormwater was collected in 10-30 litre plastic containers and stored at 5 °C for no longer than 14 days prior to analysis.

To avoid large amounts of particulate matter in the water, the water was collected in and just below the water surface in the pond, and as the algae test prescribed that the stormwater should be filtered through a 0.45 μh filter this was done with the stormwater for all the analysis.

As described earlier in this report the concentrations in stormwater are often very inconsistent and vary from very low concentrations to more extreme values under single events. Due to this and experiences from others it was chosen to spike the stormwater with known concentrations of heavy metals. To determine the background concentration and toxicity, the natural stormwater was analysed was analysed both by ICP analysis and by the toxicological test battery using the *S. capricornutum* algae, the *V. fischeri* bacteria and the *D. magna* daphnia.

Chemical analysis

The chemical analysis showed very low concentrations of copper, aluminium, iron and nickel in the natural stormwater, however the concentrations of zinc were considerable and consistent (Figure 36 and Figure 37).

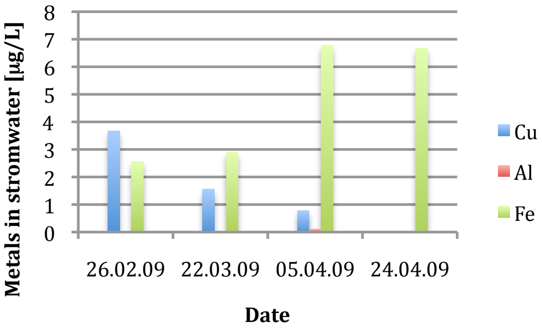


Figure 36 Concentration of copper, iron and aluminium in stormwater from Hjallerup retention pond collected at different dates. The samples were analysed by ICP analysis.

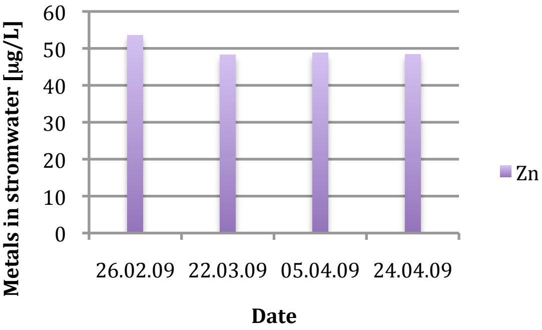


Figure 37 Concentration of zinc in stormwater from Hjallerup retention pond collected at different dates. The samples were analysed by ICP analysis.

Toxicological analysis

The toxicological tests showed different responses on the unspiked stormwater. The testing with the *S. capricornutum* green algae were encumbered with various difficulties with the growth of the algae, thus not reasonable results were produced in relation to the toxicity of the un-spiked stormwater. Only a single test were preformed on the un-spiked stormwater with the *V. fischeri* bacteria test, and this showed a 15 % inhibition of the bacteria from a sample half diluted by demineralised water.

The *D. magna* tests showed varying toxic response form the stormwater between the different dates but also great variability in the toxic response from stormwater collected the same date (Figure 38).

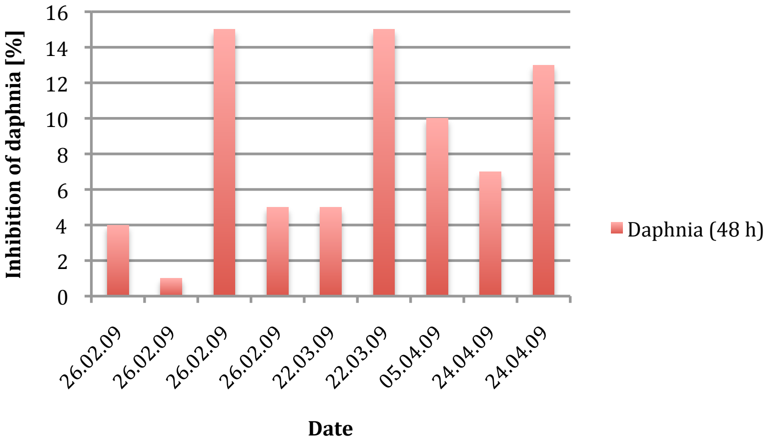


Figure 38 Toxic response from *D. magna* in relation to stormwater collected in Hjallerup detention pond at different dates.

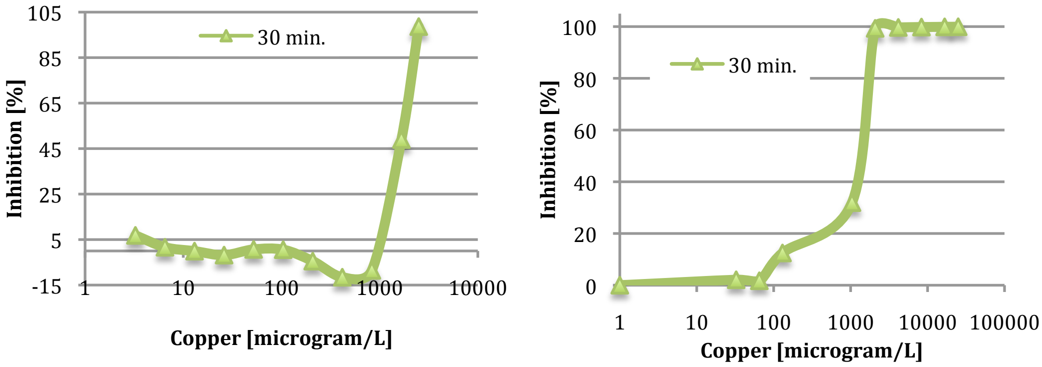
A7 - Dose-response curves

This appendix shows the results from different experiments where the toxicity of copper and benzalkonium chloride were tested by the three different toxicological tests. The toxicity of the different compounds in relation to the test organisms is presented by the dose-response curves and as LC_{50} values.

Copper

The toxicity of stormwater spiked with copper was tested by all three toxicological tests, however the *S. capricornutum* test did not show any results that were consistent enough to determine a LC_{50} values. Figure 39 shows the dose-response curves for the inhibition of *V. fischeri* and *D. magna* respectively, and Table 25 shows the estimated LC_{50} values in relation to cobber.

A) Inhibition of bacterial luminescence of *V. fischeri* (12.03.09)



B) Inhibition of mobility of *D. magna* (20.02.09)

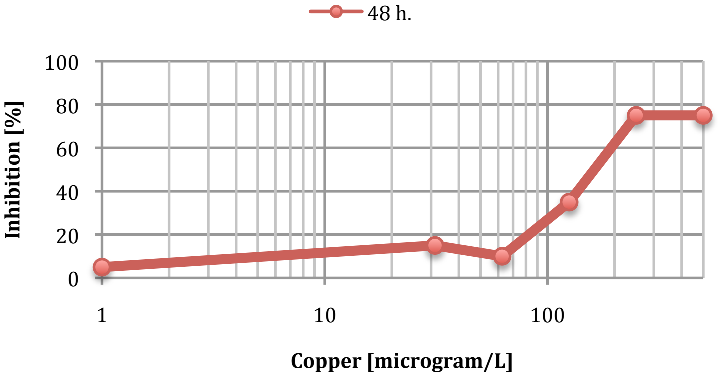


Figure 39 Dose-response curves for the bacteria *V. fischeri* (A) and the crustacean *D. magna* (B) in relation to cobber. The date of the performance of the individual tests is written in parenthesis.

Table 25 LC_{50} values of copper to the three test organisms *V. fischeri*, *S. capricornutum* and *D. magna*.

LC_{50} [µg/L] *V. fischeri* – bacteria	LC_{50} [µg/L] *S. capricornutum* - algae	LC_{50} [µg/L] *D. magna* – crustacean
1100 1)	n.d.	170 3)
1600 2)	n.d.	

Nickel and Zinc

The toxicity of the heavy metals nickel and zinc were tested in relation to the mobility of *D. magna*. The LC_{50} values were determined to 33 μg/L for nickel and 170 μg/L for zinc (Figure 40). The LC_{50} values can be estimated in a variety of ways see ISO 1997, ISO 1999 and ISO 2005. However in this case the LC_{50} values were determined by visual evaluation of the dose-response curves due the variability of the results.

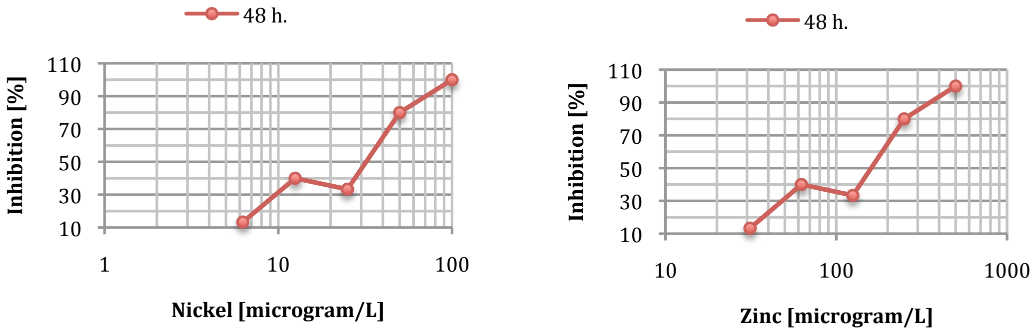


Figure 40 Dose-response curves for *D. magna* in relation to stormwater spiked with 100 g Ni/L and 500 g Zn/L (07.05.09).

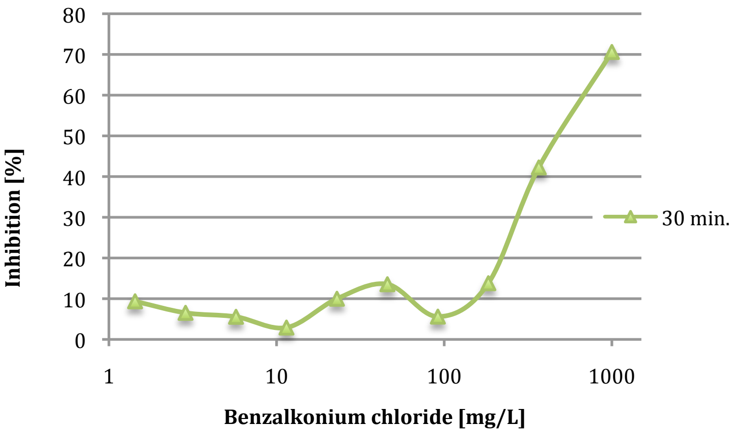
Table 26 LC_{50} values for *D. magma* towards nickel and zinc.

D. magna – crustacean	LC_{50} [μg/L]
Nickel	33
Zinc	170

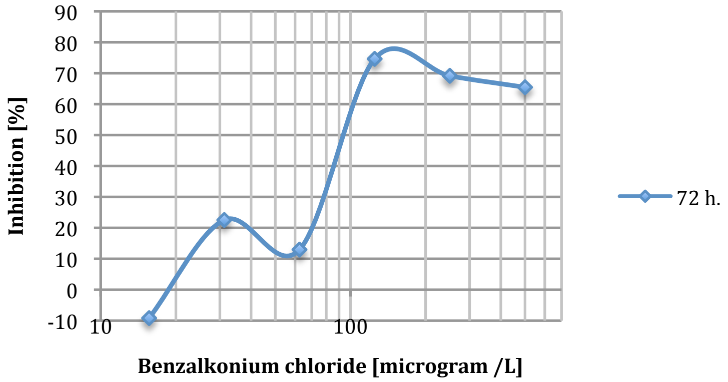
Benzalkonium chloride

The toxicity of stormwater spiked with benzalkonium chloride was determined by all three toxicological tests. Figure 41 shows the dose-response curves for the three organisms and Table 27 shows the LC_{50} values. The LC_{50} values can be estimated in a variety of ways see ISO 1997, ISO 1999 and ISO 2005. However in this case the LC50 values were determined by visual evaluation of the dose-response curves due the variability of the results.

A) Inhibition of bacterial luminescence of *V. fischeri* (17.04.09)



B) Growth rate inhibition of *S. capricornutum* (24.03.09)



C) Inhibition of mobility of *D. magna* (23.03.09)

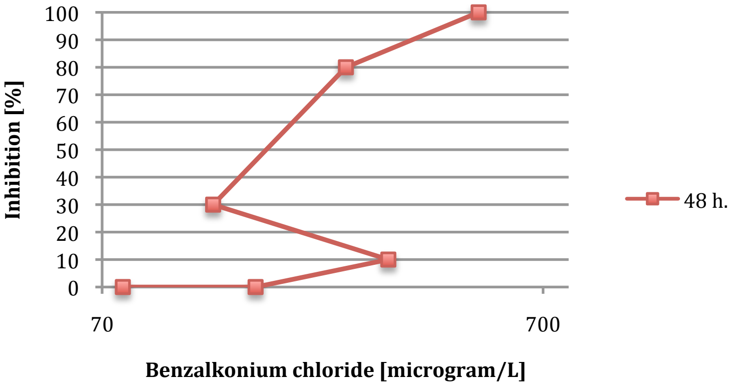


Figure 41 Dose-response curves for *V. fischeri* (A), *S. capricornutum* (B) and *D. magna* (C) in relation to stormwater spiked with benzalkonium chloride. The date of the performance of the individual tests is written in parenthesis.

Table 27 LC_{50} values of benzalkonium chloride to the three test organisms *V. fischeri, S. capricornutum* and *D. magna*.

LC_{50} [µg/L] *V. fischeri* – bacteria	LC_{50} [µg/L] *S. capricornutum* - algae	LC_{50} [µg/L] *D. magna* – crustacean
400[1)]	90[2)]	180[3)]

A8 - Algae Growth Rate Inhibition

The algae growth rate test proved to be a sensitive test the graphs below show different responses from the growth rate test. The composition of the algae nutrient media proved to be very important for sufficient algae growth.

EDTA as complex-binding-agent

The original DS/EN ISO 8692 (2005) Water quality – Freshwater algal growth inhibition test with unicellular green algae did not specify the importance of EDTA in relation to testing the toxicity of metals. Figure 42 and Figure 43 show the importance of omitting EDTA if testing the toxicity of metals. Theoretically the addition of copper would decrease the growth rate of the algae, however as shown on Figure 42 the growth rate is at the same level of the control. Only in the batch with 25 μg Cu/L the growth of the algae is decreased. However looking deeper into the theory this is not as surprising as EDTA easily forms complex bindings with metal ions and this will neutralize the copper ions and therefore not result in any decrease in the growth rate (Baird and Cann 2005).

To avoid this neutralization of the copper ions EDTA was omitted from the algae nutrient media in line with the prescription in *Standard Methods for Examination of Water and Wastewater* (American Public Health Association 2005). This resulted in a decrease in the growth rate proportional with the concentration of added copper with the highest growth rate in the control batch (Figure 43).

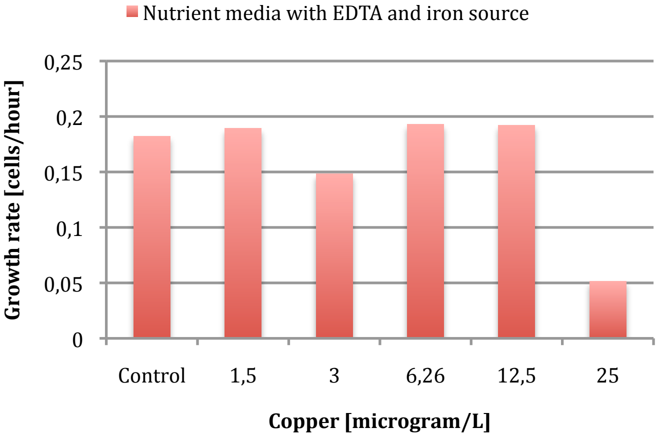


Figure 42 Toxicity of stormwater spiked with copper and cultivated in nutrient media with EDTA and iron source (13.02.09). The control batch in algae cultivated without addition of copper.

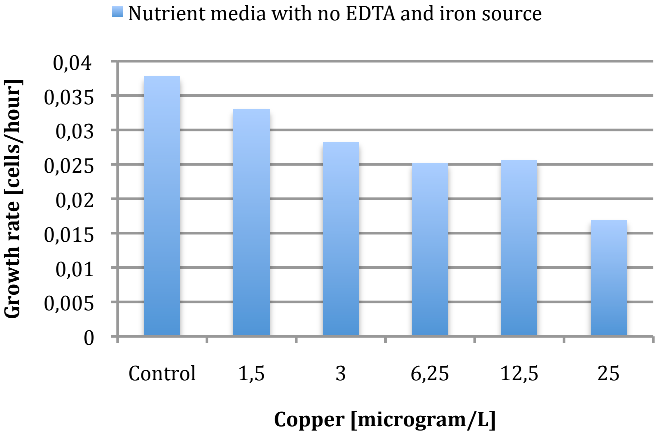


Figure 43 Toxicity of stormwater spiked with copper and cultivated in nutrient media with no EDTA and no iron source (18.02.09). The control batch in algae cultivated without addition of copper.

Iron source

As the presence of EDTA showed to have significant effect on the toxicity of copper ions, the presence of an iron source in the algae culturing proved to be important as well. The algae culturing media consists of four stock solutions. The stock solution B contains EDTA and an iron source. The stock solution is prefabricated from MicroBioTests, so to avoid EDTA, thus will the iron source also be omitted.

This omission of the iron proved to more crucial for the algae growth rate than expected even though the iron source in only is present in the algae culturing media in 64 ‰ (ISO 2004). The growth rates of the algae in the control batches decreased from a mean value of 0.14 cells/hour to 0.03 cells/hour when the iron source was omitted (Figure 44). To avoid this the stock solution B was prepared without EDTA but with an iron source of 64 ‰.

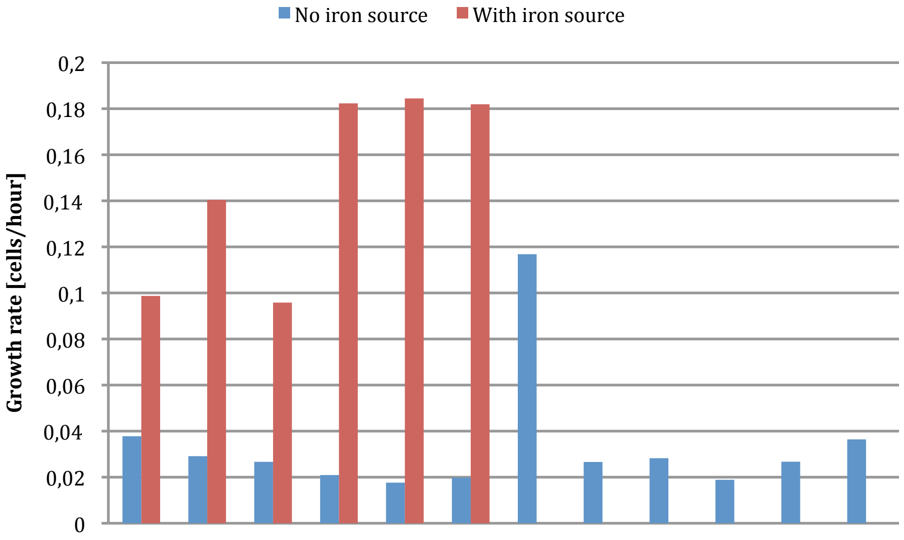


Figure 44 Overview of growth rates in relation to the composition of the algae culturing media with and without an iron source.