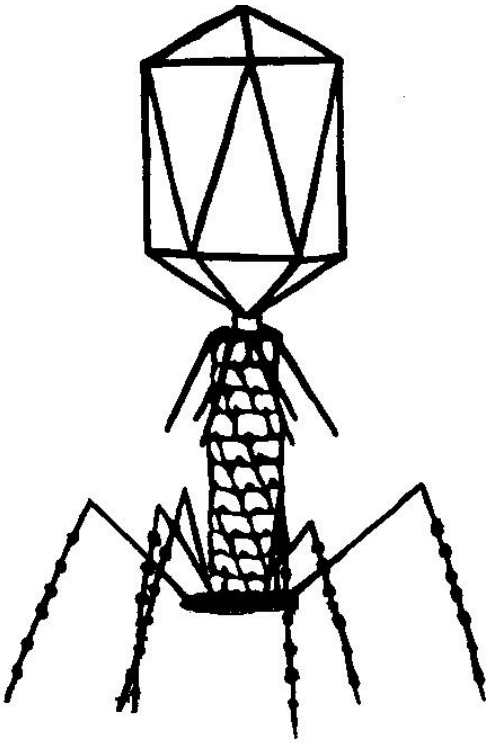
Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences

Virus removal during artificial groundwater recharge and the effects of organic matter

- Tunåsen infiltration basins, Uppsala, Sweden

Lisanne Hölting



Department of Aquatic Sciences and Assessment
Master´s Thesis – 30 ECTS
European Master in Environmental Science (EnvEuro)
Uppsala, Sweden 2015

Virus removal during artificial groundwater recharge and the effects of organic matter

- Tunåsen infiltration basins, Uppsala, Sweden

Lisanne Hölting

Supervisor: Prof. Dr. Kevin Bishop, Swedish University of Agricultural Sciences, Department for Aquatic Sciences and Assessment

Assistant Supervisors: Ph.D. Jean-Marc Mayotte, Uppsala University, Department of Earth Sciences

Prof. Dr. Ellen Kandeler, University of Hohenheim, Institute of Soil Science and Land Evaluation

Examiner: Prof. Dr. Björn Vinnerås, Swedish University of Agricultural Sciences, Department of Energy and Technology

Credits: 30 ECTS hec
Level: Second cycle, A2E
Course title: Independent Project in Environmental Science
Course code: EX0431
Programme/education: European Master in Environmental Science (EnvEuro)

Place of publication: Uppsala, Sweden
Year of publication: 2015
Cover picture: www.waksman-foundation.org
Online publication: http://stud.epsilon.slu.se

Keywords: drinking water; artificial groundwater recharge; bacteriophages; organic matter; adsorption; inactivation

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department for Aquatic Sciences and Assessment

Abstract

The contamination of drinking water with pathogenic microorganisms can lead to outbreaks of waterborne diseases, which are a major risk to human health worldwide. Even though surface waters are more vulnerable to contamination with pathogenic microorganisms than groundwater, the use of surface waters in artificial recharge systems has become an important method for the production of drinking water. This study focuses on the artificial recharge scheme Tunåsen in Uppsala, Sweden, where water from the Fyris River is infiltrated through sand in order to recharge the groundwater body and later to be used for drinking water production. The effects of organic matter on the removal of viruses during the infiltration process have been analyzed, as the levels of organic matter in the Fyris River.

Batch reactors were filled with water and sand from the Tunåsen infiltration scheme, as well as with a model virus, and were then attached to an orbital agitator that simulates the infiltration process. The batch reactors were sampled over 69 days in order to assess the decrease in virus concentration over time. Different levels of dissolved organic matter (DOM) and ionic strength (IS) have been used, which represent water chemistry variations of the Fyris River. A literature study was conducted, leading to the hypothesis that the increase in DOM in the river could lead to a lower virus removal, whereas high IS could enhance the virus removal process. Furthermore, two soils of the infiltration scheme that differ in soil organic matter (SOM) were used to test, whether high SOM reduces virus adsorption. The soil with high SOM content has been used for 9 years, whereas the soil with low SOM content has not been used for infiltration, yet.

The experiments were kept at 4°C (winter conditions in Uppsala), which creates a worst case scenario for virus removal, as virus inactivation by temperature is kept at a minimum and the virus removal is mainly dependent on adsorption to the sand. MS2 bacteriophages were used as they represent a worst-case model virus for adsorption and the double agar layer method was used for their detection. The decrease in MS2 concentration over time was modeled and a comparison between the virus removal experiments under dynamic and static conditions was done.

The results have shown that under winter conditions, inactivation is close to zero and adsorption is the main process, responsible for virus removal. The effects of DOM and IS on the virus removal process were rather small, but is has been shown that a combination of high DOM and low IS leads to the lowest inactivation rates. As assumed, a high level of SOM in the infiltration material probably suppresses adsorption almost completely, resulting in an overall low removal of viruses during the winter months. In conclusion, soil that accumulates organic matter over time could result in the increased risk of waterborne disease outbreaks.

Deutsche Zusammenfassung

Die Verunreinigung von Trinkwasser mit pathogenen Mikroorganismen kann zu epidemischen Krankheitsausbrüchen führen und stellt daher weltweit ein Risiko für die menschliche Gesundheit dar. Oberflächengewässer sind im Gegensatz zu Grundwasser anfälliger für Verunreinigungen. Trotzdem steigt die Nutzung von Oberflächengewässern zur Herstellung von Trinkwasser, aufgrund die wachsende Nachfrage der Bevölkerung. 50% des Trinkwasserkonsums in Schweden wird durch die Nutzung von Oberflächengewässern gedeckt, ein Großteil davon durch die künstliche Anreicherung von Grundwasser. In Uppsala, Schweden, wird Wasser aus dem naheliegenden Fluss Fyrisån, in sandgefüllte Infiltrationsbecken gepumpt, um die natürlichen Grundwasservorkommen anzureichern, die als Trinkwasser genutzt werden.

Diese Studie konzentriert sich auf die Adsorption und Inaktivierung von Viren während des Infiltrationsprozesses. Bodenproben stammen aus den Infiltrationsbecken und Wasserproben aus dem Pumpwerk bei Tunåsen, Uppsala. Eine Laborstudie wurde durchgeführt, in deren Fokus die Auswirkung von organischem Material auf die Adsorption und Inaktivierung der Viren steht. Flusswasser, Sand und Modelviren (MS2 Bakteriophagen) wurden in Bioreaktor-Gefäße gefüllt und ständiges Rühren simulierte den Infiltrationsprozess über 10 Wochen. Über diesen Zeitraum wurde die Konzentration der Viren anhand der „Double Agar Layer“- Methode gemessen. Die Experimente wurden zudem bei 4°C durchgeführt, um Winterbedingungen in Uppsala zu simulieren und ein Worst-Case-Szenario für Vireninaktivierung zu erschaffen.

Zwei verschiedene Böden wurden getestet, von denen einer schon mehrere Jahre als Infiltrationsmaterial genutzt wird und einer, der noch ungenutzt ist. Da die Konzentration von organischem Material in Oberflächengewässern während des letzten Jahrzehnts konstant gestiegen ist, reichert sich immer mehr organisches Material als organische Bodensubstanz (SOM) im Infiltrationsmaterial an. Die zwei Böden unterscheiden sich daher in ihrem Gehalt an organischen Kohlenstoff (TOC). Während im „alten Sand“ 0.034 % TOC gemessen wurde, wurde im „neuen Sand“ nur 0.008 % TOC gemessen.

Weiterhin unterschieden sich die Experimente in der Konzentration gelöster organischer Stoffe (DOM) und Ionenstärke (IS). Für beide Faktoren wurden jeweils eine hohe und eine niedrige Konzentration gewählt, welche die Konzentrationsschwankungen im Fluss Fyrisån wiederspiegeln.

Wie angenommen, ist die Inaktivierung der Viren während der kalten Wintermonate insgesamt sehr gering und Adsorptionsprozesse sind hauptverantwortlich für die Entfernung der Viren. Während SOM große Auswirkungen auf die Adsorption der Viren hatte, zeigten Schwankungen in IS und DOM hauptsächlich Auswirkungen auf die Inaktivierung der Viren. Eine Kombination von hohem DOM und niedriger IS führte zu den geringsten Inaktivierungsraten. Die Anreicherung von SOM in den Infiltrationsbecken führte dazu, dass Virusbindungsstellen geblockt wurden und die Adsorptionskapazität des „alten Sandes“ gleich Null war. Der „neue Sand“ hingegen, adsorbierte die Viren schnell.

Insgesamt zeigen die Ergebnisse, dass die Effizienz der Adsorptions- und Inaktivierungsprozesse von Viren in Infiltrationsbecken künstlicher Grundwasseranreicherungsanlagen mit der Akkumulation von organischem Material über die Zeit abnimmt.

Svensk Sammanfattning

Dricksvattenförorening orsakad av patogena mikroorganismer kan leda till utbrott av vattenburna sjukdomar, vilket utgör en stor risk för människors hälsa i hela världen. Ytvattens betydelse för dricksvattenproduktion växer, trots att de är mer utsatta för kontaminationsrisk. Denna studie fokuserar på det konstgjorda infiltrationssystemet vid Tunåsen i Uppsala, Sverige, där vatten från Fyrisån infiltreras genom sand för att fylla på grundvattenmagasinet. I denna laboratoriestudie har processen för borttagning av virus under infiltrationen undersökts, med fokus på effekterna av organiskt material i vattnet.

Halten organiskt material i ytvatten i Sverige ökar och det är ännu inte helt klarlagt hur det påverkar virusborttagningsprocessen. Genom en litteraturstudie formulerades hypotesen att höga halter löst organiskt material (dissolved organic matter, eller DOM) leder till en minskad borttagning av virus medan en hög jonstyrka kan förbättra virusborttagningsprocessen. Dynamiska ”batch reactor”-experiment med olika förhållanden konstruerades för att undersöka effekterna av DOM och IS på virusborttagningsprocessen.

Två jordar undersöktes, en som använts i infiltrationsbassänger under flera år och en som inte använts tidigare. Jordarna har olika halter organiskt material (SOM) och enligt hypotesen skulle den oanvända jorden med låg halt SOM avlägsna mer virus. Målet med denna studie var att bidra till utformningen av dricksvattenanläggningen på Tunåsen. Jord- och vattenprover togs från detta område. Experimenten utfördes vid en temperatur på 4°C (vinterförhållanden i Uppsala) för att skapa ett worst-case-scenario för borttagning av virus, då inaktivering minskar vid lägre temperaturer. En modell användes för att förutsäga viruskoncentrationens minskning över tid. Resultaten från de olika virusborttagningsexperimenten har sedan jämförts under dynamiska och statiska förhållanden.

Resultaten visar att borttagning av virus i infiltrationsbassänger minskar över tid, samt att effektiviteten minskar då organiskt material ackumuleras i jorden. Resultaten visar även att adsorptionsprocesser avstannar vid höga halter SOM samt att korrelationen mellan virusborttagning och DOM respektive IS är låg. Slutsatsen av denna studie är att ackumulation av organiskt material i marken över tid ökar risken för vattenburna sjukdomsutbrott.

TABLE OF CONTENTS

List of figures

List of tables

Abbreviations

BAB = Blood Agar Base plates

BCP = Bromocresol Purple Lactose Agar

BG solution = background solution

C initial = Initial virus concentration

d+/d- = High /low level of dissolved organic matter

DLVO = Derjaguin–Landau–Verwey–Overbeek theory

DOC = Dissolved organic carbon

DOM = Dissolved organic matter

e+/e- = High/low level of ionic strength

EC_{25} = Electrical conductivity at reference temperature 25°C

IS = Ionic Strength

$M_{initial,}$ $M_{final,}$ $M_{removed}$ = Initial / Final/ Removed virus mass

NaCl = Sodium Chloride

NOM = Natural organic matter

NRNOM = Reference sample “Nordic Reservoir NOM”

OM = Organic matter

PFU = Plaque forming units

pH = Potential of hydrogen

pI = Isoelectric point

std = standard deviation

SOM = Soil organic matter

TC = Total carbon

TIC = Total inorganic carbon

TOC = Total organic carbon

α = Resistivity coefficient

ΔS = Separation distance

λ = Removal rate

$\lambda 0$ = Initial removal rate

1. Introduction

1.1. Safe drinking water consumption

The quality of water has significant impacts on human health. The contamination of water with pathogenic microorganisms can lead to outbreaks of waterborne diseases, which are a major risk to human health worldwide (WHO, 2011). We use water for different purposes every day, as drinking water, for irrigation and for hygienic and recreational purposes. As a result, microbial contamination can spread fast and via different pathways. The consumption of drinking water can represents a direct and therefore fast transmission pathway between the host and the pathogen (WHO, 2011).

In order to safeguard the quality of our drinking water, the risk of infection has to be minimized by preventing microbial contamination. Safe drinking water is considered as a basic human right and is therefore a priority policy goal (WHO, 2011). The development of health-based targets, sound risk assessments, monitoring and communication systems belong to the good management of drinking water supplies (Andersson and Bohan, 2001; WHO, 2011).

Sweden has a long history of reporting and managing waterborne diseases. Cholera epidemics have firstly been reported in 1834 (Andersson and Bohan, 2001). The most common outbreaks in the beginning of the twentieth century were typhoid, shigellosis, polio and hepatitis (Andersson and Bohan, 2001). With increasing sanitation and hygiene standards, this situation changed and today, a number of regulations control the safety of drinking water. Nevertheless, the Swedish National Food Administration (Livsmedelsverket) reports that "*between 1998 and 2002, there was more than one microbial disturbance per week that lead to a boil-water recommendation*" (Lindberg and Lindqvist, 2005). Certain viruses, such as Norwalk viruses, have been diagnosed a problem in Sweden and it has been stated that the knowledge of the occurrence of viruses in Swedish drinking water is generally scarce (Andersson and Bohan, 2001; Keswick and Gerba, 1980; Lund and Lindqvist, 2004).

Viruses are worst-case pathogens, as they are small and infectious at a small doses (WHO, 2011). They originate from faecal contamination and a major source of viruses in surface waters in Sweden are probably the effluent waters of decentralized sanitation systems: about 750 000 properties in Sweden are not connected to wastewater treatment plants and therefore lack a controlled sanitation system (SEPA, 2008). Monitoring of viruses in drinking water treatment plants is generally limited to the use of bacterial indicators that detect faecal pollution (Lund and Lindqvist, 2004). It has been shown that these indicators do not reflect viral contamination adequately, limiting the potential of preventive measures (Lindberg and Lindqvist, 2005; Lund and Lindqvist, 2004). Chlorine dosing for the inactivation of viruses is limited in Sweden, which further increases the risk of waterborne outbreaks in case the water is contaminated (Hummel, 2014; Westrell et al., 2006). Finally, it has been reported that the direct consumption of tap water (unbottled and not heated up) is high in comparison to other countries (Westrell et

al., 2006), stressing the need for efficient microbial barriers in the drinking water production schemes.

1.2. Drinking water production through artificial groundwater recharge

Groundwater is usually the preferred source for drinking water, because of its higher quality: i) the water is protected through the soil from direct contamination; ii) natural cleaning mechanisms in the aquifer support the removal of pathogens and other contaminants (Bouwer, 2002; John and Rose, 2005; Keswick and Gerba, 1980).

Surface waters, in contrast, are more vulnerable to contamination, present a higher risk of carrying pathogens. The quality of surface waters varies much more, which makes it a less attractive source of drinking water (Lindberg and Lindqvist, 2005). Nevertheless, the importance of surface waters in drinking water production is growing, as the overall demand for drinking water is growing (Bouwer, 2002; Yates et al., 1987). In Sweden, 50% of the population directly uses surface water as drinking water and 25% of the population consumes groundwater that has been artificially recharged with surface water (Sundlöf and Kronqvist, 1992). Only the remaining 25% directly use groundwater sources.

The recharge of groundwater aquifers is an artificial system, where water from streams and lakes is pumped on or into the ground. The water infiltrates through basins, ditches or wells; percolates through the soil, recharges the groundwater aquifer and can be used as drinking water (Bouwer, 2002; Frycklund, 1998). The difference between natural recharge and artificial recharge is basically an enhanced and constant flow (Frycklund, 1998).

The removal of viruses and other contaminants from the surface water occurs during the soil passage and is influenced by a number of environmental factors, of which some are well known and some are not fully understood (Keswick and Gerba, 1980). Water chemistry and water flow play a role in this process and the removal efficiency is very much dependent on the type of soil and the type of virus. About 100 enteric viruses have been found in surface waters and predictions on their survival time in soil vary between single days to many months (Gerba et al., 1975). Overall, it has been reported, that the prediction of the virus removal still includes uncertainties (Schijven and Hassanizadeh, 2000).

One of the current knowledge gaps is the effect of organic matter on the process, which will be a focus of the study. For one thing, it is known that organic matter carries pathogens and for another, organic matter accumulates in the soil used for artificial groundwater recharge, which probably decreases its natural cleaning efficiency (Frycklund, 1998). Increasing organic matter levels have been proven for the catchment of the Fyris River, which serves as a source for the drinking water supply in Uppsala (Ledesma et al., 2015). In the following, the recharge scheme for Uppsala will be presented, the sources and coherent risks of viruses in drinking water will be reviewed and background on the nature, sources and trends of organic matter in Swedish streams and lakes will be given.

1.2.1. The infiltration scheme Tunåsen

In Uppsala, groundwater is extracted from the Uppsala esker, which was formed by glaciofluvial deposits from the last Ice Age (Bergström, 1986). The esker allows natural groundwater extraction rates of 300 - 400 l/s. This has not been sufficient to secure the water supply of the city (Bergström, 1986; Hummel, 2014) and the growing demand led to the installation of two artificial recharge systems in Stora Vallskog and Tunåsen, starting to produce in 1968 and 1974, respectively (Morosini, 1989). The production of water increased from 365 m^3/day in 1876, to 13500 m^3/day in 1950 and to 45000 – 50000 m^3/day today (Bergström, 1986; UppsalaVatten, 2014).

Today, Stora Vallskog is the smaller infiltration facility (9 800 m^3/day) and Tunåsen the larger one (16 200 m^3/day) (Hummel, 2014). Both sites belong to the Uppsala esker. The core consists of coarser stone material, which is overlaid with gravel, sand and postglacial clay. The esker has a length of approximately 200 km and an average thickness of 10 m, even though it is up to 50 m thick in the area of Tunåsen (Morosini, 1989).

Water from the Fyris River serves as a water source for both infiltration sites. The surface water still contains dissolved organic matter, microorganisms and inorganic substances, which should not be transported to the drinking water. By percolating through the soil, the quality of surface water increases. Natural cleaning mechanisms of the soil apply, such as mechanical filtering, sedimentation, adsorption and biochemical and bacterial activity (Frycklund, 1998). In that way, artificial recharge is an alternative for chemical treatments of drinking water. Chemical treatments remove organic compounds and disinfect, but they have several drawbacks: the use of resources for the production of chemicals and the use of energy are high; furthermore, harmful by-products from chemical treatments are released to the environment. (Frycklund, 1998)

The recharge scheme in Tunåsen works as following:

1) Water from Fyris River is taken in at Storvad, where a rapid sand filter removes larger material;
2) The filtered water from Storvad is pumped up to a distribution house at Tunåsen, where it is pumped into ten slow sand filters (open basins) that are used on a rotating basis;
3) The water infiltrates in the open basins and mixes with naturally infiltrated groundwater;
4) It flows in a general north to south gradient and is abstracted at Storvad, Galgbacken, Stadsträdgården and Sunnersta wellfields;
5) After the water is abstracted, it is pumped from Storvad and Galgbacken to the Gränby Water Treatment Plant or from Stadsträdgården and Sunnersta to the Bäcklösa Water Treatment Plant, where the water is further treated and finally distributed to the consumers. (Morosini, 1989; UppsalaVatten, 2014)

One of the main treatment effects aimed at in the infiltration basins is the removal of organic matter, which adsorbs to the sediment (Frycklund, 1998). Organic matter has to be removed from the drinking water, as it carries contaminants and pathogens, and increases taste and odor problems (Löfgren et al., 2003). It accumulates in filtering sediments, such as the infiltration basins at Tunåsen, and can be removed from the water in this way.

The infiltration basins at Tunåsen are filled with 1 m of sand and about 5 – 20 cm are scraped off after every year of operation, in order to remove the sediment with highest accumulation of organic material. When all infiltration sediment is removed, the basins are filled with new sand from a quarry in Björklinge, north of Uppsala. For this study, one sand that has been used in the infiltration basins since 2005 (9 years) and one unused sand were used in the batch experiments in order to analyze the effect of organic matter accumulation on virus removal.

1.3. Viruses in the subsurface environment

The primary concern of health hazards in regard of waterborne diseases is the microbial contamination from human excreta (Keswick and Gerba, 1980). High numbers of enteric viruses are present in wastewater and it has been proved that wastewater treatment plants cannot remove viruses completely (Bosch, 1998; Goyal and Gerba, 1979). Consequently, they are released in the environment. Other sources and pathways of enteric viruses in the subsurface are presented by Keswick and Gerba (1980): Through the land application of sewage sludge, crop irrigation with wastewater, deposition sites and landfills, as well as through leakage from septic tanks and sewer lines, viruses can reach water bodies (Keswick and Gerba, 1980).

Viruses are considered as a worst case pathogen, because they are smaller than bacteria (virus: 0.02-0.4 µm, bacteria: 0.5-5 µm) and therefore more difficult to detect and to filter (Bosch, 1998). They survive longer than bacteria and infect more people at the same exposure dose (Bosch, 1998; Gerba et al., 1975). A single virus can already have adverse effects on human health (Yates et al., 1987). Waterborne viruses of concern in developed countries are polioviruses, coxsackievirus A and B, echovirus, hepatitis A, rotavirus, Norwalk and Adenovirus (Bosch, 1998; John and Rose, 2005).

Viruses generally consist of two parts: a nucleic acid genome of RNA or DNA and a capsid of glyco- and lipoproteins proteins (Jin and Flury, 2002). Enteric viruses are transported by humans, replicate inside the living cell of their host and infect the intestinal tract (Bosch, 1998). They are primarily passed on through the ingestion of contaminated food and water. The morphological characteristics of the virus determines its survival in groundwater (Gerba, 1984; Jin and Flury, 2002), which can be long enough to be transported to the point of drinking water withdrawal (Anders and Chrysikopoulos, 2005; Keswick and Gerba, 1980).

1.4. Natural organic matter in surface waters

Natural organic matter originates from decomposed plant tissues. In soils, soil organic matter (SOM) is supporting the soil structure and productivity. A lot of SOM is produced, if the nutrient availability, temperature, moisture and pH conditions are favorable for the living conditions of plants and microorganisms (Clark et al., 2010). In waters, organic matter can

either originates from terrestrial produced plants and SOM or from the production of macrophytes, algae and bacteria in the aquatic system (Leenheer and Croué, 2003).

The concentration of natural organic matter (NOM) in surface waters is a component of water chemistry and a key factor influencing water quality (Monteith et al., 2007; Pagano et al., 2014). Natural organic matter serves as a carbon source, transports contaminants and pathogens, causes "browning" of waters, as well as taste and odor problems in drinking water plants (Löfgren et al., 2003; Pagano et al., 2014). It affects the suitability of surface water as drinking water detrimentally (Pagano et al., 2014).

A part of the NOM in water is bound to particles, but the major part is dissolved. Dissolved organic matter (DOM) largely consists of carbon (approximately 50% by weight) (Hytteborn et al., 2014). Natural organic matter in water is therefore often referred to as dissolved organic carbon (DOC) and measured in DOC or Total organic carbon (TOC) (Hytteborn et al., 2014). Dissolved organic matter can be further broken down into two chemical groups: humic and non-humic substances. Non-humic substances, such as sugars and proteins are rapidly consumed by microorganisms (Clark et al., 2010). Humic substances, such as humic acids, fulvic acids and humins are less biodegradable and their persistence and chemical properties seem to be responsible for the detrimental effects of NOM on water quality (Leenheer and Croué, 2003; Löfgren et al., 2003).

The input of organic matter into streams and lakes is increasing in many parts of the world since the 1980s (Monteith et al., 2007; Pagano et al., 2014). This is of special interest in Sweden, where surface waters are largely used for the drinking water supply (Löfgren et al., 2003). A large number of studies have been conducted in Sweden that demonstrate an increase in DOC concentrations in Swedish surface waters over the past few decades (Erlandsson et al., 2008; Futter et al., 2014; Hytteborn et al., 2014; Köhler et al., 2009; Ledesma et al., 2015). One long-term study presented by Erlandsson et al. (2007) analyzed DOC concentrations trends for twenty-eight large Swedish catchments (> 210 km²) between 1970 and 2004 (35 years). Increasing concentrations were found for the whole country and also for the Fyris River catchment (2006 km²), reported by Ledesma et al. (2012) in a long-term study between 1995 and 2011 (Appendix: Fig. 19). Figure 1 presents the increasing TOC levels, measured in the Fyris River between 1993 and 2014 (Fyrisån Klastorp Station, SLU database: Miljödata MVM).

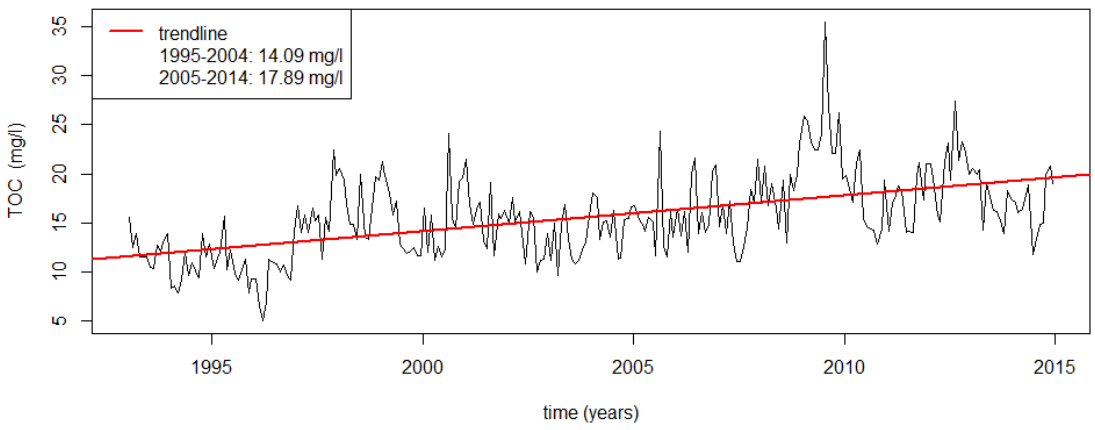


Figure 1: *TOC measurements at the Fyrisån measurement station Klastorp. The red line presents the trend line and the figures given in the plot give the average TOC values for the time periods 1995-2004 and 2005-2014.*

Different parameters are responsible for the increasing DOC trends. In general, factors that influence the production, transport and solubility of DOC have changed (Clark et al., 2010). First of all, the growth in population led to a change in land use. Higher agricultural productivity and intensified land use led to a higher production of terrestrial organic matter and a higher vulnerability to soil erosion and surface runoff at the same time (Clark et al., 2010). As a result, more SOM is transported into streams. Secondly, hydrological transport pathways have changed, resulting in a faster and more direct transport of SOM to streams (Clark et al., 2010; Köhler et al., 2009). And thirdly, the solubility of DOC in water has increased. Swedish streams and lakes recovered from acidification, leading to an increased solubility of DOC (Monteith et al., 2007; Pagano et al., 2014). There is an overall agreement that decreasing atmospheric depositions and changes in flow are the two key drivers behind the increased DOC trends in Swedish streams and lakes (Erlandsson et al., 2008; Pagano et al., 2014).

The increasing concentrations of DOM in the water have various sources as pointed out already. The high DOM levels in infiltration water could enhance the accumulation of SOM in the infiltration sediments of artificial recharge schemes and both, the increased SOM and DOC levels, could affect the removal of viruses from the water (Frycklund, 1998). The following literature review describes the virus removal process during soil passage and highlights the role of organic matter in the removal process.

1.5. Objectives of this study

The aim of this study is to review the process of virus removal for the infiltration scheme Tunåsen. Dynamic batch experiments have been conducted, using water from the Fyris River and two different sands from the Uppsala infiltration facility, in order to analyze the influence of different factors (SOM, DOM, IS) on the removal of viruses, with a focus on organic matter. The experiments were conducted as a worst-case scenario. The temperature conditions simulate winter conditions in Uppsala and a model virus was chosen that represents a worst case model virus for enteric viruses, meaning that a relatively high number of viruses survives the soil passage. The results of this study should contribute to the design of the drinking water facility in Uppsala, Sweden.

A literature study has been conducted in order to review factors influencing the virus removal process and to summarize the current knowledge and knowledge gaps. The literature review led to the following hypothesis:

- During the winter, virus inactivation in the infiltration basins is suppressed due to low temperatures.
- The accumulation of soil organic matter (SOM) in the infiltration basins and the increasing levels of dissolved organic matter (DOM) in the Fyris River may lead to lower adsorption of viruses in the infiltration basins.
- Furthermore, low levels of ionic strength (IS) may lead to a lower adsorption of viruses in the infiltration basins.

The overall research question of this study is: “How does each factor (SOM, DOM, IS) affect the removal of viruses in the sand of the infiltration basins of Tunåsen under winter conditions, using a worst-case model virus?”

2. Virus removal process: a literature review

2.1. Adsorption and Inactivation

The removal of viruses from groundwater aquifers is defined as the disappearance of viruses from water (Keswick and Gerba, 1980; Schijven and Hassanizadeh, 2000; Yates et al., 1987). It can be expressed in a quantitative way by defining the logarithmic reduction of virus concentrations over time (Schijven and Hassanizadeh, 2000). The two main processes that lead to the removal of viruses from groundwater aquifers are inactivation and adsorption (Schijven and Hassanizadeh, 2000; Yates et al., 1987):

INACTIVATION:

Viruses can travel a certain time and distance through the soil without losing their ability to infect other organisms, but they cannot maintain their metabolism forever (Schijven and Hassanizadeh, 2000; Walshe et al., 2010; Yates et al., 1987). Protein disruptions at their outer coat and the degradation of the nucleic acid lead to the inactivation of viruses (Grant et al., 1993; Jin and Flury, 2002). If a virus is inactivated, it cannot infect its host anymore (Gerba, 1984). A virus can be inactivated if it is attached to the soil particle surface or if it is free in the fluid phase (Grant et al., 1993) as demonstrated in Figure 2.

ADSORPTION:

The adsorption of suspended viruses is seen as a removal process, because it disappears from the water. If a virus is adsorbed to the soil surface, it is no longer mobile and will degrade over time. Adsorption is a two-step process that starts with the transport of the viruses to the soil particle surface (Schijven and Hassanizadeh, 2000). This step is mainly dependent on the mass flow of the infiltrating water. The second step is the immobilization of the virus at the soil surface (Grant et al., 1993). This step is mainly dependent on the binding forces and the rates of attachment and detachment (Schijven and Hassanizadeh, 2000). If the attachment is permanent and no detachment occurs, the immobilization at the soil surface is enhanced (Schijven and Hassanizadeh, 2000).

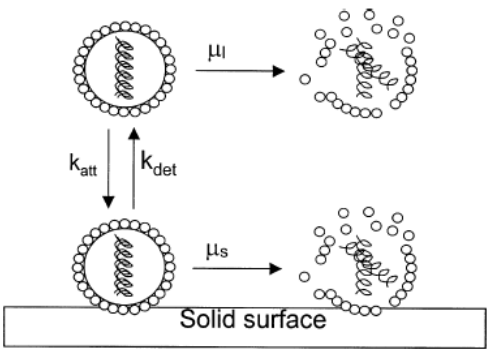


Figure 2: *Inactivation and adsorption of viruses at the solid surface (Grant et al., 1993)*

The total removal of viruses, as combination of adsorption and inactivation, is presented in Figure 2, where k_{att} is the attachment rate coefficient, k_{det} is the detachment rate coefficient, μ_s is the inactivation rate coefficient of attached viruses and μ_l is the inactivation rate coefficient of detached viruses. In the end, the rates of detachment, attachment and inactivation determine the virus-soil interactions and will decide how many viruses could possibly end up in the effluent (Grant et al., 1993; Schijven and Hassanizadeh, 2000). The different factors that lead to changes in adsorption and inactivation will be presented in the following.

2.2. The DLVO theory

The classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory describes the mobilization of solid colloids and explains the interactions between colloids by changes in their environment. The DLVO theory has been used to explain virus adsorption to surfaces and provides a framework to understand the virus-surface interactions (Gerba, 1984; Ryan and Elimelech, 1996).

Suspended viruses can be described as colloids that interact with soil particles (Gerba, 1984; Ryan and Elimelech, 1996; Yates et al., 1987). Both, the virus and the soil particles, have certain surface charges that induce attractive and repulsive forces between them. Two types of forces are generally described to control virus-soil interaction: a) attractive van der Waals forces and b) repulsive double layer forces (Gerba, 1984; Ryan and Elimelech, 1996).

Van der Waals forces (a) exist between two instantaneously induced dipoles (Gerba, 1984; Hermansson, 1999). An attraction occurs if the surfaces of the colloids are oppositely charged. The double layer forces (b) originate from two layers of ions (Stern layer, Gouy layer) that surround a colloid. These ions are attracted to the colloid in order to balance its surface charge and to keep the colloidal system electrically neutral (Gerba, 1984; Ryan and Elimelech, 1996). The first layer is called Stern layer and consists of ions that are oppositely charged to the colloid. The second layer is a diffuse cloud of ions, called Gouy layer (Gerba, 1984).

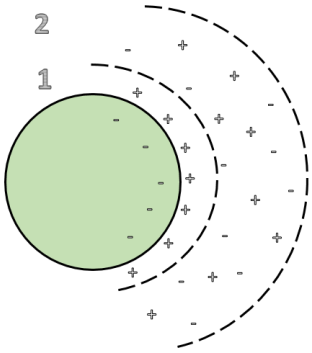


Figure 3: *Double layer of ions. 1 = Stern layer, 2 = Gouy layer*

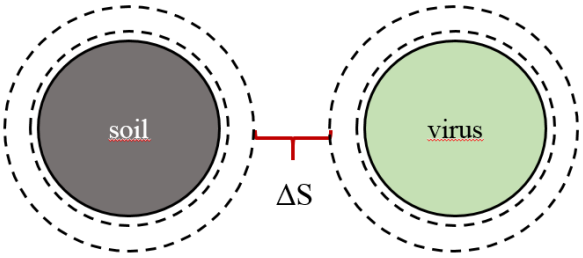


Figure 4*: Soil particle and virus particles as colloids, separated by the separation distance ΔS.*

If the double layers of two colloids are relatively thick, they overlap each other. Then, the separation distance ΔS between the two double layers is small and the repulsion between the virus and the soil colloids is maximal. At large separation distances ΔS (thin double layers), the repulsion is minimal. In conclusion, the double layer force is a form of repulsive energy between two colloids and the thickness of the double layers is the decisive parameter. The overall forces (ϕTotal) can be either attractive or repulsive. Figure 5 shows the total energy ϕTotal as a function of the separation distance ΔS.

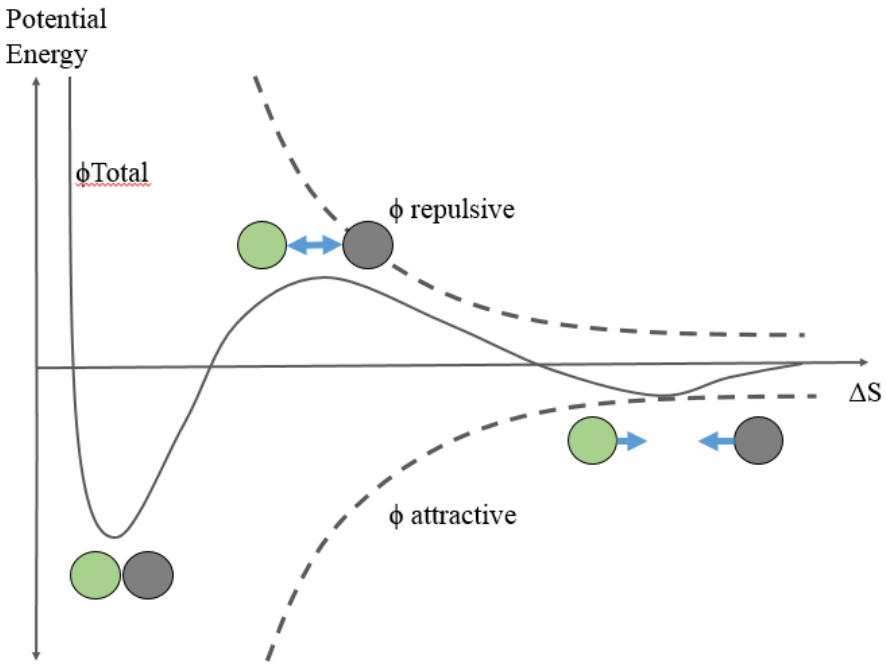


Figure 5: *DLVO energy as a function of the separation distance ΔS between two colloids. ϕTotal = Tota forces, ϕ repulsive = repulsive double layer forces, ϕ attractive = attractive van der Waals forces.*

At very small ΔS, there is an initial attraction well. In case of thick double layers (small ΔS) and maximal repulsion, the overall forces are repulsive. In case of thin double layers (large ΔS) and minimal repulsion, the attractive van der Waals forces lead to an overall attraction between the colloids (Schijven and Hassanizadeh, 2000).

2.3. Factors affecting Adsorption

In order to achieve a high removal of viruses from the water, an attachment to the soil is favored. If the thickness of the double layers of virus and soil particles is reduced, van der Waals attraction can lead to an adsorption of the virus to the soil surface, as shown in Figure 5 (Gerba, 1984). Factors that influence the thickness of the double layers (pH, IS) and other factors that influence adsorption (virus and soil type, organic matter, flow velocity) will be presented in the following.

2.3.1. Ionic strength and pH

The thickness of the double layer of colloids is mainly altered by a change in ionic strength (IS) or pH (Ryan and Elimelech, 1996). In general, the addition of IS leads to an increased level of counterions in the bulk solution (Ryan and Elimelech, 1996; Yates et al., 1987). Less volume is required to neutralize the surface charge of the colloids and the thickness of the diffuse layer is reduced (Gerba, 1984; Ryan and Elimelech, 1996). In this case, adsorption is more likely to happen (Gerba, 1984; Ryan and Elimelech, 1996).

In streams and lakes, a change in IS is linked to a change in flow (US EPA, 2012). Ions dissolve in waters through the weathering of minerals. As a result, groundwaters have a generally higher IS than surface waters (Morosini, 1989). The base flow in a river represents the inflow of ion-rich groundwater and contributes to a large part of the IS in natural streams. Precipitation on the other hand is low in ions and dilutes the IS in natural streams (Yates et al., 1987).

A change in pH influences the thickness of the double layers in a different way. The change in pH leads to an ionization of surface groups on the colloid (Gerba, 1984), such as carboxyl and amino groups of the viruses protein coatings, which can be ionized to COO^- and NH_3^+ (Fermin and Riley, 2010). The virus surface is consequently more negatively charged at a high pH, resulting in higher repulsive forces. At a low pH, the surface charges become less negative, resulting in lower repulsive forces (Gerba, 1984; Jin and Flury, 2002; Schijven and Hassanizadeh, 2000; Walshe et al., 2010). Different types of viruses have significantly different protein coatings and the relation between pH and adsorption rates is therefore virus specific (Schijven and Hassanizadeh, 2000).

2.3.2. Virus and soil type

Viruses and soil particles are colloids of a certain electrical charge and hydrophobicity (Jin and Flury, 2002). The charge of viruses and soil particles is not constant. At a defined pH value, called the isoelectric point (pI), every virus and soil particle has a net charge of zero. If the pH

around the colloid changes, the electrical charge of its surface changes accordingly (Gerba, 1984). The colloid is positively charged, if the pH is below its pI and it is negatively charged, if the pH is above its pI.

It has been reported that a larger number of enteric viruses have low pI values (Gerba, 1984; Jin and Flury, 2002):

Table 1*: Characteristics of selected enteric viruses.*

Virus	Diameter (nm)	Isoelectric point
Reovirus	81	3.9
Poliovirus	28 – 30	4.5 – 8.2
Echo 1	27	5.0 – 6.4
Influenza A	80 – 120	5.3
Coxsackie	27	4.8
Norwalk	~ 27	5.0

Enteric viruses are consequently more electronegatively charged in environments with neutral pH, such as rivers, lakes and groundwater and the attraction to positively charged soils is enhanced, due to strong van der Waals forces. There is still attraction to neutrally charged surfaces, but the overall forces to a negatively charged surface are repulsive.

The attachment of viruses is limited by the type of soil. Positively charged sites on soil surfaces that favor attachment might be per example sites with iron, aluminum or manganese oxide coatings (Loveland et al., 1996; Ryan and Elimelech, 1996; Schijven and Hassanizadeh, 2000). Sand, as in the sand filters of infiltration basins and as being used in this study, has been reported to have neutral charge, whereas organic matter contributes to a net negative charge (Chrysikopoulos and Aravantinou, 2012; Zhuang and Jin, 2003).

Furthermore, the electrical charge can be high or low, leading to stronger or weaker forces between the colloids. Accordingly to the DLVO theory, high surface charges lead to higher repulsive forces, because more counter-ions are needed to balance the surface charge and the double layer of counter-ions becomes ticker (Gerba, 1984).

Another indicator for virus adsorption is the degree of hydrophobicity of viruses and soils. Hydrophobicity of viruses arises from the coat proteins, which consist of hydrophobic lipids and amino acids (Jin and Flury, 2002; Ryan and Elimelech, 1996). Viruses differ in hydrophobicity, because of differences in the structures of virus coats. If a virus has high hydrophobicity, such as MS2 bacteriophages used in this study, it is more repelled from water molecules and more likely to interact with hydrophobic groups of soil colloids, such as hydrocarbons and aromatic structures in organic matter particles (Gerba, 1984). The hydrophobic interactions lead to hydrophobic bindings between the colloids. In that way, hydrophobicity may support adsorption if the soil surface offers hydrophobic binding sites (Gerba, 1984).

In summary, viruses are generally negatively charged in neutral environments and adsorb strongly to positively or neutrally charged soil surfaces. Hydrophobic groups on the soil surface may support the attachment by hydrophobic bindings, which is enhanced if the virus has a strong hydrophobicity. Nevertheless, the DLVO theory is a simplified theory, assuming that viruses and soil surfaces are homogenous, which is not true. In reality the colloids have different surface charges and different pIs, which originate from the heterogeneity of their surfaces (Ryan and Elimelech, 1996). The DLVO theory can only be used to explain some interactions, but the

interactions are very virus- and soil-specific and highly dependent on the environmental conditions.

2.3.3. Organic matter

Many studies suggest that the organic matter content of the infiltration sediment and the infiltrating water influences the adsorption of viruses (Bradford, 2006; Foppen et al., 2006; Pieper et al., 1997; Powelson et al., 1991; Zhuang and Jin, 2003). Organic matter is more negatively charged than most viruses at the pH of neutral water (pI ≤ 3) (Gerba, 1984; Walshe et al., 2010), which leads to a competition between viruses and particles of organic matter for the same positively charged binding sites (Gerba, 1984; Jin and Flury, 2002; Walshe et al., 2010). Especially, if the concentration of DOM is high or if the binding sites are rare, particles of organic matter are preferentially adsorbed and this competition can lead to an increased level of detached viruses in the effluent (Ryan and Elimelech, 1996). Binding sites on the soil surface will be blocked progressively and virus adsorption becomes lower. This phenomenon is called blocking (Schijven and Hassanizadeh, 2000).

In contrast to this, it is proposed that organic matter that is already bound to the sediment (SOM) may offer hydrophobic binding sites for viruses and could therefore enhance virus adsorption (Jin and Flury, 2002). As long as the effect of blocking is not bigger than the effect of hydrophobic binding, the adsorption of viruses could increase (Schijven and Hassanizadeh, 2000).

From the above considerations, it is to conclude that the effects of organic matter on virus adsorption may be either enhancing or reducing. Regarding the interpretation and predictions of virus adsorption in general, organic matter considerably increases uncertainties (Schijven and Hassanizadeh, 2000).

Zhuang and Jil (2003) studied the effect of organic matter on adsorption with two bacteriophages. They found that MS2 bacteriophages were significantly more adsorbed to soils in the presence of organic matter than ΦX174 bacteriophages. MS2 bacteriophages have a lower surface charge and a higher hydrophobicity than ΦX174 bacteriophages (Zhuang and Jin, 2003). Again, adsorption is a virus- and soil-specific process. They concluded that the effect of organic matter on virus adsorption (enhancing or attenuating) seems to depend on the dominant mechanism of colloidal interaction (electrostatic or hydrophobic). If electrostatic interactions play a dominant role in the system, as with ΦX174, which is low in hydrophobicity, but has a strong surface charge, virus adsorption will be reduced. If hydrophobic interactions are the dominant process, as with MS2, virus adsorption will be enhanced (Zhuang and Jin, 2003). Different authors suggest that electrostatic interactions are usually more dominant than hydrophobic interactions (Gerba, 1984; Torkzaban et al., 2006; Zhuang and Jin, 2003). This means that in most cases, virus adsorption via hydrophobic bindings do not play an important role. It is more likely that virus adsorption is reduced when organic matter levels are increasing, because of the competition for binding sites.

2.3.4. Flow velocity

Virus adsorption was widely studied under both, static and dynamic conditions. Under dynamic conditions it is possible to include the effect of flow on the virus removal process, which has been suggested as the most influential factor regarding virus adsorption in porous media, when compared with the effects of pH, ionic strength and organic matter (Walshe et al., 2010). A main difference between static and dynamic conditions are the collision efficiencies between viruses and soil particles, which are higher for dynamic experiments than for static experiments (Kretzschmar et al., 1997). The adsorption of viruses is generally limited by the collision frequency and the fraction of collision that actually results in attachment (Kretzschmar et al., 1997).

Under flow conditions, viruses that enter the soil passage are initially rapidly adsorbed, as the system aims for an equilibrium between attached and free viruses (Schijven and Hassanizadeh, 2000). Attachment rates are relatively high and detachment rates relatively low ("kinetic adsorption"). The time to reach equilibrium adsorption is estimated to vary between 20 min and 24 hours, depending on virus type, initial virus concentration, particle size, agitation, as well as on the number of adsorption sites (Jin and Flury, 2002). After equilibrium has been reached, the attachment and detachment rates are constant over time ("equilibrium adsorption") (Schijven and Hassanizadeh, 2000).

In the environment, flow rates in an aquifer can be increased by rain events or artificial recharge. Kretschmar et al. (1997) studied the effect of different flow velocities on virus transport in porous media. With high flow velocities, the collision efficiencies between viruses and soil particle decreased, resulting in lower attachment and higher detachment rates (Kretzschmar et al., 1997; Walshe et al., 2010).

2.4. Factors affecting Inactivation

Viruses can be inactivated, if they are free in the bulk solution or if they are adsorbed (Fig. 2). The removal of viruses by inactivation mainly depends on temperature and environmental factors that influence the microbial activity.

2.4.1. Temperature and microbial activity

Temperatures between 4 °C and 37 °C have a significant effect on virus survival (John and Rose, 2005). Inactivation rates increases with increasing temperature above a threshold of 4°C. The main reason for this is the thermal degradation of the proteins in the virus capsid (John and Rose, 2005). At temperatures below 4°C, the inactivation by thermal degradation is insignificant and viruses survive longer. The sensitivity of the inactivation rate on temperature is virus-specific, as it depends on the proteins of a virus (Yates et al., 1987), but the inactivation of attached and free viruses probably reacts similar to temperature changes (Gordon and Toze, 2003).

Inactivation also increases with increasing microbial activity, for both free and attached viruses (Gordon and Toze, 2003; John and Rose, 2005; Schijven and Hassanizadeh, 2000b; Yates et al., 1987). Microorganisms, living in the groundwater aquifer, can have deleterious effects on virus survival as they produce different enzymes and substances are virus-inactivating (Gordon and Toze, 2003). Microorganisms in the subsurface environment form a biofilm on the sediment, which can contribute to the inactivation of viruses through selective predation by bacteria (Engblom and Lundh, 2006; Skraber et al., 2005). If the sediment layer which contains the biofilm is replaced regularly, the formation of a biofilm can contribute to the overall removal of viruses.

2.4.2. The presence of soil and water

The inactivation rates of free and adsorbed viruses differ. If the viruses are already adsorbed to the soil surface, inactivation will be reduced, as adsorbed viruses are generally more protected against virus-inactivating enzymes and substances than free viruses (Schijven and Hassanizadeh, 2000). On the other hand, it has been found that inactivation of free viruses will be enhanced in the presence of soil, because the collision efficiency between the particles increases (Schijven and Hassanizadeh, 2000). Especially larger aggregates of viruses break faster in the presence of soil.

Many studies have examined the effect of soil and water interfaces on inactivation. Chrysikopoulos and Aravantinou (2003) state that the effect of "protection through soil" is particularly true for unsaturated conditions, where an air-water interface is formed. The air-water interface is associated with physical forces that enhance inactivation (Chrysikopoulos and Aravantinou, 2012; Schijven and Hassanizadeh, 2000). Especially under agitated and unsaturated conditions, inactivation rates increase tremendously, because the contact between the suspended viruses and the air-water interface increases (Chrysikopoulos and Aravantinou, 2012; Syngouna and Chrysikopoulos, 2010). The adsorption to soil particles protects the viruses against the inactivation at the air-water interface. Under saturated conditions, there is no air-water-interface and inactivation is lower.

2.5. Model viruses

Model viruses are used in laboratory studies, in order to study the fate of pathogenic viruses in the subsurface environment. It is not safe to use pathogenic viruses in experimental studies, because of an increased risk of infection and adverse health effects (Keswick and Gerba, 1980). Model viruses are non-pathogenic, that fulfil several requirements to be representative for enteric viruses. The model virus has to have: i) a similar ecology to the pathogens ecology, ii) a similar resistance as the pathogens resistance and iii) simple laboratory methodologies must be applicable (Bosch, 1998; IAWPRC, 1991).

Bacteriophages fulfil these criteria and are widely used as model viruses, as summarized by Schijven and Hassanizadeh (2000). They are not pathogenic to humans, but use exclusively

bacteria as host organisms. Bacteriophages infect bacteria cells by injecting their nucleic acid into the cell, replicating within the cell and releasing the new phages by lysis of the bacteria cell (IAWPRC, 1991). A large number of bacteriophages (10^{10} to 10^{20} PFU/m^2) can be seeded; a reduction up to 11 $\log_{10}$ can be shown; and the enumeration of bacteriophages is easy in comparison with pathogenic viruses (Schijven and Hassanizadeh, 2000).

The IAWPRC investigated the potential of using bacteriophages as model viruses with a focus on three groups of bacteriophages: somatic coliphages, F-specific bacteriophages and phages of the *Bacteroides fragilis* bacterium (IAWPRC, 1991). F-specific bacteriophages were found to be good indicators for enteric viruses. They show a great resistance against environmental stresses and their behavior in treatment processes was similar to the behavior of enteric viruses (Havelaar et al., 1993). The use of somatic coliphages as model viruses was found not to be the best choice, because they can multiply in unpolluted waters, their resistances vary a lot between different strains and the laboratory counts tend to overestimate the number of viruses. Next to F-specific bacteriophages, phages of the *Bacteroides fragilis* bacteria are another good indicator, especially for contamination with human feaces (IAWPRC, 1991).

2.5.1. MS2 bacteriophages

The bacteriophages used in this project are MS2 bacteriophages, which are F-specific bacteriophages. They belong to the *Leviviridae* family, are single-stranded RNA-phages with a cubic capsid and a diameter of 24-26 nm (Gerba, 1984; IAWPRC, 1991). As a host bacteria, *Salmonella typhimurium* (WG49 strain) can be used, which is a modified *E.Coli* strain (Walshe et al., 2010).With a pI of 3.9 it is negatively charged in neutral environments, such as a large number of enteric viruses (Table 1) (Durán et al., 2002). MS2 has a hydrophobic protein coat, containing lipids, which could lead to bindings with hydrophobic groups, such as organic matter compounds (Gerba, 1984; Schijven and Hassanizadeh, 2000).

Different characteristics of MS2 bacteriophages makes it a model virus for worst-case scenarios, meaning that a high number of viruses survives and possibly ends up in the effluent water after the soil passage (Schijven and Hassanizadeh, 2000). These characteristic include:

- their small size in comparison to other enteric viruses (Table 1),
- a relatively low sticking efficiency and low attachment rates in most soils,
- a relatively low stability regarding inactivation,
- a high sensitivity on temperature changes (high inactivation at high temperatures, very low inactivation at temperatures below 7°C). (Schijven and Hassanizadeh, 2000)

Due to its characteristics listed above, it is to expect that the bacteriophage does not adsorb well to many soils, except for those which have a high degree of hydrophobicity, such as organic soils.

3. Materials and Methods

3.1. Experimental setup

In order to test the inactivation of viruses under different conditions, a complex setup of dynamic batch experiments was installed with a full experimental design. The factorial design contains three factors with 3, 2 and 2 levels, giving a total of 12 experiments (3 x 2 x 2 = 12). The factors tested were ionic strength (IS), dissolved organic matter (DOM) and soil organic matter (SOM) with 3 levels of SOM ("high", "low", "no") and 2 levels for both, DOM and IS ("high", "low").

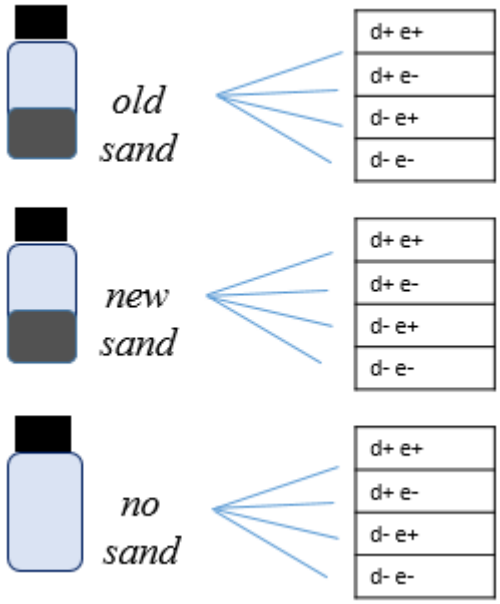


Figure 6: *The experimental setup: Three soil treatments were conducted with four different water chemistry treatments. ("d+" = high DOM, "d-" = low DOM, "e+" = high ionic strength and "e-" = low ionic strength)*

Figure 6 presents the setup of the twelve experiments. The "old sand", "new sand" and "no sand" represent the SOM levels high, low and no SOM, respectively. Each of the three soil treatments has four experiments that differ in IS and DOM, leading to a number of 12 experiments in total. For each of the 12 experiments, 13 batch reactors were prepared in the exact same way, in order do the analysis over 13 time steps. Furthermore, two replicates for each experiment was set up. Pyrex disposable culture tubes (11.5 ml) were used as batch reactors. In total 312 vials have been used (12 experiments *13 batch reactors * 2 replicates = 312 glass vials). The filling of the batch reactors, as well as details on the preparation of the background solutions is presented in Section 3.3.

In order to test the virus removal under flow conditions, an orbital batch agitator (length 1.10 m) was installed, which rotated at a speed of 4-4.2 rpm (Appendix: Fig. 20). The individual batch reactors were attached to it and the agitated conditions allowed a constant mixing of the sediment, water and bacteriophages. The agitator ran for 10 weeks and was kept in a Phytotron cabinet at a temperature of 4°C in the dark to prevent biological growth and the inactivation of phages by UV light or temperature. During this time period, individual batch reactors were taken off the agitator at random in order to enumerate the bacteriophages.

A static experiment has previously been conducted by Stacy Sutcliff-Johansson (M.Sc. Student, Uppsala University). It has been carried out under the same conditions, but without agitation. Figure 7 gives another overview over the experimental setup.

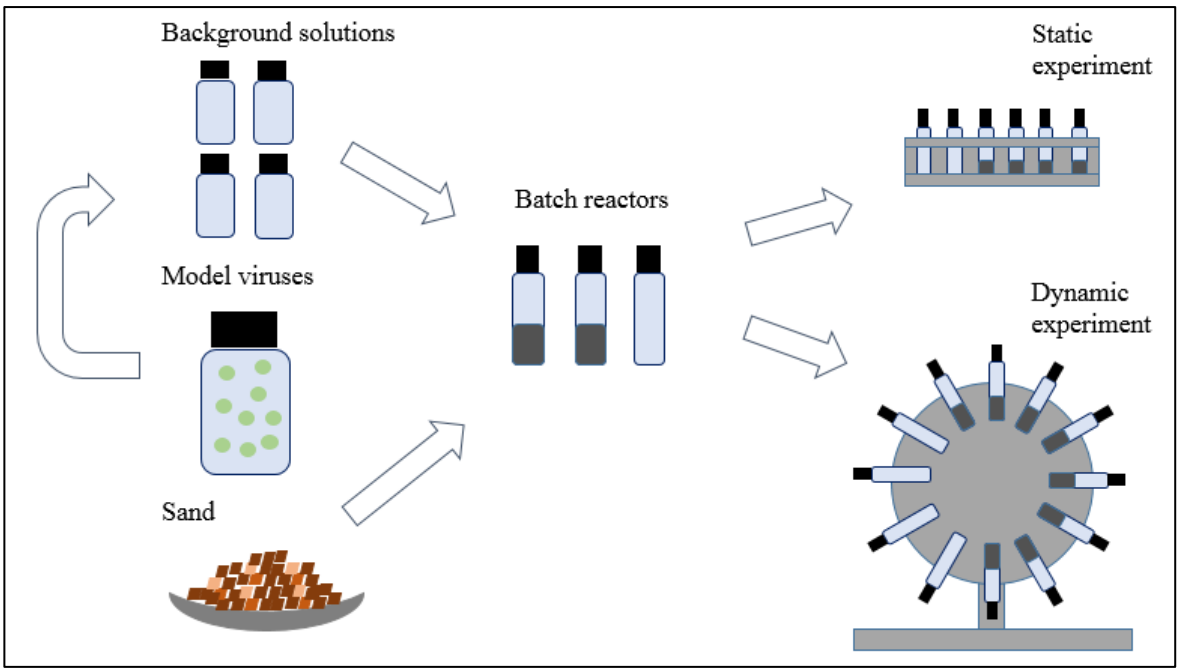


Figure 7: *Schematic of the experimental setup. Dynamic and static experiments are shown. Only one batch reactor for each soil treatment is presented (Chrysikopoulos and Aravantinou, 2012).*

3.2. Sampling and Sampling sites

Twenty liters of water were collected on the 9th February 2015 and stored in the dark at a constant temperature of 4°C. The cold and dark environment prevents changes in the water chemistry. The water was taken from the distribution house of the artificial recharge scheme (Fördelningskammare) located at the top of the Tunåsen esker wherein the infiltration basins lie. At this point, the water, having originated in the Fyris River, has passed through a rapid sand filter before being pumped up to the distribution house. The collected water was then filtered again, first through a 1.6 µm glass microfiber filter (VWR 691) in order to remove larger particles and then through a 0.45 µm glass microfiber filter (Supor 450) to remove undissolved natural organic matter. The pH, conductivity and DOC concentrations of the final, "raw water" sample are pH 8.0 ± 0.3, 414.0 ± 1.2 µS/cm and 17.4 ± 3.6 mg/l respectively (other measurements: Appendix, Table 20).

Two sand samples were taken on the 19th December 2014, one from a used infiltration basin at Tunåsen (basin 10) and the other one from the quarry where UppsalaVatten purchases their new sand for the infiltration basins. This quarry is located in Björklinge, north of Uppsala and the sediment that are being purchased for the infiltration basins is sand. The sand from the used infiltration basin, will be further referred to as "old sand". The sand from the quarry will be further referred to as "new sand". After the purchase of "new sand", 1m of "new sand" is put down in the infiltration basins and 20 cm is taken off the top after every year of operation. This is done in order to mitigate the detrimental effects of sediment blocking by particles and to guarantee efficient cleaning mechanisms. The "old sand" of this study has been used as infiltration sediment since 2005 (9 years) and is the last layer of sand to be used for infiltration.

The organic carbon content of both sand samples has been measured in order to test how much organic material accumulated in the used sediment over time and how much can be found in the new sediment. Through dry combustion of the sand samples with a LECO TruMac instrument, the Total Carbon (TC), Total Inorganic Carbon (TIC) and Total Organic Carbon (TOC) was measured. The TOC content can be used as an estimate for the level of organic matter (Hytteborn et al., 2014). The results show that much more organic matter was accumulated in the "old sand":

Table 2: *Soil analysis on TC, TIC, TOC*

	TC %	**TIC %**	**TOC %**
Old sand	0.045	0.011	0.034
New sand	0.048	0.040	0.008

In order to lessen the effect of grain-size distribution from the test results, both sand samples were air-dried for one week on a plastic sheet with the sand spread to a thickness of ~ 1 cm. Two articulating fans were in operation while the sand was drying. Each sand sample was then ordered accordingly to its grain-size distribution, separated and remixed in order to match the grain-size characteristics between the samples. The sand samples were then stored in a Polyethylene box at room temperature.

3.3. Background solution preparation

All background solution were prepared by using 1 l of the raw water sample, collected on the 9th February 2015 from Fyris River. According to the experimental setup, four background solutions were needed. They differ in DOM and IS as following: 1) high DOM and high IS, 2) high DOM and low IS, 3) low DOM and high IS, 4) low DOM and low IS.

The high DOM-background solutions were prepared by adding NOM to the raw water, in form of an IHSS Standard NOM sample. The high IS-background solutions were prepared by adding Sodium Chloride (NaCl). No NOM was added for the low DOM-background solutions; no NaCl was added to the low IS background solutions. The electrical conductivity (EC_{25}), pH, TOC (Sievers 900 portable TOC analyzer) and initial virus concentration (C initial) were measured after the preparation of the background solutions. Table 3 presents the additives and the measurement results.

Table 3: *Background (BG) solution preparation ("d+"= high DOM, "d-"= low DOM, "e+"= high ionic strength and "e-"= low ionic strength)*

BG solution	NOM added to 1.2 l (g)	TOC (mg/l)	NaCl added to 1.2 l (g)	EC_{25} (uS/cm)	pH	Final BG solution volume (mL)	C initial (PFU/ml)
d+e+	0.0584	31.24	0.0972	630	7.96	1000.1	$4.03*10^7$
d+e-	0.0603	30.57	-	440	8.06	1000.0	$1.24*10^7$
d-e+	-	17.71	0.0980	612	8.03	1000.1	$3.82*10^7$
d-e-	-	17.23	-	420	8.07	1000.2	$1.05*10^8$

The increase in IS is indicated by an increase in conductivity, as the conductivity of a solution depends on the number of ions present. The measured conductivity of the raw water samples was ~ 430 µS/cm, representing "low IS" conditions. By a stepwise titration experiment, it was found that an addition of ~ 0.975 g NaCl resulted in a conductivity of ~ 620 µS/cm, representing high conductivity levels measured in the Fyris River (SLU database: Miljödata MVM, Fyrisån Klastorp station: 1984 – 2014).

Natural organic matter was added in form of a NOM reference sample, provided by the International Humic Substances Society (IHSS). In this study, the "Nordic Reservoir NOM" (NRNOM) was used, which works as a reference sample for sites that resemble the sampling site. The NRNOM sample was isolated from Lake Vallsjøen in Skarnes, Norway, and was then homogenized (IHSS, 2013). The geographical location, land-use and the water chemistry of the Norwegian sample site resemble the study site in Uppsala (IHSS, 2013):

Lake Vallsjøen, Skarnes, Norway	Fyrisån, Uppsala, Sweden
60°15'23.7"N 11°53'01.1"E, 225 m above sea level, boreal landscape, pH = 5.6, EC = 21.0 µS/cm, DOC = 10.7 mg/L	59°53'40.2"N 17°37'19.4"E, 20 m above sea level, boreal landscape, pH = 8.0, EC_{25} = 41.4 µS/cm, DOC =17.4 mg/l

In order to get an even distribution of NOM in the background solutions, the 2.4 l raw water for the high DOM-background solutions were filled in 12 smaller flasks of 200 ml. 0.01 g NOM reference sample was added to each flask, and the flasks were agitated for 24 hours, to dissolve the NOM and to get an even distribution. Due to the experimental error, the concentrations vary slightly between the two high DOM-background solutions (Table 3). The average NOM addition was 0.06 g per 1.2 l, resulting in TOC levels of 30-31 mg/l, which represent high TOC levels in the Fyris River (SLU database: Miljödata MVM, Fyrisån Klastorp station: 1984 – 2014).

For the propagation of the virus titer MS2 bacteriophages and *Salmonella typhimurium* (WG49 strain), as their host bacterium, were removed from the "SLU stock library". The virus titer should have a high concentration of ~ 10^{10} PFU/ml. First of all, Salmonella colonies that were infected by MS2 bacteriophages were chosen and incubated to increase their number. It is possible to identify MS2 infected Salmonella colonies, by the use of Bromocresol Purple (BCP) Lactose Agar plates, which work as a colorimetric indicator. MS2 phages do only infect the "male" Salmonella bacteria, which produce acids that yield a color change on BCP plates. At a high pH, the BCP Lactose Agar remains purple, meaning that the Salmonella colonies are not infected by MS2 phages.

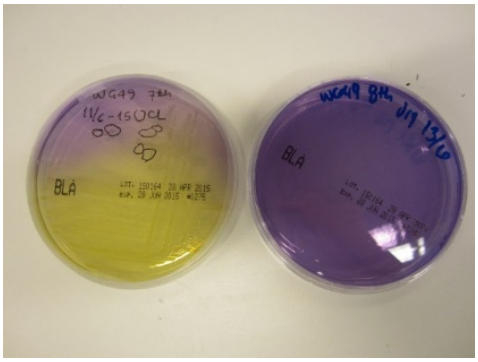


Figure 8: *Lactose Agar plates for MS2 propagation. Left plate: MS2 phages identified. Right plate: no MS2 phages.*

In order to get a high concentration (~ 10^7 PFU/ml) of the Salmonella host bacteria, a yellow colony was isolated from a BCP plate, using an inoculation loop of 10 um. The colony was put into nutrient broth (~ 300 ml) and incubated for 90 min at 37°C under agitated conditions. Phages from the "SLU stock library" were now added to the incubated host-nutrient broth. In order to multiply the phages, the new titer was again incubated at 37°C under agitated conditions for 24 hours. Afterwards, the titer was centrifuged in 2000 g for 15-20 min, filtered through a sterile filter, to remove aggregated virus and bacteria and the concentration was tested. The MS2 concentration in the titer was $3.36*10^{10}$ PFU/ml.

Then, 1 ml of the virus titer was added to each background solution (1000 ml) in order to achieve an initial virus concentration of ~ 10^7 PFU/ml. Bacteriophages were enumerated by the use of the double agar layer method, which is described in detail in Section 3.4. Due to the experimental error, the four background solutions had slightly different initial virus concentrations (C initial), as presented in Table 3. The average was $4.91*10^7$ PFU/ml, with a variation between $1.24*10^7$ PFU/ml and $1.05*10^8$ PFU/ml.

The individual batch reactors were filled with the according background solutions and the according sand in a soil-water ratio of 1:2 (appr. 5 g sand and 10 ml BG solution). For the "no sand" experiments, batch reactors were only filled with the according background solution (appr. 13 ml). The weight for each batch reactor was taken, which is presented in the Appendix (Table 14). In order to create saturated conditions, the vials were filled until the top to avoid any air in the vials.

3.4. Sample analysis

Two replicates of each experiment were sampled twice a week. The bacteriophages were enumerated by using the double agar layer method. In this technique, agar is plated together with a dilution of the phage´s host bacterium. Single viruses and virus colonies are not visible to the human eye, but the organisms appear as a hole in a colony of bacteria host cells. Each hole presents a plaque that can be counted visually as Plaque-forming units (PFU/ml) according to the ISO 10705-1 standard.

Dilution series were used, as the number of bacteriophages in undiluted samples was too numerous to count. First of all, the supernatant from each sample was taken with a 10 ml syringe and filtered with a 0.45 µm filter, in order to remove residual soil particles. A ten-fold dilution series was then prepared with Peptone buffered NaCl solutions in 16.5 ml vials.

For the plating, Agar warmed up to 48°C in individual glass vials on a Grant QBD4 heating block, in order to keep it liquid. The bacteria host broth (1 ml), the diluted sample (1 ml) and the Agar (2 ml) were then plated on Blood Agar Base (BAB) plates. Additionally, a positive and a negative control plate were prepared on each sampling day in order to control whether the host was working correctly. All plates were incubated at 37°C for at least 12 hours and Plaque-forming units were counted.

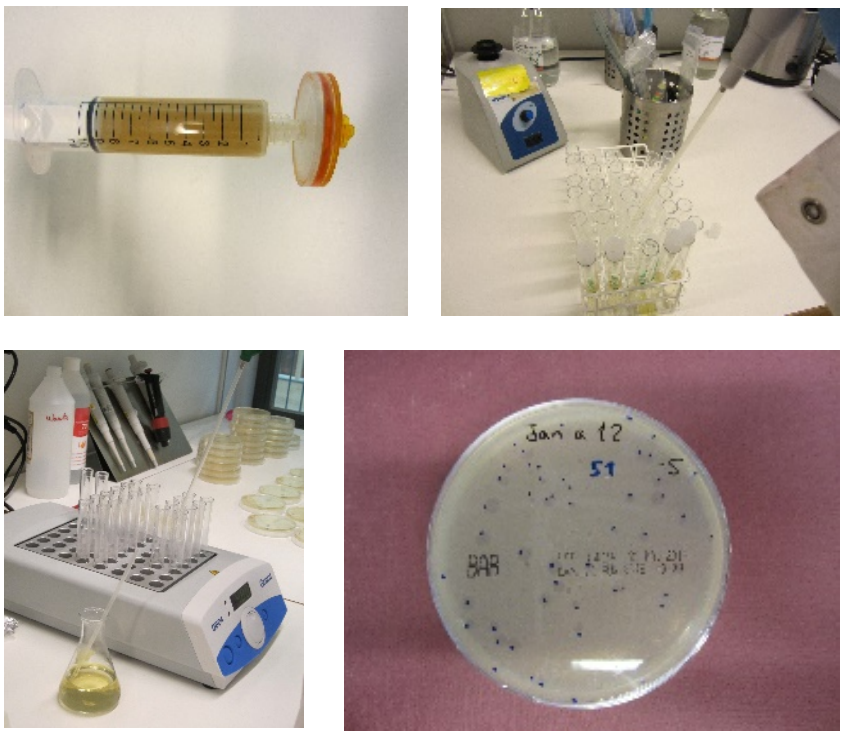


Figure 9: *a) filtering the supernatant, b) dilution series, c) agar heating and plating, d) enumeration of incubated plates*

Microbiological methods, such as the double agar layer method, include several uncertainty components, originating from the inoculum volume and the dilution series, the random scatter of particles (e.g. viruses) and the reading of the results (e.g. plates) (Niemelä, 2002). The different uncertainty components are further discussed in Section 5.5. The total uncertainty of the study results has been estimated by a Type A evaluation of uncertainty, following the ISO uncertainty guide (Niemelä, 2002). As a replicate of each experiment was taken, the standard deviation of each experiment and time step was computed, divided by the average value of both measurements, giving the relative standard deviation for each experiment and time step (Appendix: Table 21). The relative standard deviation, also known as the coefficient of variance, describes the precision of the experimental method as a dispersion of the measurements around the arithmetical mean value (US EPA, 2001). The total uncertainty of the individual experiments ranged between 27.06 and 74.72 % with an average of 43.33 %.

This high level of uncertainty is due to the small number of replicates (Niemelä, 2002). As the concentration of the virus titer has been measured more often, the titer can be seen as a better measure of the total uncertainty. Six independent parallel measurements have been taken, with a standard deviation of $1.36*10^{10}$ PFU/ml (mean: $2.43*10^{10}$ PFU/ml), resulting in an uncertainty of 55.92 %, which is even higher than the average total uncertainty of all individual experiments. The uncertainty of the experimental method strongly influences the interpretation of the study results, which is further discussed in Section 5.5.

3.5. Data Analysis

The observed virus concentration were decreasing over time. This decrease can be described as a first-order function by Equation 1, which has been previously used to explain the relation between virus concentrations and time (Chrysikopoulos and Aravantinou, 2012). The observed virus concentration presents the response variable; the measured time presents the predictor variable.

$$dC(t)/dt = -\lambda(\mathrm{t}) * C(t) \qquad \text{Equation 1}$$

C = concentration of suspended viruses
t = time
λ = removal rate coefficient of the suspended viruses.

The model uses a time-dependent removal coefficient λ(t), which is an important parameter for the comparison of the virus removal between different treatments. The relation between virus concentration and time can be linear or non-linear. In case of a linear decrease, the removal rate λ remains constant over time (λ(t) = λ) and the following linear model results from Equation 1:

$$\ln \frac{C(t)}{C0} = -\lambda * \mathrm{t} \qquad \text{Equation 2}$$

C0 = initial concentration of suspended viruses
λ = removal rate coefficient of the suspended viruses.

In case of a non-linear decrease, the removal rate λ has an initial value λ0 that changes over time, due to a certain resistivity against virus removal. The following equation describes the changing removal rate. The resistivity is described by the resistivity coefficient α and stays constant over time. A high resistivity α leads to a lower removal rate for the specific time step.

$$\lambda(\mathrm{t}) = \lambda 0 * e^{-\alpha t} \qquad \text{Equation 3}$$

λ0 = initial removal rate of suspended viruses
α = resistivity coefficient.

For each experiment, a linear and a non-linear model was fitted to the experimental data. The following non-linear model results from Equation 1 and 3:

$$\ln \frac{C(t)}{C0} = -\frac{\lambda 0}{\alpha} * (e^{-\alpha \mathrm{t}} - 1) \qquad \text{Equation 4}$$

Both models were fitted to the experimental data by the use of non-linear least squares algorithms. In this method, the squared differences between observed and predicted virus concentrations are calculated and minimized. It implies the assumption that the data is normally distribution. The experimental data and the residues of the models were therefore tested for normality. A graphical test (R: qqplot) and Anderson-Darling tests have been used. As shown in Table 19 (Appendix) the p-values < 0.01 indicate whether there is a significant deviation from the normal distribution.

3.5.1. Theoretical considerations: model fit

The unknown model parameters λ0, λ and α were estimated, parameter λ by fitting the linear model (Eq.2) to the experimental data and parameters λ0 and α by fitting the non-linear model (Eq.4) to the experimental data. The two models were compared in order to find the model that fits best to the data. The following graphs illustrate the procedure of fitting the different regression lines to the data.

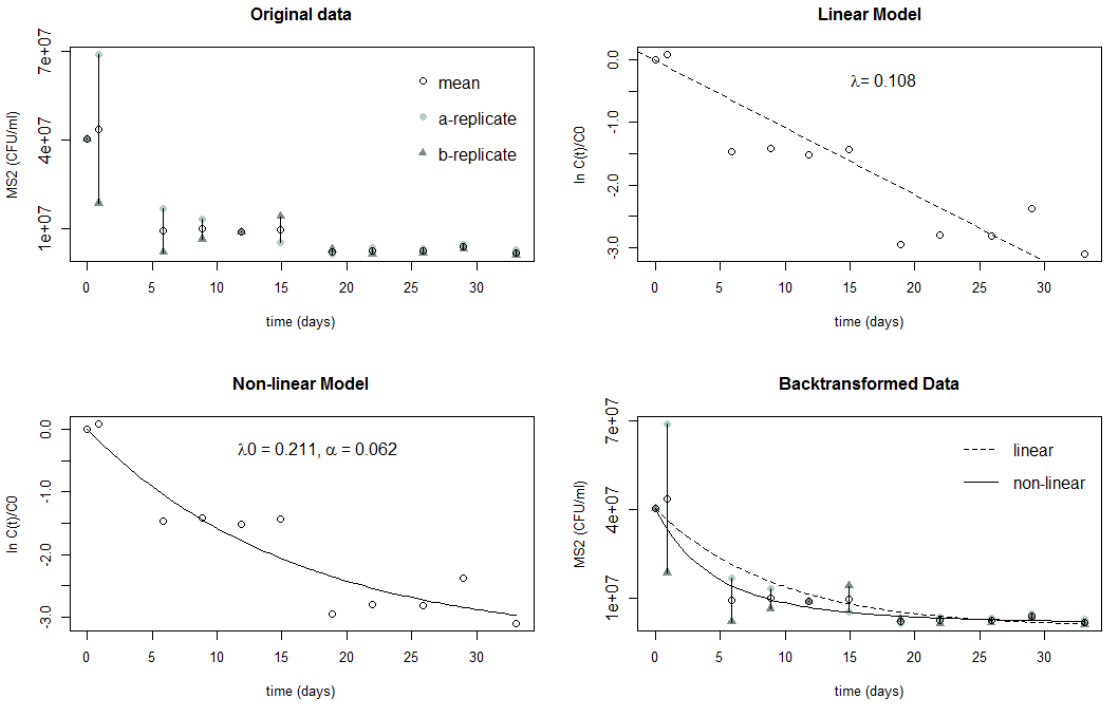


Figure 10: *10.1 data points (treatment: Old sand, high DOM and high IS (d+e+)), 10.2 linear regression, 10.3 fitted non-linear regression, 10.4 back transformed data points and regression lines.*

At first, the virus concentrations of each replicate was plotted against time. The average value and the standard deviation of both replicates was computed for each time step, as shown in Figure 10.1. The linear model was fit to the average values of the data and a regression line was added (Figure 10.2). The slope represents an estimate for the removal coefficient λ. In the next step, the non-linear model was computed by using Equation 4 and an initial guess of the parameters (λ0 = 0.1, α = 0.01). The regression line of the non-linear model fitted to the data is shown in the third graph (Fig. 10.3). Again, average values were taken in the model. In the end, the data points were transformed back and plotted on the original scale with both regression lines (Fig. 10.4).

In order to compare the fit of each model, two things are important: 1) whether the model parameters are significant and 2) whether the goodness of the overall model fit is high. In order to test the model parameters, each estimated parameter has been compared with its standard deviation and tested on significance (t-tests). Different significance levels have been chosen and compared. The resulting p-values of the t-tests are shown in Table 4. In order to test the overall model fit, a R^2 has been computed for the linear model, as well as the residual standard error and the sum-of-squared-residuals (SSR) for both types of models.

4. Results

4.1. Agitated conditions

4.1.1. MS2 removal: Time dependent vs. time independent removal

Table 4 presents the model results for the MS2 removal under agitated conditions. The parameters λ, λ0 and α are depicted with their standard deviation. The p-value for each parameter is shown, as well as the SSR for each model and the R^2 for the linear model.

Table 4: *Model fit and parameters, agitated conditions. ("d+" = high DOM, "d-" = low DOM, "e+" = high ionic strength and "e-" = low ionic strength)*

	Linear model				**Non-linear model**				
	λ (day^{-1})	p (λ)	R^2	SSR	λ0 (day^{-1})	α (day^{-1})	p (λ0)	p (α)	SSR
Old sand									
d+e+	0.108 ± 0.008	***	0.94	2.82	0.211 ± 0.048	0.062 ± 0.025	**	*	1.38
d+e-	0.061 ± 0.008	***	0.82	2.81	0.057 ± 0.033	- 0.005 ± 0.042	x	x	2.88
d-e+	0.118 ± 0.012	***	0.90	5.70	0.200 ± 0.073	0.046 ± 0.037	*	x	4.48
d-e-	0.148 ± 0.010	***	0.95	4.17	0.213 ± 0.055	0.031 ± 0.024	**	x	3.47
New sand									
d+e+	0.639 ± 0.088	***	0.83	299.77	15.071 ± 3.179	1.063 ± 0.229	**	**	10.80
d+e-	0.579 ± 0.075	***	0.84	219.09	10.976 ± 2.680	0.858 ± 0.213	**	**	11.21
d-e+	0.659 ± 0.094	***	0.81	345.60	26.530 ± 7.733	2.830 ± 0.545	**	**	15.58
d-e-	0.705 ± 0.098	***	0.82	375.10	21.056 ± 4.896	1.352 ± 0.321	**	**	15.08
No sand									
d+e+	0.113 ± 0.011	***	0.90	4.89	0.055 ± 0.019	- 0.049 ± 0.019	*	*	2.78
d+e-	0.062 ± 0.011	***	0.76	4.33	0.022 ± 0.016	- 0.068 ± 0.037	x	x	3.02
d-e+	0.129 ± 0.007	***	0.97	1.81	0.097 ± 0.019	- 0.021 ± 0.013	***	x	1.45
d-e-	0.179 ± 0.014	***	0.94	7.95	0.212 ± 0.068	0.013 ± 0.026	*	x	7.75

*P-values: x = ($p \geq 0.1$); xx = ($p \leq 0.1$); * = ($p \leq 0.05$); ** = ($p \leq 0.01$); *** = ($p \leq 0.001$)*

A linear model generally has a good overall fit, if the slope of the regression line is significantly different to zero. In this case, the removal rate coefficient λ represents the slope of the linear regression line. As shown in Table 4, the removal rate coefficient λ is significant for all twelve experiments with a significance level of $p \leq 0.001$. The R^2-values are high for all twelve experiments, varying between 0.76 and 0.97. All in all, the linear model describes the relation between virus concentration and time adequately for all experiments.

For the non-linear model, both parameters ($\lambda 0$, α) should be significantly different from zero. The resistivity coefficient α plays a major role of importance: if α does not differ significantly from zero, Equation 3 results in $\lambda(t) = \lambda 0$ and the removal rate λ remains constant over time. In this case, the linear model should be used instead of the non-linear one. As shown in Table 4, the significance of the model parameters varies between the different experiments.

- For all "old sand" and "no sand" treatments, except the treatment "high DOM and high IS (d+e+)", the resistivity coefficient α is not significantly different to zero ($p(\alpha) \geq 0.1$). Furthermore, negative estimates for the resistivity coefficient α have been found in four cases (Table 4). In these cases, the non-linear model cannot predict the decrease in virus concentration over a longer time.
- For all "new sand" treatments, both, the resistivity coefficient α and the initial removal coefficient $\lambda 0$ are significantly different to zero ($p \leq 0.01$).

In order to analyze the overall fit of the model further, the sum-of-squared-residuals (SSR) has been computed for each model. The SSR describes the deviation of the predicted values from the experimental values. The smaller this deviation, the smaller the discrepancy between the fitted model and the experimental data. First of all, it is noticeable that the SSR of all non-linear model outcomes is smaller than the SSR of the linear model outcomes, except the "old sand, high DOM and low IS (d+e-)" treatment. A t-test has been computed to test the significance of the reduction in SSR for each soil treatment.

- For all "old sand" and "no sand" experiments, the SSR of the linear models were not significantly reduced by the non-linear models ("old sand SSR": $p = 0.42$, "no sand SSR": $p = 0.29$, significance level: $p \leq 0.01$).
- For all "new sand" experiments, the SRR was significantly reduced by the non-linear model ("new sand SSR": $p = 0.003$, significance level: $p \leq 0.01$).

The residual standard error has also been computed for each model and lead to the same conclusion: by using the non-linear model, the residual standard error was significantly reduced for the "new sand" experiments (p-value: 0.0006, significance level: $p \leq 0.001$). Furthermore, the tests for normality (Appendix, Table 19) indicate, that the linear models can be used for the "old sand" and "no sand" experiments, where the model residues are normally distributed, whereas the non-linear works better for the "new sand" data points.

Overall, the non-linear model does not describe the relation between virus concentration and time adequately for the "old sand" and "no sand" treatments. This means that the removal of viruses in these treatments is a time independent process. The non-linear model with time-dependent removal rates can only be used for the "new sand"-treatments. It is to conclude that the time-independent removal rates λ can be used for a comparison between all twelve experiments, as the linear model has a good fit for all twelve experiments. For predictions and other modeling purposes the non-linear model should be used for the "new sand" treatments, as it describes the decrease in viruses even better than the linear model.

In the following figures, the decrease in MS2 concentration over 33 days is presented. Linear regression lines are added to the "old sand" and "no sand" experiments (Figure 11, Figure 13). Non-linear regression lines are added to the "new sand" experiments (Figure 12). The four different experiments within each soil treatment are plotted together and the experimental data was normalized. Normalized MS2 concentrations over 1 are due to the measurement error of the double agar layer method, which is further explained in Section 5.5.

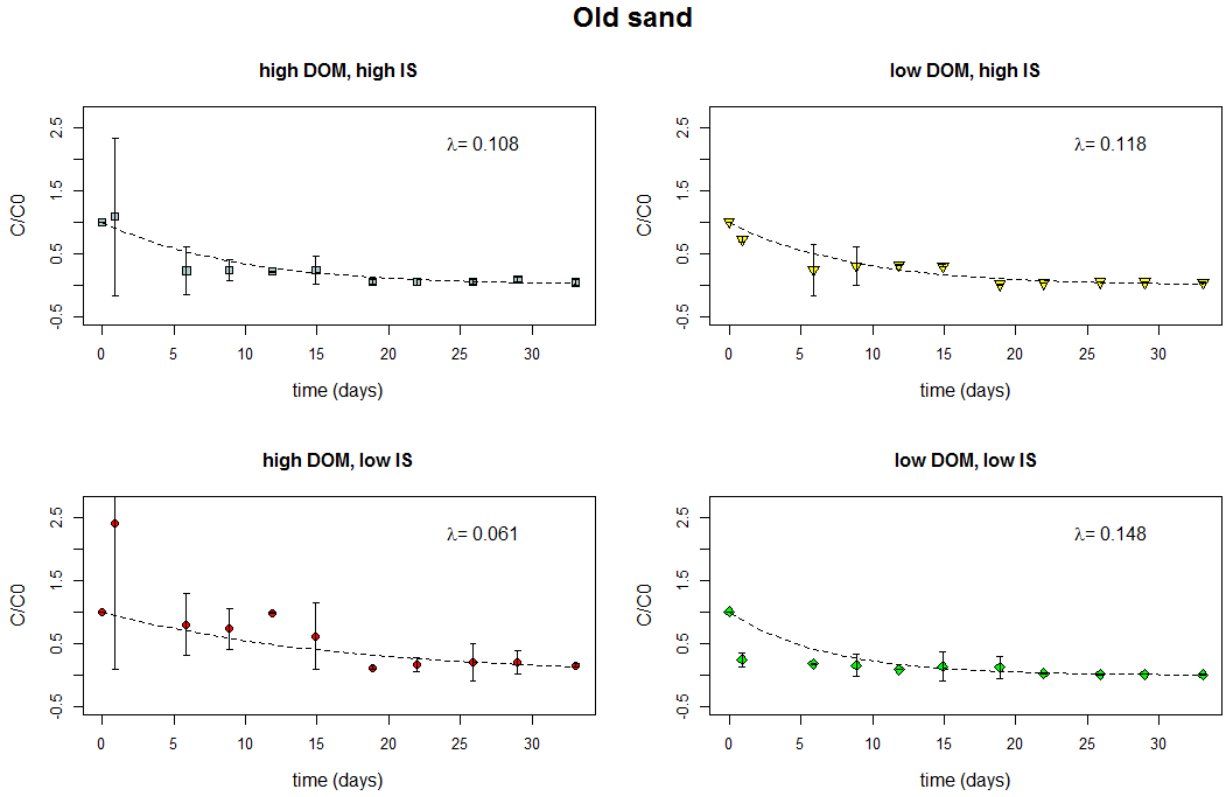


Figure 11: *MS2 removal in the "Old sand"- experiments. The four graphs represent experiments with altering levels of DOM and IS. Average values of the normalized experimental data are plotted with error bars representing the two replicates. The dashed line represents the modelled MS2 concentrations (linear model) and the modeled removal rate λ is given.*

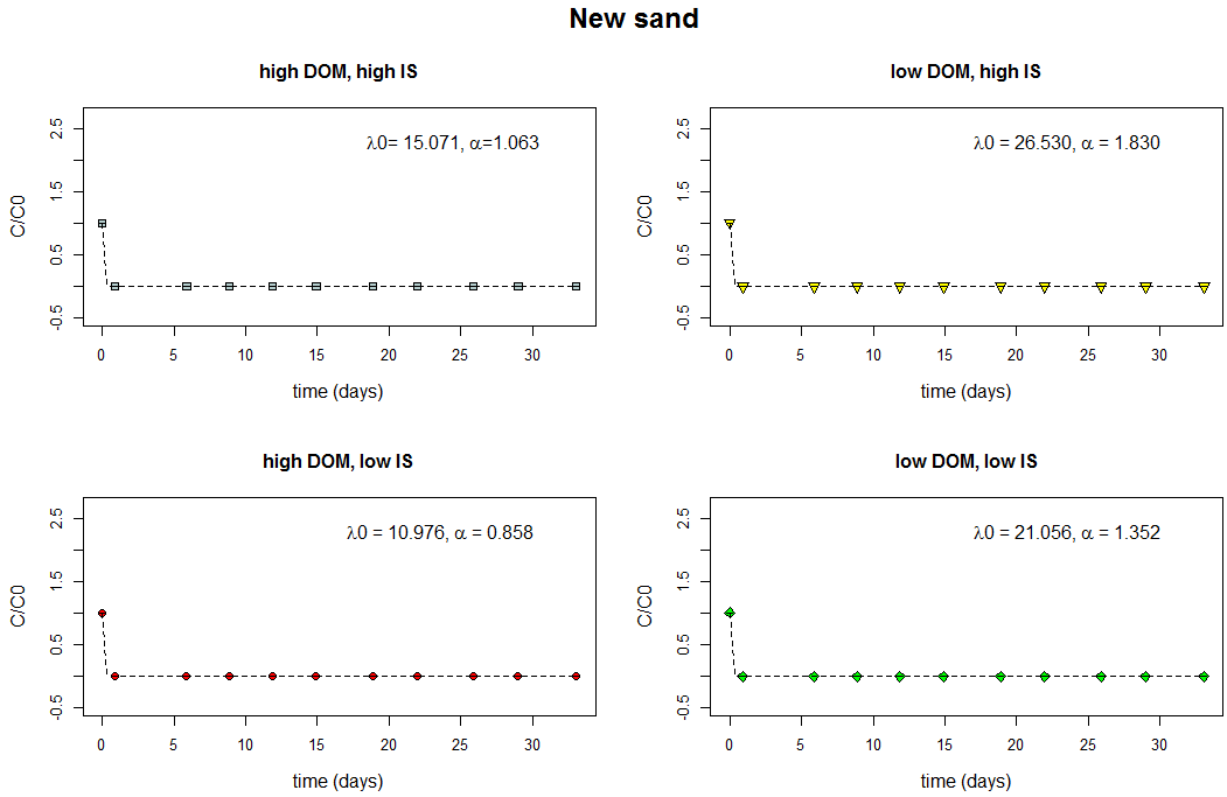


Figure 11: *MS2 removal in the "New sand"- experiments. The four graphs represent experiments with altering levels of DOM and IS. Average values of the normalized experimental data are plotted with error bars representing the two replicates. The dashed line represents the modelled MS2 concentrations (non-linear model) and the modeled initial removal rate λ0 and resistivity coefficient α is given.*

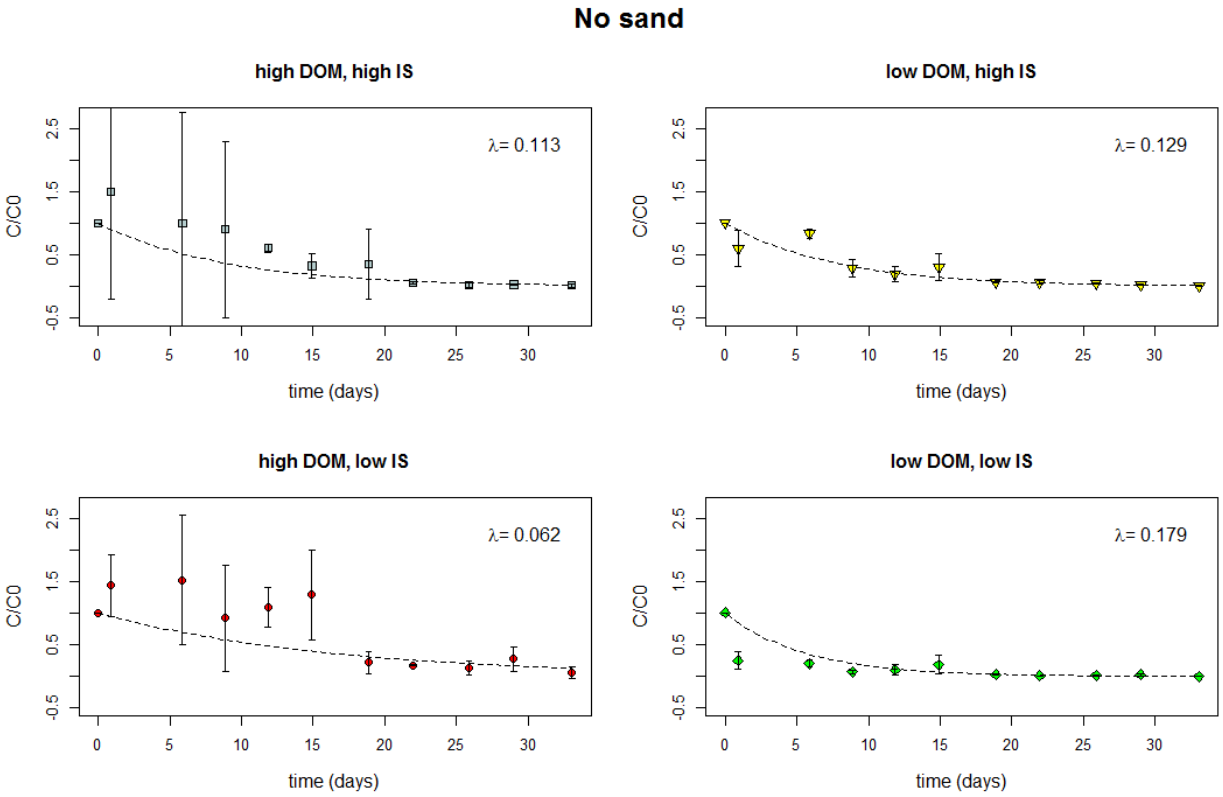


Figure 12: *MS2 removal in the "No sand"- experiments. The four graphs represent experiments with altering levels of DOM and IS. Average values of the normalized experimental data are plotted with error bars representing the two replicates. The dashed line represents the modelled MS2 concentrations (linear model) and the modeled removal rate λ is given.*

The decrease in MS2 concentration in all "old sand" experiments is rather constant over the time of the whole experiment (Fig. 11). It is more rapid in the beginning and slows down after approximately 10 days. The total drop in MS2 concentration between day 0 to day 33 went from $4.91*10^7$ PFU/ml on average to $1.63*10^6$ PFU/ml on average. The removal rates λ are shown for each treatment, with values varying between 0.061 day^{-1} and 0.148 day^{-1} (mean: 0.109, std: 0.031). The highest removal rate has been observed for the "low DOM and low IS" treatment and the lowest removal rate for the "high DOM and low IS" treatment.

The decrease in MS2 concentrations in all "new sand" experiment (Fig.12) is markedly different from the "old sand" experiment (Fig. 11). The concentrations dropped significantly during the first day of the experiment, decreased rather rapidly until day 6 and kept decreasing more slowly thereafter. From an initial concentration of $4.91*10^7$ PFU/ml on average, the MS2 concentrations decreased down to a level of $4.55*10^3$ PFU/ml on average on day 1 and down to 5.88 PFU/ml on average on day 33.

The initial removal rates λ0 and resistivity coefficients α for the "new sand" experiments are shown in the plots (Fig. 12). The initial removal rates λ0 are generally high with 18.41 day^{-1} on average, but the variation between the different treatments is quite large (std: 4.62). The highest removal was observed for the "low DOM and high IS"- treatment and the lowest removal for the "high DOM and low IS"- treatment. Accordingly, the resistivity coefficient α was highest for "low DOM and high IS" - treatment and lowest for the "high DOM and low IS"- treatment.

The decrease in MS2 concentration for all "no sand" experiments is shown in Figure 13. It resembles the decrease in the "old sand" experiments (Fig.11) and the average removal rate is only a bit higher than the average removal rate of the "old sand" experiment ("no sand": 0.121 day^{-1}, "old sand": 0.109 day^{-1}). As Figure 13 shows, the decrease is more rapid in the beginning,

but rather constant over the whole timespan. The removal rate λ reflects this linear relation between MS2 concentration and time. It varies between 0.062 day^{-1} and 0.179 day^{-1} (std: 0.042), with a the highest rate for the "low DOM and low IS" - treatment and the lowest rate for the "high DOM and low IS" - treatment. From the initial virus concentrations of $4.91*10^7$ PFU/ml on average, the virus concentration decreases down to $4.49*10^5$ PFU/ml on average after 33 days.

In conclusion, it is easy to see that similar decrease in MS2 concentrations was observed for the "no sand" and "old sand" experiments, whereas the decrease in the "new sand" experiments looks markedly different. Furthermore, the lowest removal rates in all experiments was observed for the "high DOM and low IS" - treatments. The treatment "low DOM and low IS" was responsible for the highest removal rates in both, the "no sand" and "old sand" experiments, whereas a different treatment was responsible for the highest removal in the "new sand" (low DOM and high IS).

For all twelve experiments, a larger drop in concentration was observed between day 15 and day 19 after the start of the experiment. During this time the Phytotron cabinet broke down and the temperatures increased for an unknown time (up to 12 hours). Between these days, the virus concentrations went down due to inactivation by temperature. For the "old sand" experiments they decreased from $1.09*10^7$ PFU/ml on average to $4.37*10^6$ PFU/ml on average. For the "new sand" experiments they decreased from $4.90*10^1$ PFU/ml on average to $2.13*10^1$ PFU/ml on average. For the "no sand" experiments they decreased from $1.50*10^7$ PFU/ml on average to $5.55*10^6$ PFU/ml on average. The MS2 concentrations for each individual experiment are shown in Table 16 (Appendix).

As the decrease was observed for each experiment on the same magnitude, a comparison of the experimental results is still possible and the measured values after day 15 were further used for the analysis of the data. For modeling purposes, such as predictions, it would be better to only use the experimental data of day 0 until day 15. The parameters λ, α and λ0 have been computed by the use of the experimental data between day 0 until day 15 only and results are presented in Table 13 (Appendix). For some experiments, it was not possible to obtain the model parameters, due to the limited number if data points.

4.1.2. Removal coefficients

In order to compare the different treatments with each other, the removal rates λ for each time step were computed for the "new sand" experiments by the use of Equation 3 (Appendix, Table 18). For the "old sand" and "no sand" experiments, the constant removal rates λ from the linear model was used for each time step. The change in removal rates λ over time is presented in Figure 14.

It clearly shows the difference in removal rates between the different experiments. The removal of MS2 bacteriophages in the "new sand" experiments was highest during day 0 with an average removal rate λ of 18.41 day^{-1} (std: 5.09). After the first day, the removal rates λ decreased down to 5.50 day^{-1} on average (std: 0.48). Towards the end of the experiment, the removal rates λ had decreased towards zero, with 0.027 day^{-1} (std: 0.027) on day 6 and $1.34*10^{-12}$ day^{-1} (std: $2.31*10^{-12}$) on day 33 (Appendix: Table 18).

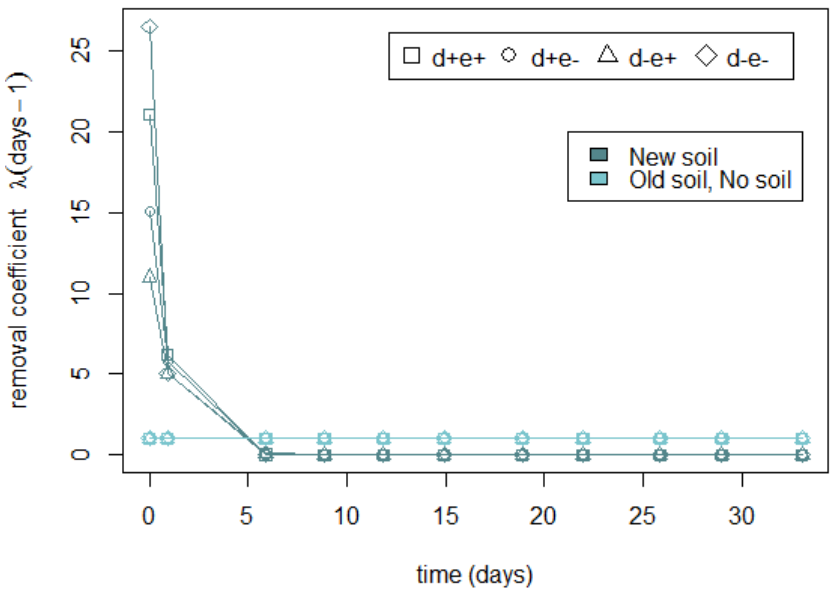


Figure 13: *Removal coefficients λ (day-1) over time. ("d+" = high DOM, "d-" = low DOM, "e+" = high ionic strength and "e-" = low ionic strength)*

The constant removal rates of the "old sand" and "no sand" experiments are very similar to each other. The "old sand" experiments had an average removal rate λ of 0.120 day^{-1} (std: 0.031) and the "no sand" experiments had an average removal rate λ of 0.108 day^{-1} (std: 0.042). The removal of MS2 bacteriophages in the "old sand" experiments were only a bit higher than the removal in absence of sand and the difference between both soil treatments was not significant (t-test: p = 0.705). As expected, the difference between the "new sand" removal rates and the "old sand" removal rates was significant (t-test: $p = 7.753*10^{-6}$), as well as the difference between the "new sand" removal rates and the "no sand" removal rates (t-test: $p = 6.403*10^{-6}$).

4.1.3. Total removal of MS2

The total removal of viruses in Plaque-forming units (PFU), percent (%) and log_{10} was calculated for each of the twelve experiments. The total volume of water (V) that used for all samples of one experiment was summed up and multiplied with the initial virus concentration ($C_{initial}$) measured on day 0, resulting in the initial virus mass ($M_{initial}$) in PFU.

$$M_{initial}\ (PFU) = V\ (ml) * C_{initial}\ (PFU/ml) \qquad \text{Equation 5}$$

Similarly, the final virus concentration (C_{final}) measured on day 33 was multiplied with the volume of water (V) of all samples used for that experiment, resulting in the total virus mass remaining (M_{final}). The total final virus mass (M_{final}) was subtracted by the total initial virus mass ($M_{initial}$), resulting in the total virus mass removed ($M_{removed}$) during the experiment. The results are presented in Table 5:

Table 5: *Total removal of viruses; remaining viruses after 33 days and 69 days after the start of the experiment. ("d+" = high DOM, "d-" = low DOM, "e+" = high ionic strength and "e-" = low ionic strength)*

	$M_{initial}$ [PFU]	M_{final} (day 33) [PFU]	$M_{removed}$ (day 33) [PFU]	[log_{10}]	M_{final} (day 69) [PFU]	$M_{removed}$ (day 69) [PFU]	[log_{10}]
Old sand							
d+e+	$5.54*10^9$	$2.51*10^8$	$5.29*10^9$	1.3	$2.19*10^8$	$5.32*10^9$	1.4
d+e-	$1.70*10^9$*	$2.69*10^8$*	$1.43*10^9$*	0.8	$9.78*10^7$*	$1.60*10^9$*	1.2
d-e+	$5.26*10^9$	$2.23*10^8$	$5.04*10^9$	1.4	$8.25*10^7$	$5.18*10^9$	1.8
d-e-	$1.44*10^{10}$	$1.53*10^8$	$1.43*10^{10}$	1.9	$1.23*10^8$	$1.43*10^{10}$	2.1
New sand							
d+e+	$5.36*10^9$	$1.13*10^3$	$5.36*10^9$	6.7	0	$5.36*10^9$	9.5
d+e-	$1.67*10^9$*	$1.62*10^3$*	$1.67*10^9$*	6.0	0*	$1.67*10^9$*	9.2
d-e+	$5.18*10^9$	$2.03*10^2$	$5.18*10^9$	7.4	0	$5.18*10^9$	9.5
d-e-	$1.44*10^{10}$	$2.04E*10^2$	$1.44*10^{10}$	7.8	0	$1.44*10^{10}$	10.1
No sand							
d+e+	$6.41*10^9$*	$1.02*10^8$*	$6.31*10^9$*	1.8	$2.28*10^7$*	$6.38*10^9$*	2.5
d+e-	$1.97*10^9$	$1.20*10^8$	$1.85*10^9$	1.2	$6.96*10^6$	$1.96*10^9$	2.5
d-e+	$6.08*10^9$	$4.27*10^7$	$6.04*10^9$	2.2	$2.96*10^4$	$6.08*10^9$	5.3
d-e-	$1.68*10^{10}$	$2.05*10^7$	$1.68*10^{10}$	2.9	$1.36*10^4$	$1.68*10^{10}$	6.1

** = missing information on volume V. In each of the three soil treatments, the information on volume for one experiment was accidentally not noted. The volume for these experiments was estimated as the average volume of the three remaining experiments of the same soil treatment (Appendix: Table 14).*

The final virus mass removed during the experiment, is significantly different between the experiments. The virus mass remaining (M_{final}) in the "new sand" experiments after 33 days is low (10^2 - 10^3 PFU), whereas the virus mass remaining (M_{final}) in the "old sand" and "no sand" experiments is still high (10^7 - 10^8 PFU). A log_{10} removal of 6 – 8 was achieved in the "new sand", whereas a log_{10} removal of 1 – 2 was achieved in the "old sand" and "no sand" experiments.

Day 33 represents the end of the regular measurements, but a final measurement has been conducted on day 69 in order to see the effects of the different factors on virus removal in a long-term perspective. The results are included in Table 5, showing that no viruses are left in the "new sand" after 69 days (9 – 10 log_{10} removal). In the "old sand" and "no sand" experiments, a final virus mass of 10^4 – 10^8 PFU remains after 69 days (1 – 6 log_{10} removal).

Within each of the three soil treatments, a variation in the total removal can be observed. In the "old sand" and "no sand" experiments, both high DOM-treatments have a lower total removal than the low DOM-treatments (Table 5). This difference is most markedly for the "no soil" experiments (Table 5). For the "new sand" experiments the parameters λ0 and α have to be compared (Table 4) for analyzing the variance between the four different treatments, as all experiments had a 100% removal. It is noticeable that the high DOM-treatments have a lower λ0 and α than the low DOM-treatments and the high IS-treatments have a higher λ0 and α than the low IS-treatments (Table 4). All in all, DOM and IS seem to have some influence on the total removals, but the choice of the soil treatment (SOM) seems to be the most influential factor.

4.1.4. Effect of DOM, SOM, IS and combined effects

In order to test the effect of the different factors tested, the relation between each factor and the removal rates has been examined, by the use of simple regression models. The removal rates of the linear model were taken as the response variable for this analysis. The individual factors (SOM, DOM, IS) present categorical predictor variables. Furthermore, the initial virus concentration ($C_{initial}$) has been tested as a predictor variable. First of all, models with only one categorical variable were computed as shown in Table 6. The R^2 and the standard error of each model can be used to analyze the influence of each individual factor on the virus removal.

Table 6: *Effect of individual factors (SOM, DOM, IS, C initial) on the removal rates*

Model	**λ ~ SOM**	**λ ~ DOM**	**λ ~ IS**	**λ ~ C initial**
R^2	0.975	0.015	0.0001	0.024
Standard error	0.046	0.276	0.278	0.307

It was found that SOM explains the different removal rates adequately, whereas DOM and IS could not explain the different removal rates in a good way. For DOM and IS, the R^2 was smaller than 0.1 and the residual standard error was high. Another factor which could have had an effect on the removal of viruses was the initial concentration of viruses ($C_{initial}$). Four different background solutions have been used for the twelve experiments (Section 3.3.) and each background solution had a slightly different initial virus concentration, due to the experimental error. Its effect on the removal rates was tested, but it was proven that the factor did not affect the removal rate significantly (R^2: 0.024, RSE: 0.307). Still, it is noticeable that the effect is higher than the effect of DOM and IS.

Next to the effect of each individual factor, interactions between the factors might have had an effect on the virus removal efficiency. All individual factors and possible interactions between SOM, DOM and IS were included into a more complex regression model. For the factor $C_{initial}$ no interactions with other factors were expected and therefore no interactions between $C_{initial}$ and other factors were included. An Analysis Of Variance (ANOVA) was used to test the significance of the model estimates. Results are presented in Table 7. The degrees of freedom (df), the estimated parameters with its standard errors and p-values of the estimates (F-test) are included in the table.

Table 7: *Analysis of Variance Table. Response variable = removal rates*

Effect	**df**	**Estimate**	**Standard error**	**p-value**
SOM	2	0.75148	0.37574	***
DOM	1	0.01178	0.01178	**
IS	1	0.00009	0.00009	x
C initial	1	0.00672	0.00672	**
SOM : DOM	2	0.00032	0.00016	x
SOM : IS	2	0.00004	0.00002	x
DOM : IS	1	0.00672	0.00672	**
Residuals	2	0.00012	0.00006	
Total	**11**	**0.77055**		

*P-values: x = ($p \geq 0.1$); xx = ($p \leq 0.1$); * = ($p \leq 0.05$); ** = ($p \leq 0.01$); *** = ($p \leq 0.001$)*

It was found that the twelve removal rates were best explained by the individual factors SOM, DOM, C initial and an interaction of DOM and IS. The following model can be used:

$$\lambda = SOM + DOM + IS + DOM{:}IS \qquad \text{Equation 6}$$

In total, 99.94% (R^2) of the variance in removal rates can be explained by this regression model, whereby the effect of SOM (partial R^2 = 97.53%) is the strongest. The other factors are of minor importance: only about 1% of the variance is explained by each of the factors DOM, the C initial and the interaction DOM:IS (partial R^2: DOM = 1.53%, C initial = 0.87%, DOM:IS = 0.87%). The effect of IS, as well as the two other interactions are no significant estimates in the model and explain less than 1% of the variance. It is noticeable, that IS is not a significant factor itself, but it remains in the model as a factor of the interaction DOM:IS.

The two non-significant interactions were stepwise removed from the regression model and it was found that models without the interactions were statistically not different from the original model (ANOVA-test, p-values: 0.7687 (SOM:IS) and 0.1067 (SOM:DOM), significance level: $p \leq 0.01$). A model without the DOM:IS-interaction though, was significantly different from a model with the interaction (ANOVA: p-value: $9.412*10^{-5}$). All three interactions are plotted in Figure 15. The predictor variables are interacting, if the lines of the plots have different slopes; if all lines are parallel to each other, there is no interaction. Again, one can see that DOM:IS is the only important interaction.

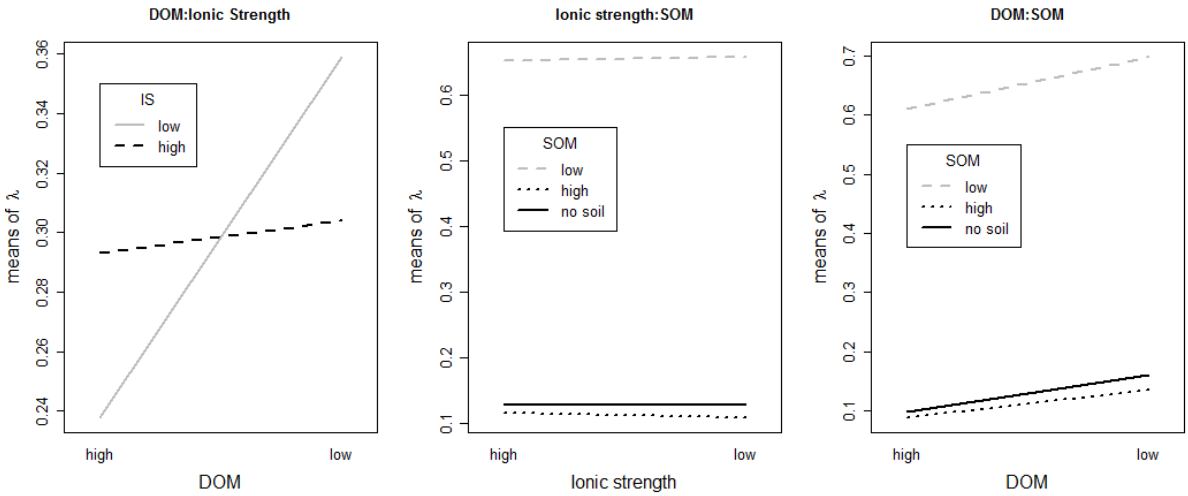


Figure 14: *Interaction plots (One predictor variable is plotted on the x-axis and its impact on the response variable (removal rate λ) depends on the level of the second predictor variable, which is presented in the interaction plots as broken, unbroken and dotted lines.)*

The interaction between DOM and IS was further analyzed and a contingency table was created, which provides a picture of the interrelation between the two factors.

Table 8: *Contingency tables. Removal rates or change in removal rates in day^{-1}.*

		DOM	
		high	low
Ionic strength	high	0.104	+ 0.015
	low	- 0.053	+ 0.095

The table shows the change in removal rates, estimated by the regression model (Eq.6). If DOM and IS are high, the estimated regression removal rate is 0.104. The change to low DOM induced a change of plus 0.015. The change to low IS induced a change of minus 0.053, but the combination of a change to low IS with low DOM lead to an overall increase of the removal

rates of 0.095. This pattern is applied to a three-way contingency table in the following (Table 9).

Table 9: *Three way contingency table of modeled removal rates in day^{-1}. (Values in brackets give the removal rates of the linear models of Table 2 in day^{-1}).*

	Old sand		**New sand**		**No sand**	
	High DOM	Low DOM	High DOM	Low DOM	High DOM	Low DOM
high IS	0.104	0.119	0.641	0.656	0.116	0.131
low IS	0.051	0.214	0.588	0.751	0.063	0.173

From this table it is easy to see, that for each soil treatment, the removal rates behave in the same way, when being modeled with the constant removal rates λ. They increase with low DOM, decrease with low IS and have the highest increase with low DOM and low IS.

4.2. Non-agitated conditions

Table 10: *Model fit and parameters, non-agitated conditions. ("d+" = high DOM, "d-" = low DOM, "e+" = high ionic strength and "e-" = low ionic strength)*

	Linear model				**Non-linear model**				
	λ (day^{-1})	p (λ)	R^2	SSR	λ0 (day^{-1})	α (day^{-1})	p (λ0)	p (α)	SSR
Old sand									
d+e+	0.173 + 0.015	***	0.93	9.52	0.315 + 0.086	0.044 ± 0.025	**	x	6.58
d+e-	0.182 + 0.017	***	0.93	11.20	0.409 + 0.119	-0.039 ± 0.035	*	x	5.72
d-e+	0.219 + 0.171	***	0.95	11.57	0.348 + 0.097	0.014 ± 0.027	**	x	8.81
d-e-	0.133 + 0.009	***	0.95	3.78	0.120 + 0.037	0.030 ± 0.024	*	x	3.72
New sand									
d+e+	0.023 + 0.016	***	0.96	9.84	0.343 + 0.081	-1.071 ± 0.233	**	x	7.45
d+e-	0.274 + 0.019	***	0.96	13.94	0.412 + 0.099	-0.870 ± 0.219	**	x	10.21
d-e+	0.339 + 0.016	***	0.98	10.14	0.279 + 0.053	-1.864 ± 0.572	**	x	8.86
d-e-	0.289 + 0.022	***	0.95	18.44	0.173 + 0.046	-1.378 ± 0.343	**	x	10.95
No sand									
d+e+	0.188 + 0.012	***	0.96	5.89	0.056 ± 0.019	0.125 + 0.031	**	x	4.08
d+e-	0.211 + 0.008	***	0.99	2.58	0.009 ± 0.009	0.229 + 0.035	***	x	2.49
d-e+	0.223 + 0.011	***	0.98	4.64	0.066 ± 0.017	0.297 + 0.049	***	x	3.24
d-e-	0.148 + 0.009	***	0.96	3.64	0.158 ± 0.063	0.093 + 0.021	**	*	2.05

*P-values: x = (p ≥ 0.1); xx = (p ≤ 0.1); * = (p ≤ 0.05); ** = (p ≤ 0.01); *** = (p ≤ 0.001)*

Similar to the results of the agitated experiments, the linear model has a good fit to the data of the non-agitated experiments. The removal rate coefficient λ is significantly different to zero for

all twelve treatments with a significance level of $p \leq 0.001$. The R^2-values are very high, with $R^2 \geq 0.93$ for all experiments. This model can be used for all treatments.

The non-linear model does not fit well to the experimental data in general. As shown in Table 10, the resistivity coefficient α does not differ significantly from zero, except for one experiment ("no sand: low DOM and low IS"). In some cases, negative values for the resistivity coefficient α have been modeled, which reflect that the model should not be used for predictions. The SSR has been computed for all models. The SSR of all non-linear models is smaller than the SSR of the linear models and a t-test has been computed to test the significance of this reduction. It showed that the reduction was not significant for all twelve experiments ("old sand SSR": $p = 0.238$, "new sand SSR": $p = 0.162$, "no sand SSR": $p = 0.202$, significance level: $p \leq 0.01$). Furthermore, the residual standard error was not significantly reduced by the use of the non-linear model for all twelve experiments.

Only the experiment "no sand: low DOM and low IS" can be modeled adequately by the non-linear model. For all other experiments, the non-linear model cannot model the decrease in virus concentration over time adequately and the linear model should be used. For a further comparison of the experiments, the removal rates λ (linear model) are presented in a three way contingency table below.

Table 11: *Three-way contingency table for non-agitated conditions.*

	Old sand		**New sand**		**No sand**	
	High DOM	Low DOM	High DOM	Low DOM	High DOM	Low DOM
high IS	0.173	0.219	0.023	0.339	0.188	0.223
low IS	0.182	0.133	0.274	0.289	0.211	0.148

First of all it is noticeable, that the differences between the removal rates are not as big as previously observed for the agitated experiments, where the "new sand" removal rates were significantly higher than the removal rates of the "old sand" and "no sand" experiments (Table 8). Under non-agitated conditions, the removal rates only vary between 0.023 and 0.399 (Table 11) (mean: 0.200, std: 0.078). Secondly it is noticeable, that the "new sand" treatments have higher removal rates on average than the other experiments. The average removal rates for "old sand", "new sand" and "no sand" experiments are 0.177 (std: 0.031), 0.231 (std: 0.122) and 0.193 (std: 0.029) respectively. As said, even though the "new sand" experiments removed more viruses, the difference is not as big as under agitated conditions (Table 8).

The individual and combined effects of the experimental factors (SOM, DOM, IS, C initial) were further analyzed. Simple models between the individual factors, as categorical variables, and the removal rates λ were build. Additionally, more complex models were build, including interactions and ANOVA-tests were computed to analyze the differences between these models. It was found that the individual factors cannot explain the differences in removal rates adequately. The R^2 of the simple models were very low (SOM: $R^2 = 0.086$, DOM: $R^2 = 0.102$, IS: $R^2 = 0.006$). The initial virus concentration, varying due to the preparation of different background solutions, was found to be the most influential factor ($R^2 = 0.385$), even though it was not the intention of the experiment to test this factor. Interactions between the individual factors have also been found to be insignificant (ANOVA: SOM:IS: p-value = 0.370, SOM:DOM: p-value = 0.233, DOM: IS: p-values = 0.126).

5. Analysis and Discussion

This study was conducted with the overall aim of analyzing the virus removal efficiency for the infiltration scheme Tunåsen, Uppsala. The main question in the analysis of the results, relating to the initial research question, should therefore be: "Which treatment worked best in order to remove viruses and how well can the results be related to the Tunåsen scheme?"

By keeping the experiment at 4°C, winter conditions in Uppsala were simulated, suppressing the removal by inactivation. Still, we observed that adsorption was not the only process removing viruses. In the absence of soil, the concentration of viruses did decrease, which must be the result of inactivation processes. Inactivation and adsorption processes will therefore be discussed in the following.

The literature suggested that SOM, DOM, IS and flow have major impacts on the removal of viruses and the literature review lead to the hypothesis that high IS and flow lead to a higher virus removal, whereas high DOM leads to a lower virus removal. Furthermore it was assumed that the accumulation of organic matter in the infiltration basins, as SOM, leads to lower adsorption of viruses, but may offer hydrophobic binding sites. The different factors were tested in the experiment and the hypothesis will be discussed in detail. A discussion on the uncertainties in the results and the linkage between the experiment and the field conditions at Tunåsen is included at the end.

5.1. Adsorption

5.1.1. The effect of SOM

The effect of blocking was of major importance for this experiment. As described by various authors, SOM covers positively charged binding sites and results in decreased attractive forces between the virus and soil particles (Ryan and Elimelech, 1996). The treatments that removed the least amount of viruses were the "old sand" treatments, where the sand had a high level of TOC (0.034%). Besides the blocking of binding sites, the high amount of negatively charged SOM could have additionally led to repulsive forces between the virus and sand particles, as suggested by Zhuang and Jin (2003). Treatments that removed most viruses during the experiment were the "new sand" treatments, which had a low SOM level (TOC = 0.008%). A large initial drop in MS2 concentration was observed in the "new sand", because the binding sites for viruses were not blocked by SOM.

One hypothesis was that SOM offers positively charged hydrophobic bindings sites for viruses and that the total adsorption could therefore be higher with high SOM (Schijven and Hassanizadeh, 2000). In this study, the opposite was true and therefore it is to assume that hydrophobic interactions play only a minor role in the adsorption process. Electrostatic interactions were most likely primarily responsible for the adsorption process in the "new sand"

experiments. Soil organic matter may have offered additional hydrophobic binding sites. These findings are coherent with the results of other authors, stating that, overall, hydrophobic interactions are of minor importance for adsorption, and electrostatic interactions, either repulsive or attractive, are crucial (Torkzaban et al., 2006; Zhuang and Jin, 2003).

As already mentioned, adsorption to soil particles was not possible in the "no sand" experiments and we assume that viruses in were exclusively removed due to inactivation of free viruses. The level of removal in this experiment was similar to the level of removal in the "old sand" experiments (old sand λ: 0.120 day^{-1} ± 0.03; no sand λ: 0.108 day^{-1} ± 0.04), meaning that the adsorption capacity of the "old sand" must be close to zero. It is likely that inactivation processes are primarily responsible for the total removal in these experiments, whereas the removal in the "new sand" is a result of combined inactivation and adsorption.

In total, the inactivation processes, as observed in the "old sand" and "no sand" experiments led to a rather small removal (1-3 log_{10} removal after 33 days), whereas the combination of adsorption and inactivation in the "new sand" led to a high removal (6-8 log_{10} removal after 33 days). All in all, the adsorption to unblocked binding sites is most likely of major importance for an efficient virus removal in the sand of the Tunåsen infiltration scheme.

Another difference between the three soil treatments was the change in removal rates over time. The virus concentrations in the "new sand" treatments dropped significantly during the first 22 hours of the experiment (Fig. 12, Fig. 14), whereas this large initial drop has not been observed in the "old sand" or "no sand" experiments. The initial drop in virus concentration is consequently mainly a result of adsorption. If most of the binding sites are unblocked, a high number of viruses can rapidly adsorb to the soil surface. Regarding modeling of virus concentrations over time, it was found that non-linear models only fit to the experiments with an initial concentration drop.

All in all, the most efficient way to remove viruses during the soil passage is achieved by choosing an infiltration sediment with low SOM. As described in Section 1.2., the infiltration basins are filled with sand, that originally contains low SOM, and every year approximately 10-20 cm of the sand are scraped off every year. This is done to remove the sediment with the highest accumulation of organic matter. The results of this study show, that the adsorption of viruses to sand that has been used for many years is negligibly low. Viruses are though still removed through inactivation and the question whether there is a definite risk of viruses entering the groundwater cannot directly be answered by this study. First of all, the initial concentration of model viruses in the experiments was $4.91*10^7$ PFU/ml on average, which does not represent the actual concentration of viruses in the river water. Secondly, even in the "old sand" experiments, $1.74*10^7$ PFU/ml on average were removed during the first 22 hours of the experiment. The possible risk of viruses entering the drinking water, the risk of infection and the management of risk assessments are further being discussed in Section 5.3.

5.1.2. The effect of flow

Similarly to the "old sand" or "no sand" experiments, no larger initial drop in virus concentration has been observed under static conditions. Non-agitated systems are generally kinetically limited and collision efficiencies are smaller than under agitated conditions (Ryan and Elimelech, 1996; Schijven and Hassanizadeh, 2000). The low interactions between viruses and soil particles in the static experiments might have led to slow attachment rates and consequently, no large initial drop was observed. Agitation, on the other hand, simulated the

flow of the infiltrating water, which transports free viruses to the soil particles and generally leads to increased collision efficiencies (Ryan and Elimelech, 1996; Schijven and Hassanizadeh, 2000). It was observed that a high number of viruses were removed rapidly, most likely through the attachment to soil particles, as the system was aiming for an equilibrium between free and attached viruses (Schijven and Hassanizadeh, 2000). Flow is probably important for rapid adsorption and results in the observed drop in virus concentration.

It is to assume that inactivation is the main removal process under non-agitated conditions, because the removal of viruses in the "no sand" experiments was close to the removal in the other two soil treatments (old sand λ: 0.231 $day^{-1} \pm 0.12$; new sand λ: 0.193 $day^{-1} \pm 0.03$, no sand λ: 0.177 $day^{-1} \pm 0.03$). Regarding the adsorption process, it has been reported that equilibrium adsorption occurs under non-agitated conditions (Schijven and Hassanizadeh, 2000). In this case, attachment and detachment rates are constant over time and linear models should be used to model the virus removal over time, as found in Section 4.2. During transport conditions on the other hand, it has been reported that there is no equilibrium, but attachment rates are relatively high and detachment rates are relatively low (Schijven and Hassanizadeh, 2000).

At the artificial recharge scheme Tunåsen, water is pumped into the infiltration basins and percolates to the groundwater aquifer. Agitated conditions clearly model this process better than non-agitated, but the rate of flow at the Tunåsen scheme might be very different from the induced flow rate of the agitator (4 - 4.2 rpm). An assumption could be that the range of removal between the results of both experiments could be taken as a range in which the real removal rates vary. The upper level of virus removal at the Tunåsen scheme would be the removal rates of the agitated experiment. The lower level would be presented by the removal rates of the agitated experiment.

5.2. Effects of DOM, IS and $C_{initial}$

The effects of DOM and IS on the total virus removal process, as a combination of adsorption and inactivation, were much lower than the effect of SOM (Table 6, Table 7). Dissolved organic matter was found to explain 1.53% and IS less than 1% of the variance in the regression model for removal rates (Eq. 6). Nevertheless, a combined effect of DOM and IS was found influential (Table 7).

In regard of DOM and IS effects on virus removal, literature studies mainly focus on the adsorption process. In this study, the effects of DOM and IS have been analyzed for all experiments and it was found that DOM and IS affect the "no sand" experiments in the same way as the experiments with sand, when using first order decay models (linear) (Table 8). It is to assume that the effects of DOM and IS on the inactivation process only can be analyzed by these result. The following table therefore shows the levels of removal rates, modeled in Section 4.1.4., as levels of inactivation rates for each treatment and gives an overview over the combined effect of DOM and IS. Effects of DOM and IS on the whole virus removal process, including adsorption processes, can be interpreted by looking at the "new sand" experiments only.

Table 12: *Interaction between DOM and IS and its effect on the removal rates. ("d+" = high DOM, "d-" = low DOM, "e+" = high ionic strength and "e-" = low ionic strength)*

	DOM		Inactivation rates	Lowest
Ionic	high	low		Low

strength	high	d+e+	d-e+		High
	low	d+e-	d-e-		Highest

In general, viruses are inactivated when the protein coatings are disrupted and the nucleic acids are degraded (Gerba, 1984).Yates et al. (1987) reported, that certain cations and organic matter may either protect viruses against inactivation or support the inactivation process. In this study, all treatments with higher DOM had lower removal rates (Table 12), leading to the assumption that MS2 bacteriophages were in some way protected against inactivation by DOM particles. Following this assumption, low DOM would not protect the viruses against inactivation and lead to higher removal rates, which is in line with the study results (Table 12).

Gerba (1984) reported that functional groups of organic matter affect the inactivation of viruses. Basic groups, such as amino groups may favor the inactivation, whereas carboxylic group may hinder the inactivation (Gerba, 1984). Organic matter has hydrophobic fractions (humic and fulvic acids, aromatic acids, phenols, proteins) that may interact with hydrophobic regions of the bacteriophage (Gerba, 1984). Increased amounts of DOM might therefore lead to hydrophobic bindings between the virus and the DOM particles, protecting the virus coatings and proteins against disruption and denaturation.

Similarly, it can be assumed that high IS protects viruses against inactivation, because the removal rates in this study decreased, when changing from low IS to high IS at low DOM levels (Table 12). At high IS and high DOM, on the other hand, the removal rates do not decrease with higher IS (Table 12) and the previous assumption must be rejected. This pattern of inactivation levels, as shown in Table 12, is the result of the combined effect of DOM and IS.

Previous studies have shown that IS has a strong effect on the coagulation of DOM. The solubility of DOM increases with lower IS (Monteith et al., 2007; Pagano et al., 2014), which could be one possible explanation for the combined effect of DOM and IS. A change from high to low IS at high DOM would increase the solubility of DOM. There would be no protection by ions, but an increased amount of truly dissolved DOM particles (humic acids, fulvic acids, etc.), protecting viruses against inactivation.

Regarding the protective effects by IS, it has been proven that an increase of IS promotes virus aggregation (Gerba, 1984; Walshe et al., 2010). Salts, such as Sodium Chloride used in this study, are referred to as anti-chaotropic agents, that promote hydrophobic effects within a solution (Gerba, 1984). MS2 bacteriophages have hydrophobic regions on the viral capsid that are repulsive to water molecules and lead to a certain protein folding on the viral capsid (Jin and Flury, 2002). Anti-chaotropic agents increase the net hydrophobic effect in the solution, meaning that the proteins of the bacteriophage are well protected from denaturation. Moreover, salt bridges between the virus colloids may evolve and the separation distance between the colloids decreases, resulting in overall attractive forces (Gerba, 1984). Aggregated viruses in the end are protected against denaturation.

The lowest level of virus inactivation has been found for the “low DOM and low IS“-treatment. No protective effect through IS is expected and viruses will probably be less coagulated, but the solubility of DOM is enhanced. The protective effect of truly dissolved DOM seems to be higher than the protective effect of colloidal DOM. The protective effect might be highest in this treatment, because the hydrophobic net effect is enhanced with truly dissolved DOM or because the protective effect is higher when viruses are less coagulated.

Overall, the interaction between DOM and IS could be explained by the increase in DOM solubility at low IS. The high amount of organic compounds dissolved in the water, might lead to a protection against inactivation and to a high decrease in virus inactivation. In the future,

changes towards more DOM and lower IS are expected (Ledesma et al., 2015; Monteith et al., 2007). As shown in this study, this change would lead to lowest removal rates and potentially more viruses transported to the groundwater body. In comparison to the effect of SOM, the effect of DOM and IS was still small, which is coherent with the finding of others, that changes in the water chemistry are not influencing the total removal processes a lot (Schijven and Hassanizadeh, 2000).

As Yates et al. (1987) reported, it seems to be very virus specific, whether the inactivation is enhanced or attenuated by changes in the water chemistry (Yates et al., 1987). It is to emphasize that, in regard of DOM and IS effects, the findings of this study are limited to MS2 bacteriophages. This model virus is quite hydrophobic and its interactions with organic matter compounds might be quite high in comparison to other enteric viruses (Schijven and Hassanizadeh, 2000). The effects of DOM and IS are most likely different for other model viruses and other enteric viruses.

Regarding the effects of DOM and IS on adsorption, it was expected that the removal would be lower at high DOM, because of the competition for the binding sites and that the removal would be higher at high IS, due to thin double layers (DLVO theory). Effects of DOM and IS on the adsorption process only could not be detected by this study, but by looking at the "new sand" experiments only, the whole removal process, including adsorption processes can be evaluated. Overall, the hypothesis regarding adsorption are in line with the study results: 1) the high DOM treatments had lower removal rates than the low DOM treatments (Table 4), most likely because DOM particles blocked positively charged binding sites on the sand particles and less viruses could adsorb; 2) the high IS treatments had higher removal rates than the low IS treatments (Table 4), most likely because high IS lead to thin double layers, small separation distances and more adsorption.

Another factor that influenced the removal of viruses was the initial virus concentration in the batch reactors. The factor was found to be significant, even though it explained only a small part of the variation in the removal and had a high standard error for the agitated experiments (Table 6, Table 7). Different authors have found that high initial concentrations of viruses lead to virus aggregation and lower removal rates, as virus aggregates protect individual viruses against inactivation (Chrysikopoulos and Aravantinou, 2012; Gerba, 1984; Yates et al., 1987). The results of this study, showed the opposite: under both static and agitated conditions, higher $C_{initial}$ clearly led to higher removal rates. It is to assume, that in both experiments, no larger aggregates were formed and simply that more individual viruses were inactivated when the initial virus concentration was higher. In case of the dynamic experiments, the constant mixing of the sediment and the viruses might have suppressed the development of larger virus aggregates.

The variation of the initial virus concentration between the four background solutions was relatively large (relative standard deviation: 69.93 %) and its influence on the removal rates was evident (Table 6, Table 7). A normalization of the initial virus concentrations was therefore not considered, as each change of raw data will falsify the model outputs.

5.3. Risk evaluation: Virus removal in the infiltration basins

Regarding the risk of waterborne disease outbreaks, it has been reported that the occurrence of different enteric viruses in water bodies, especially Noroviruses, is an emerging threat in Sweden (Andersson and Bohan, 2001; Ansker et al., 2013). Weekly microbial disturbances led to an increase number of boil-water recommendations (Lindberg and Lindqvist, 2005) and the current methods used for the detection of viruses in water works have been found to be insufficient (Ansker et al., 2013; Gerba et al., 1975; Lund and Lindqvist, 2004).

A large part of Sweden´s population (42%) directly consumes tap water and less people than in other countries buy bottled water or heat up the tap water (e.g. for coffee and tea) (Westrell et al., 2006). Tap water in Sweden is generally considered unpolluted and people do not fear to become ill, when consuming tap water (Westrell et al., 2006). This direct consumption pathway increases the potential risk of infection and stresses the importance of risk assessments to prevent larger microbial disease outbreaks. The land application of sewage sludge in Sweden, imposes another pressure on surface waters. Pre-treated sewage sludge still contains infectious viruses, which might be transported to rivers and lakes with stormflows or leach to the groundwater (Keswick and Gerba, 1980). Risk assessments are an important tool to estimate the effect of different microbial barriers in a drinking water production scheme.

The sand filters in the artificial groundwater recharge scheme at Tunåsen present one microbial barrier as they filter a large amount of organic materials that transport viruses and furthermore, they adsorb and inactivate suspended viruses, as shown in this study. Regarding the scope of a risk assessment, it is to clarify that with the results of this study a risk assessment is limited to assess the removal capacity within the infiltration basins. Other microbial barriers within the drinking water production in Uppsala contribute to prevent the transport of viruses to the drinking water, which is distributed to the municipality. These barriers include a rapid sand filter at Storvad, a 1.9 km flow through the esker to the pumping wells at Galgbacken and the chlorination and aeration in the waterworks at Gränby before distribution (UppsalaVatten, 2014).

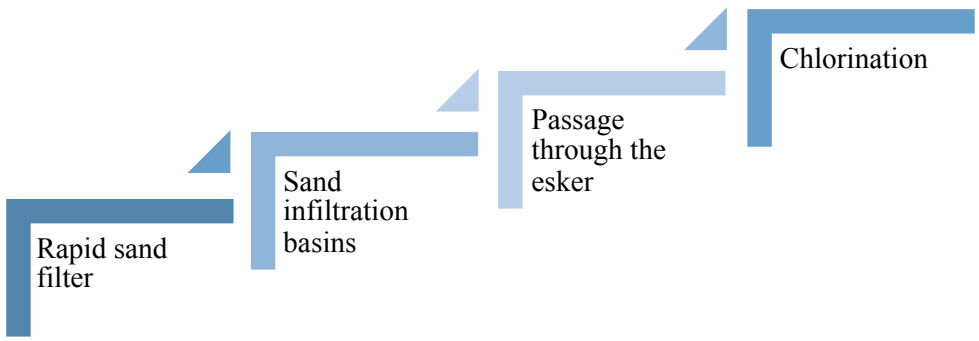


Figure 15: *Microbial barriers within the drinking water production, Uppsala.*

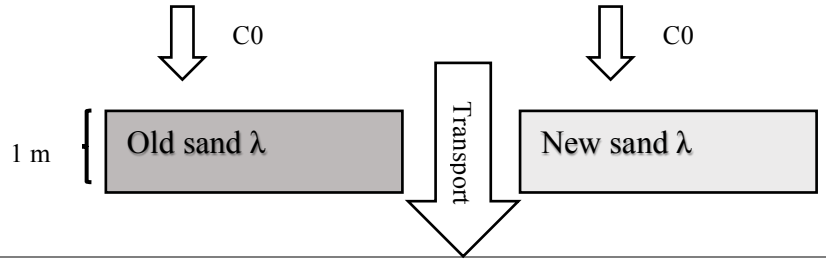
Regarding the risk assessment for the infiltration basins only, it is possible to model the concentration of viruses removed in the infiltration basins. The infiltration basins can be modeled as sand columns of 1 m height. With an estimate of the retention time in the basins, the virus load entering the basins and the removal rate it is possible to calculate the virus removal in the infiltration basins.

Infiltration rates in a similar infiltration basin to the Tunåsen basins have been reported to be about 2.9-3.8 m/day (Frycklund, 2001). The water would therefore need 6.3-8.3 hours to move through the infiltration basin of 1 m height. As the removal of viruses in rapid sand filters is generally negligible (Huisman and Wood, 1974), it is to assume that the level of viruses expected in raw surface waters (usually given in PFU/100 ml or in PFU/l) is the same as the

level of viruses entering the infiltration basins. Studies in the Fyris River are not available, but the Swedish Water and Wastewater Association reported to have found $2.6*10^4$ to $1.3*10^6$ infectious virus particles per liter, with maximum values of $2.6*10^7$ virus particles per liter (Ansker et al., 2013).

The occurrence of viruses in other rivers throughout Europe can be used as a comparison to assess the level of viruses found in raw surface waters. A study in the Netherlands determined the concentration of human viruses in two large rivers. Average concentrations for F-specific bacteriophages and somatic coliphages were $7*10^3$ – $2*10^4$ PFU/l, for noroviruses and rotaviruses $2*10^2$ PFU/l and for reoviruses and enteroviruses 1-6 PFU/l (Lodder and de Roda Husman, 2005). Similar numbers of viral contamination in surface waters were presented by Hot et al. (2003), who took 68 samples in four French rivers. All samples were tested positively for somatic phages and 60 samples were positively for enteroviruses. Most of the viruses were detected at a concentration of $> 10^4$ PFU/l with a range of range $4*10^2$ PFU/l to $1.6*10^5$ PFU/l (Hot et al., 2003).

A virus concentration of 10^4 PFU/l should be a good estimate in this study, for the viruses entering the infiltration basins. With a virus load of 10^4 PFU/l and a transport time of 6.3-8.3 hours the resulting $\log_{10}$ removal would depend on the removal rate in the sand basins. With the small removal rates of the "old sand" ($\lambda = 0.11$ day^{-1} on average, Table 4) only a reduction of 0.29-0.38% ($\log_{10}$ reduction of 0.001-0.002) could be achieved. With the higher initial removal rates in the "new sand" (λ (0) = 18.4 day^{-1} on average, Table 18) a higher reduction of 48.3-63.6% ($\log_{10}$ reduction of 0.29-0.44) could be achieved. Furthermore, the initial removal rate in the "new sand" decreases over time. On the second day (λ (1) = 5.5 day^{-1} on average, Table 18) the reduction would already be lowered to 14.4-19.0% ($\log_{10}$ reduction of 0.07-0.09).



	λ (PFU/ml*day)	Transport time (hours)	Reduction (%)	Reduction ($\log_{10}$)
Old sand	0.11	6.3 – 8.3	0.29 - 0.38	0.0013 - 0.0017
New sand day 1	18.4	6.3 – 8.3	48.30 - 63.63	0.287 - 0.439
New sand day 2	5.5	6.3 – 8.3	14.44 - 19.02	0.068 - 0.092

Figure 16: *Schematic of the process*

If the assumptions (C0 = 10^4 PFU/l, t = 6.3-8.3 h) are correct, the total removal would be much less than 1 $\log_{10}$ reduction in both sands. The Swedish Water and Wastewater Association reports that a log reduction of 5-7 is required for the production of safe drinking water (Ansker et al., 2013). The study results show that the infiltration basins have a limited capacity for virus removal and that more microbial barriers would be needed for the production of safe drinking water. The sand that is currently used in the basins does not remove viruses efficiently, because of the high amount of organic matter that accumulated in the sand over the time. The new sand has a higher removal capacity, but the risk of viruses entering the groundwater remains. The risk could further increase with the predicted increase of DOM in the river water.

In order to reduce the risk, more research is needed to evaluate, whether changes in the management of the infiltration basins, such as a more frequent removal of the sand or the implementation of other microbial barriers at this stage of the water treatment would be reasonable. It is to emphasize that this study present a worst case scenario, as the experiment was conducted under winter conditions and a worst-case model virus was chosen. Furthermore, a high level of uncertainty remains in the above risk assessment, since the initial virus concentration and the transport time were not measured. The removal rates could be different under field conditions and vary for different viruses. Statements about the actual risk of virus occurrence in the drinking water are limited, as there are more microbial barriers after the infiltration basins.

The further removal of viruses occurs during the transport of the water through the esker and in the waterworks in Gränby. The groundwater level is about 22 m below the infiltration basins and the total transport from Tunåsen to the pumping wells in Galgbacken is about 1.9 km and takes up to 232.8 days (Hummel, 2014). During this transport, a larger amount of viruses is probably removed by inactivation and adsorption to the esker material. Chlorination and aeration at the Gränby water works further removes viruses (UppsalaVatten, 2014). Additionally, it is reported, that new methods for the detection of viruses are being developed, per example within the NORVID project. This project was started in 2009 by the Swedish Water and Wastewater Association and aims at developing advanced analysis methods for virus detection in Swedish waters (Ansker et al., 2013). Risk assessments for human pathogenic viruses in Swedish surface waters is further developed by this project, as well as by other project, such as the VISK project (Viruses in water – Scandinavian bank of knowledge, 2010-2013) (Ansker et al., 2013). Further research is highly needed as the current knowledge of the risk of human pathogenic viruses in Swedish waters is limited.

5.4. Discussion of the assumptions

Within the setup of the experiment, different assumptions were made that influence the outcome of the experiment.

Assumptions:
The experimental IS levels simulate high and low IS levels in the Fyris River
The experimental DOM levels simulate high and low DOM levels in the Fyris River
Water temperature in the winter is at 4°C
Agitated experiments simulate the water flow in the sand basins at Tunåsen

In order to get a low and high level of IS, NaCl was added to the background solutions, as described in Section 3.3., and the conductivity of the different background solutions was measured as an estimate of IS. The resulting level for low IS was EC_{25} = 420 - 440 µS/cm and EC_{25} = 612 - 630 µS/cm for high IS (Table 3). The average conductivity measured in the Fyris River between 1984 and 2014 is EC_{25} = 404.8 µS/cm and the maximum conductivity measured is EC_{25} = 663 µS/cm (SLU database: Miljödata MVM). The values chosen for low and high IS do therefore rather represent the average and maximum levels of IS in the Fyris River.

If different IS levels would have been chosen, such as the 25% and 75% quantiles (EC_{25} = 368.75 µS/cm, EC_{25} = 441.25 µS/cm, SLU database: Miljödata MVM), the results would

represent the lower and upper levels of IS in the Fyris River better. As the high level of IS can be seen as an extreme value, the effects of IS on the removal of viruses as observed in this study, give an indication of how removal rates change with a change in IS, but the change would probably not happen in the observed magnitude.

The following graph presents the conductivity measurements (EC_{25}) and TOC-measurements taken in the Fyris River (Fyrisån Klastorp station) between 1984 and 2014 (SLU database: Miljödata MVM).

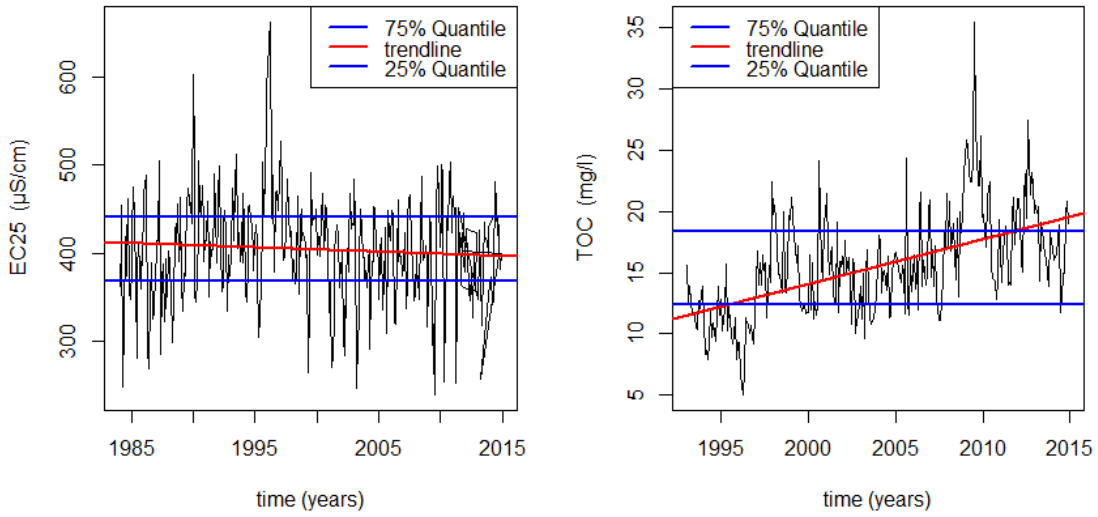


Figure 17: *Long-term measurements of EC_{25} and TOC in the Fyris River (SLU database: Miljödata MVM, Fyrisån Klastorp station, 1984 – 2014)*

The average amount of NOM added to the "high DOM" background solutions was 0.06 g per 1.2 l, resulting in "high DOM" levels of TOC = 31 mg/l (Table 3). The TOC measurements for the raw water samples, presenting "low DOM" levels, was TOC = 17 mg/l. The long-term TOC measurements in the Fyris River showed that these levels do rather represent average and maximum DOM levels (average 17.9. mg/l, maximum 35.5 mg/l) measured in the last decade between 2005 and 2014 (SLU database: Miljödata MVM, Fyrisån Klastorp station). In order to simulate the lower and higher DOM levels in the river, it would be better to use the lower and upper quantile TOC values (25% Quantile: 14.60 mg/l, 75% Quantile: 20.43 mg/l). Again, the interpretation of the study results should rather be drawn to the average and maximum DOM conditions in the river. They indicate how DOM can affect the removal of viruses, but the high DOM level is still an extreme and the effect of increasing organic matter levels in the river water on the removal of viruses is of a lower magnitude.

Furthermore, it was assumed that the average water temperature of the infiltration water is 4°C, which should be a good estimate, at least for the months November - March. The average water temperature measured during these 5 months during 2011-2014 was 1.68°C (SLU database: Miljödata MVM, Fyrisån Klastorp station).

It was also assumed that the agitation of the batch reactor (4-4.2 rpm) simulates the flow at Tunåsen adequately. As being said before, the flow in the infiltration basins might be quite different from the flow in the agitated batch reactors, but the agitation simulates the conditions

in the basins better than the static experiments. Additionaly, it was assumed that the infiltration basins are saturated during the time of infiltration. Therefore the batch reactors were filled to the top, without air space, simulating the saturated conditions in the sand basins. During the infiltration process, the infiltrating water might be in contact with air-water interfaces, which is not taken into account in this study. Such air-water interfaces probably occur in the Tunåsen esker beneath the infiltration basins until the groundwater level.

5.5. Discussion of the experimental method

In regard of the experimental set up, it was decided to use only MS2 bacteriophages as model viruses. Sand was the only adsorption material investigated and no other water chemistry parameter than DOM and IS were considered. For further studies, it would be interesting to use different model viruses, as MS2 bacteriophages are worst-case model viruses regarding their strong survivability in the environment and their low adsorption levels (Schijven and Hassanizadeh, 2000). As sand is being used in the infiltration basins by UppsalaVatten, it would not be recommended to use another adsorption material, but the effect of organic matter could further be studied by OM-coated sand. Water chemistry parameters that are known to alter virus adsorption are positively charged mineral oxides. It would therefore be interesting to analyze the sediment on mineral oxides, such as Iron oxides (Schijven and Hassanizadeh, 2000).

In regard of the experimental error, the double agar layer method is extensively used to analyze phages, but an experimental error remains. The total measurement uncertainty averaged for all experiments has been estimated to be 43.33 % (Section 3.4.), which is much higher than the European Pharmacopoeia “Precision requirement” for microbial quantification methods of 10-15 % uncertainty or the often accepted uncertainty of 30 % (Sutton, 2011). Uncertainty arises from dilution to pipetting, plating and counting. First of all, dilution errors include that different volumes of buffer solution are initially put in the dilution series and that different volumes of the sample and the diluted samples are taken, which falsifies the number of phages plated. Plating errors include that different volumes are put on the final plates or that the plate is being contaminated. The counting in the end, adds considerable uncertainty to the results. Plaques, formed by phages can be too small and turbid to detect and count them accurately. More errors evolved, as two different persons conducted the experiment and because the samples were kept at room temperature for a certain time before they were diluted and plated. Different operators have been working with the experiments, which plays an important role in the uncertainty that arises from pipetting and counting. The room temperature could have led to inactivation of some phages. These errors should be considered, when evaluating the results. For further studies it would be recommended to take more replicates per experiment.

A drop in virus concentration has been observed between day 15 and day 19, in which the Phytotron cabinet broke and the temperature did increase for some time. In total, the temperature did increase up to 12 hours and was probably close to room temperature, before the experiment was put to 4°C again. The final virus concentration, as well as the total removal of viruses would probably be higher than measured in this experiment. As the drop was observed in all experiments, the data was still used for comparison of the different treatments.

6. Conclusion

The infiltration basins Tunåsen present one of the microbial barriers in the artificial recharge scheme in Uppsala. Changes in the water chemistry are predicted, that could negatively influence the efficiency of virus removal in the infiltration basins, especially due to increasing levels of DOM in the infiltration water. This study proved, that the removal of viruses during winter months is generally low. Inactivation of viruses during cold winter conditions is close to zero and the total removal of viruses depends primarily on adsorption processes.

The adsorption capacity of sand that has been used as infiltration sediment for 9 years, is close to zero. With increasing levels of DOM in the river water, the adsorption sites in the sand will be increasingly blocked by accumulating SOM and the risk of further transport of viruses to the groundwater table will increase.

The effects of DOM and IS were low in comparison to the effects of SOM, but it was shown that high levels of DOM combined with low levels of IS probably increase the solubility of the organic particles and lead to very low inactivation rates.

All in all, it has been proved that the infiltration basins do not remove viruses efficiently during winter months and the risk may increase with rising levels of DOM. There are more microbial barriers in the drinking water production scheme, reducing the risk of viruses entering the drinking water distribution system of the municipality. But as it remains difficult to detect viruses via the methods used today, further action should be taken to secure the quality of the drinking water, such as the development of better detection methods or the introduction of further microbial barriers.

7. Popular Summary

Freshwater resources

Uppsala is the fourth largest municipality in Sweden with approximately 140.000 inhabitants. Drinking water in Uppsala has long been obtained from groundwater wells, as groundwater is usually safer to drink than the water from rivers and lakes. New groundwater is produced naturally, when rain infiltrates into the soil and recharges the groundwater body. It has been calculated, that the amount of groundwater recharge in Uppsala is about 300-400 l/s, but as the population of Uppsala grew a lot in the 18th and 19th century, the natural groundwater resources were not enough to produce drinking water for everybody. The idea of using the water of River Fyris got more and more popular.

Today, 50% of the drinking water in whole Sweden and most of the drinking water in Uppsala is surface water. In comparison to groundwater, surface waters are more vulnerable to pollution, because they are open waters and not protected by a soil cover such as groundwater bodies.

Viruses and organic matter

Surface water can be contaminated with viruses. Challenges with viruses include that i) one single pathogenic virus can infect a human and cause severe diseases, ii) the disease spreads easily, because water is used in all parts of our daily life and iii) the detection of outbreaks is often difficult and too late to contribute to the prevention of the disease. It is important to secure safe drinking water and to prevent microbial contamination.

Organic matter can be seen as another contaminant in surface waters that tremendously influence water quality. The levels of organic matter in Swedish streams and lakes have been increasing during the last decade, leading to high production of biomass, reduced light attenuation in the lake and finally eutrophication problems. It has also been proved that chemical pollutants and pathogenic microorganisms bind to organic matter and lead to other problems.

Natural cleaning mechanisms of the soil

In order to produce safe drinking water from surface water, a barrier for contaminants, such as viruses and organic matter has to be created. One way to do this, is to filtrate the surface water through soil. The water gets filtrated and "natural cleaning mechanisms" of the soil apply. Viruses that enter this soil passage are removed from the water, because they adsorb to certain "binding sites" on the soil particles.

Organic matter influences the removal of viruses during the soil passage, but it is not certain whether it enhances or attenuates the removal. On one hand, organic matter that is dissolved in the water (dissolved organic matter = DOM) can bind to the soil particles in the same way as viruses. Consequently, there is a competition for binding sites between viruses and DOM. On the other hand, organic matter, which is bound to the soil (soil organic matter = SOM) can offer such binding sites and therefore increases the adsorption of viruses and their removal from the water.

The experimental design

The question that has been studied in this project is: How fast do the viruses adsorb to the soil and die off in the infiltration basins that are used for artificial groundwater recharge in Uppsala? The infiltration basins in Uppsala are 1 m deep and consist of sand. Water from the Fyris River is pumped into these basins. In this study, sand was taken from the infiltration basins and river water was taken in the pump house. Glass vials were filled with sand, water and model viruses and the concentration of the model viruses were measured over several weeks.

In order to test different water chemistry conditions, the levels of dissolved organic matter and ionic strength differed between the experiments and additionally another sand, which has not been used for infiltration, yet, was tested under the same conditions. Furthermore, we kept the experiment at 4°C (winter conditions in Uppsala), because we assumed that hardly any viruses would be removed by natural inactivation. In this way we could focus on the effect of adsorption.

This study has shown that…

The infiltration basins that are currently used for the drinking water production have a high level of SOM and do therefore have a low adsorption capacity. The SOM blocks the binding sites for viruses and viruses remain in the water, when passing through the basins. The total removal capacity is therefore low during the winter months, when inactivation due to temperature is neglectable.

Changes in the water chemistry did not affect the removal process a lot, but the increasing levels of DOM in the Fyris River could further influence the efficiency of virus removal in the infiltration basins negatively. It has been showed that low levels of ionic strength, combined with high levels of DOM lead to low inactivation rates. With increasing levels of DOM in the river water, the adsorption sites will be increasingly blocked by accumulating SOM and the risk of further transport of viruses to the groundwater table will increase.

…and this means that…

In winter months, the virus removal in the infiltration basins is low, if the sand is being used for a long time and the risk may increase with rising levels of DOM. In the end, the infiltration basins present only one of the microbial barriers in the artificial recharge scheme in Uppsala and other barriers reduce the risk of viruses entering the drinking water distribution system of the municipality.

8. Acknowledgements

Thanks to Jean Marc Mayotte for the supervision during this project. In my opinion, this project is quite unique and I am glad that I had the opportunity to work with it. Your support during the laboratory work was great and I also want to thank the team of the Microbiological Laboratory from the Department of Energy and Technology: Björn Vinnerås, Annika Nordin, Jenna Senecal-Smith and Cecilia Lalander.

Thanks to Kevin Bishop and Ellen Kandeler for further supervision at the Swedish University of Agriculture (SLU) and the University of Hohenheim (UHOH) and thanks to my examiner, Björn Vinnerås (SLU).

I would like to thank Mikael Andersson Franko from the Department of Applied Mathematics and Statistics, for thorough support during the analysis of the results; Dimitry Wintermantel for the opposition in the Thesis defense; Philip McCleaf from Uppsala Vatten och Avfall AB; Inger Juremalm from the Department of Soil and Environment for the soil analysis; Emma Lannergård and Jelena Rakovic for the help with the "Svensk Sammanfattning"; and Stacy Sutcliff-Johansson, for the exchange of ideas during the entire project.

9. References

Anders, R., Chrysikopoulos, C.V., 2005. Virus fate and transport during artificial recharge with recycled water. Water Resour. Res. 41, W10415.

Andersson, Y., Bohan, P., 2001. Disease surveillance and waterborne outbreaks, in: Water Quality: Guidelines, Standards and Health. Assessment of Risk and Rik Management for Water-Related Infectious Disease. Fewtrell, L. and Bartram, J. (Eds.) IWA Publishing, London, pp. 115–133.

Ansker, J., Athley, E., Ericsson, P., Häggström, P., Lindgren, P.-E., Nyström, F., Pott, B.-M., 2013. NORVID – Risk Assessment With MRA and GDP Based on a Long-term Study of Norovirus Occurrence in Swedish Surface Waters (No. Rapport Nr 2013–20). Svenskt Vatten Utveckling, Sweden.

Bergström, R.B., 1986. Uppsalaåsen och Vattholmaåsen. En hydrogeologisk undersökning av delen Galgbacken – Svista/Fullerö.

Bosch, A., 1998. Human enteric viruses in the water environment: a minireview. Int. Microbiol. Off. J. Span. Soc. Microbiol. 1, 191–196.

Bouwer, H., 2002. Artificial recharge of groundwater: hydrogeology and engineering. Hydrogeol. J. 10, 121–142.

Bradford, Y.F.T., 2006. Transport of coliphage in the presence and absence of manure suspension. J. Environ. Qual. 35, 1692–701.

Chrysikopoulos, C.V., Aravantinou, A.F., 2012. Virus inactivation in the presence of quartz sand under static and dynamic batch conditions at different temperatures. J. Hazard. Mater. 233–234, 148–157.

Clark, J.M., Bottrell, S.H., Evans, C.D., Monteith, D.T., Bartlett, R., Rose, R., Newton, R.J., Chapman, P.J., 2010. The importance of the relationship between scale and process in understanding long-term DOC dynamics. Sci. Total Environ. 408, 2768–2775.

Durán, A. e., Muniesa, M., Méndez, X., Valero, F., Lucena, F., Jofre, J., 2002. Removal and inactivation of indicator bacteriophages in fresh waters. J. Appl. Microbiol. 92, 338–347.

Engblom, K., Lundh, M., 2006. Microbiological barrier in artificial recharge of ground water – a review.

Erlandsson, M., Buffam, I., Fölster, J., Laudon, H., Temnerud, J., Weyhenmeyer, G.A., Bishop, K., 2008. Thirty-five years of synchrony in the organic matter concentrations of Swedish rivers explained by variation in flow and sulphate. Glob. Change Biol. 14, 1191–1198.

Fermin, D., Riley, J., 2010. Colloid Science: Principles, Methods and Applications: Chapter 2: Charge in Colloidal Systems. John Wiley & Sons.

Foppen, J.W.A., Okletey, S., Schijven, J.F., 2006. Effect of goethite coating and humic acid on the transport of bacteriophage PRD1 in columns of saturated sand. J. Contam. Hydrol. 85, 287–301.

Frycklund, C., 2001. Colloids in infiltration water and their retention in filter sand.

Frycklund, C., 1998. Artificial recharge of groundwater for public water supply : potential and limitations in boreal conditions, Trita-AMI. PHD, 1400-1284 ; 1020. Stockholm.

Futter, M.N., Valinia, S., Löfgren, S., Köhler, S.J., Fölster, J., 2014. Long-term trends in water chemistry of acid-sensitive Swedish lakes show slow recovery from historic acidification. Ambio 43, 77–90.

Gerba, C.P., 1984. Applied and Theoretical Aspects of Virus Adsorption to Surfaces, in: Laskin, A.I. (Ed.), Advances in Applied Microbiology. Academic Press, pp. 133–168.

Gerba, C., Wallis, C., Melnick, J., 1975. Viruses in Water: the problem, some solutions. Environ. Sci. Technol. 9, 1122–1126.

Gordon, C., Toze, S., 2003. Influence of groundwater characteristics on the survival of enteric viruses. J. Appl. Microbiol. 95, 536–544.

Goyal, S.M., Gerba, C.P., 1979. Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. Appl. Environ. Microbiol. 38, 241–247.

Grant, S.B., List, E.J., Lidstrom, M.E., 1993. Kinetic analysis of virus adsorption and inactivation in batch experiments. Water Resour. Res. 29, 2067–2085.
Havelaar, A.H., van Olphen, M., Drost, Y.C., 1993. F-specific RNA bacteriophages are adequate model organisms for enteric viruses in fresh water. Appl. Environ. Microbiol. 59, 2956–2962.
Hermansson, M., 1999. The DLVO theory in microbial adhesion. Colloids Surf. B Biointerfaces 14, 105–119.
Hot, D., Legeay, O., Jacques, J., Gantzer, C., Caudrelier, Y., Guyard, K., Lange, M., Andréoletti, L., 2003. Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. Water Res. 37, 4703–4710.
Huisman, L., Wood, W.E., 1974. Slow Sand Filtration. WHO.
Hummel, A., 2014. Skattning av den integrerade hydrauliska konduktivitetens variation kring Tunåsens infiltrationsanläggning : En utredning av påverkan från möjliga osäkerheter i befintlig information.
Hytteborn, J.K., Futter, M.N., Temnerud, J., 2014. Evaluating common drivers for color, iron and organic carbon in Swedish watercourses. Ambio 43 Suppl 1, 30–44.
IAWPRC, 1991. Bacteriophages as model viruses in water quality controlag. Water Res. 25, 529–545.
IHSS, 2013. Source Materials for IHSS Samples [WWW Document]. URL http://www.humicsubstances.org/sources.html (accessed 6.14.15).
Jin, Y., Flury, M., 2002. Fate and Transport of Viruses in Porous Media, in: Sparks, D.L. (Ed.), Advances in Agronomy, Advances in Agronomy. Academic Press, pp. 39–102.
John, D.E., Rose, J.B., 2005. Review of Factors Affecting Microbial Survival in Groundwater. Environ. Sci. Technol. 39, 7345–7356.
Keswick, B.H., Gerba, C.P., 1980. Viruses in groundwater. Environ. Sci. Technol. 14, 1290–1297.
Köhler, S.J., Buffam, I., Seibert, J., Bishop, K.H., Laudon, H., 2009. Dynamics of stream water TOC concentrations in a boreal headwater catchment: Controlling factors and implications for climate scenarios. J. Hydrol. 373, 44–56.
Kretzschmar, R., Barmettler, K., Grolimund, D., Yan, Y., Borkovec, M., Sticher, H., 1997. Experimental determination of colloid deposition rates and collision efficiencies in natural porous media. Water Resour. Res. 33, 1129–1137.
Ledesma, J.L.J., Grabs, T., Bishop, K.H., Schiff, S.L., Köhler, S.J., 2015. Potential for long-term transfer of dissolved organic carbon from riparian zones to streams in boreal catchments. Glob. Change Biol. n/a–n/a.
Ledesma, J.L.J., Köhler, S.J., Futter, M.N., 2012. Long-term dynamics of dissolved organic carbon: Implications for drinking water supply. Sci. Total Environ. 432, 1–11.
Leenheer, J.A., Croué, J.-P., 2003. Peer Reviewed: Characterizing Aquatic Dissolved Organic Matter. Environ. Sci. Technol. 37, 18A–26A.
Lindberg, T., Lindqvist, R., 2005. Dricksvatten och mikrobiologiska risker. Livsmedelsverket Natl. Food Adm. Swed. Rapport 28.
Lodder, W.J., de Roda Husman, A.M., 2005. Presence of noroviruses and other enteric viruses in sewage and surface waters in The Netherlands. Appl. Environ. Microbiol. 71, 1453–1461.
Löfgren, S., Forsius, M., Andersen, T., 2003. The color of water - Climate induced water color increase in Nordic lakes and streams due to humus. Nord. Counc. Minist. Brochur.
Loveland, J.P., Ryan, J.N., Amy, G.L., Harvey, R.W., 1996. The reversibility of virus attachment to mineral surfaces. Colloids Surf. Physicochem. Eng. Asp., A collection of papers presented at the Symposium on Colloidal and Interfacial Phenomena in Aquatic Environments 107, 205–221.
Lund, F., Lindqvist, R., 2004. Virus in food and drinking water in Sweden. Livsmedelsverket Natl. Food Adm. Swed. Rapport 22.
Monteith, D.T., Stoddard, J.L., Evans, C.D., de Wit, H.A., Forsius, M., Høgåsen, T., Wilander, A., Skjelkvåle, B.L., Jeffries, D.S., Vuorenmaa, J., Keller, B., Kopácek, J., Vesely, J.,

2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature 450, 537–540.
Morosini, M., 1989. The artificial recharge of Tunåsen, Uppsala. A hydrochemical consideration.
Niemelä, S.I., 2002. Uncertainty of Quantitative Determinations Derived by Cultivation of Microorganisms. Centre for Metrology and Accreditation.
Pagano, T., Bida, M., Kenny, J.E., 2014. Trends in Levels of Allochthonous Dissolved Organic Carbon in Natural Water: A Review of Potential Mechanisms under a Changing Climate. Water 6, 2862–2897.
Pieper, A.P., Ryan, J.N., Harvey, R.W., Amy, G.L., Illangasekare, T.H., Metge, D.W., 1997. Transport and Recovery of Bacteriophage PRD1 in a Sand and Gravel Aquifer: Effect of Sewage-Derived Organic Matter. Environ. Sci. Technol. 31, 1163–1170.
Powelson, D.K., Simpson, J.R., Gerba, C.P., 1991. Effects of organic matter on virus transport in unsaturated flow. Appl. Environ. Microbiol. 57, 2192–2196.
Ryan, J.N., Elimelech, M., 1996. Colloid mobilization and transport in groundwater. Colloids Surf. Physicochem. Eng. Asp., A collection of papers presented at the Symposium on Colloidal and Interfacial Phenomena in Aquatic Environments 107, 1–56.
Schijven, J.F., Hassanizadeh, S.M., 2000. Removal of Viruses by Soil Passage: Overview of Modeling, Processes, and Parameters. Crit. Rev. Environ. Sci. Technol. 30, 49–127.
SEPA, 2008. Wastewater treatment in Sweden. Swedish Environmental Protection Agency, Stockholm.
Skraber, S., Schijven, J., Gantzer, C., Husman, A.M. de R., 2005. Pathogenic viruses in drinking-water biofilms: A public health risk? Biofilms 2, 105 – 117.
Sundlöf, B., Kronqvist, L., 1992. Konstgjord grundvattenbildning: nulägesbeskrivning av tjugo svenska anläggningar. Svenska vatten- och avloppsverksfören. (VAV).
Sutton, S., 2011. Accuracy of plate counts. J. Valid. Technol. 42–46.
Syngouna, V.I., Chrysikopoulos, C.V., 2010. Interaction between Viruses and Clays in Static and Dynamic Batch Systems. Environ. Sci. Technol. 44, 4539–4544.
Torkzaban, S., Hassanizadeh, S.M., Schijven, J.F., van den Berg, H.H.J.L., 2006. Role of air-water interfaces on retention of viruses under unsaturated conditions. Water Resour. Res. 42, W12S14.
UppsalaVatten, 2014. Vattenforsorjning i Uppsala stad.
US EPA, 2001. Method 1602 Malespecific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure.
US EPA, O., 2012. Flow introduction | CADDIS: Sources, Stressors & Responses | US EPA [WWW Document]. URL http://www.epa.gov/caddis/ssr_flow_int.html (accessed 5.15.15).
Walshe, G.E., Pang, L., Flury, M., Close, M.E., Flintoft, M., 2010. Effects of pH, ionic strength, dissolved organic matter, and flow rate on the co-transport of MS2 bacteriophages with kaolinite in gravel aquifer media. Water Res., Transport and Fate of Colloids and Microbes in Granular Aqueous Environments 44, 1255–1269.
Westrell, T., Andersson, Y., Stenström, T.A., 2006. Drinking water consumption patterns in Sweden. J. Water Health 04, 511–522.
WHO, 2011. Guidelines for drinking-water quality, fourth edition [WWW Document]. WHO. URL http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/ (accessed 4.28.15).
Yates, M.V., Yates, S.R., Wagner, J., Gerba, C.P., 1987. Modeling virus survival and transport in the subsurface. J. Contam. Hydrol. 1, 329–345.
Zhuang, J., Jin, Y., 2003. Virus Retention and Transport as Influenced by Different Forms of Soil Organic Matter. J. Environ. Qual. 32, 816.

10. Appendix

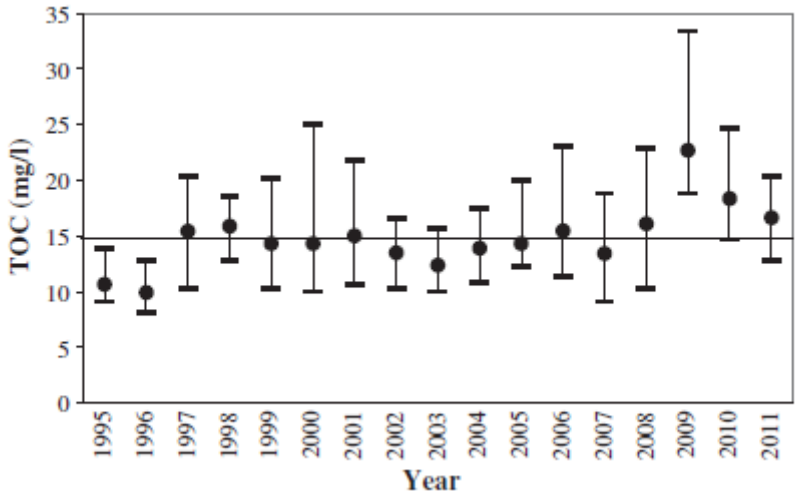


Figure 18*: Annual range of TOC concentrations measured at the outlet of Fyrisån catchment. Black circles represents annual means and horizontal lines maximum and minimum values. Long horizontal line represents the mean value for the whole time series. (Ledesma et al., 2012)*

Table 13: *Model fit and parameters obtained from the experimental data between day 0 and day 15.Agitated conditions*

	Linear model				**Non-linear model**				
	λ	p (λ)	R2	SSR	λ0	α	p (λ0)	p (α)	SSR
Old sand									
d+e+	0.127 ± 0.019	*	0.90	0.84	0.394 ± 0.180	0.248 ± 0.145	xx	x	0.23
d+e-	0.022 ± 0.019	x	0.20	0.91	-	-	-	-	-
d-e+	0.105 ± 0.019	**	0.86	0.90	0.606 ± 0.223	0.488 ± 0.194	xx	xx	0.08
d-e-	0.179 ± 0.033	**	0.86	2.59	2.722 ± 0.989	1.370 ± 0.521	xx	xx	0.26
New sand									
d+e+	1.184 ± 0.214	**	0.86	108.68	16.056 ± 2.486	1.216 ± 0.197	**	**	2.19
d+e-	1.045 ± 0.178	**	0.87	75.78	11.946 ± 1.647	1.022 ± 0.147	**	**	1.37
d-e+	1.192 ± 0.263	**	0.80	165.19	31.629 ± 5.833	2.384 ± 0.458	**	**	1.35
d-e-	1.278 ± 0.263	**	0.83	165.50	23.136 ± 2.307	1.614 ± 0.169	***	***	0.89
No sand									
d+e+	0.049 ± 0.015	*	0.67	0.56	0.003 ± 0.005	0.317 ± 0.140	x	xx	0.18
d+e-	0.014 ± 0.011	x	0.26	0.29	-	-	-	-	-
d-e+	0.004 ± 0.034	x	0.01	2.83	0.001 ± 0.033	0.254 ± 2.752	x	x	2.79
d-e-	0.179 ± 0.039	**	0.80	3.79	-	-	-	-	-

*P-values: x = (p ≥ 0.1); xx = (p ≤ 0.1); * = (p ≤ 0.05); ** = (p ≤ 0.01); *** = (p ≤ 0.001),*

"-" = modeling was not possible, due to a limited number of data points

Table 14: *Soil and water filling of the vials*

	Number of vials	Empty vials [g]	Sand added [g]	Water added [g]	Final weight [g]	Water per vial (average) [g]	Sand per vial (average) [g]
Old sand							
d+e+	26	548.59	130.63	297.82	977.04	11.45	5.02
d+e-	26	548.91	x	x	x	11.44*	5.00*
d-e+	26	549.19	129.63	297.98	976.80	11.46	4.99
d-e-	26	696.51	129.95	296.17	1122.63	11.39	5.00
New sand							
d+e+	26	559.43	130.32	288.13	977.88	11.08	5.01
d+e-	26	x	x	x	x	11.24*	5.08*
d-e+	26	550.13	133.60	293.68	977.41	11.30	5.14
d-e-	26	548.89	132.00	295.26	976.15	11.36	5.08
No sand							
d+e+	26	x	x	x	x	13.25*	0.00*
d+e-	26	548.52	0.00	343.53	892.05	13.21	0.00
d-e+	26	549.62	0.00	344.72	894.34	13.26	0.00
d-e-	26	549.45	0.00	344.88	894.33	13.26	0.00

x = missing data, * = the values were estimated as the average value of the 3 remaining experiments of the same soil-treatment

Table 15: *MS2 concentrations measured for in PFU/ml for each time step and replicate*

Old sand	d+e+		d+e-		d-e+		d-e-	
Replicate	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
C(0)	4.03E+07	4.03E+07	1.24E+07	1.24E+07	3.82E+07	3.82E+07	1.05E+08	1.05E+08
C(1)	6.89E+07	1.86E+07	4.43E+07	1.55E+07	2.85E+07	2.68E+07	3.13E+07	1.99E+07
C(2)	1.69E+07	1.86E+06	1.30E+07	6.91E+06	1.71E+07	1.75E+06	1.99E+07	1.87E+07
C(3)	1.32E+07	6.41E+06	1.11E+07	7.12E+06	5.67E+06	1.73E+07	7.43E+06	2.54E+07
C(4)	8.94E+06	8.73E+06	1.22E+07	1.23E+07	1.23E+07	1.19E+07	9.82E+06	9.54E+06
C(5)	5.25E+06	1.40E+07	4.43E+06	1.09E+07	1.21E+07	1.15E+07	2.27E+06	2.64E+07
C(6)	1.45E+06	2.79E+06	1.66E+06	1.34E+06	8.29E+05	5.17E+05	4.26E+06	2.21E+07
C(7)	3.58E+06	1.32E+06	2.80E+06	1.41E+06	1.37E+06	1.29E+06	3.14E+06	2.26E+06
C(9)	3.21E+06	1.61E+06	4.33E+06	6.78E+05	2.34E+06	2.11E+06	2.03E+06	1.04E+06
C(10)	4.42E+06	3.13E+06	1.39E+06	3.70E+06	1.84E+06	2.30E+06	2.02E+06	2.17E+06
C(11)	2.68E+06	9.67E+05	1.80E+06	2.12E+06	1.77E+06	1.47E+06	1.39E+06	8.43E+05
C(12)	2.06E+06	1.13E+06	6.95E+05	7.30E+05	6.02E+05	5.97E+05	1.00E+06	8.06E+05
New sand	**d+e+**		**d+e-**		**d-e+**		**d-e-**	
Replicate	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
C(0)	4.03E+07	4.03E+07	1.24E+07	1.24E+07	3.82E+07	3.82E+07	1.05E+08	1.05E+08
C(1)	5.37E+03	6.37E+03	6.46E+03	1.41E+04	2.03E+02	4.06E+02	4.06E+02	3.05E+03
C(2)	2.48E+02	1.10E+02	1.31E+02	3.02E+02	1.61E+02	1.11E+02	1.11E+02	9.06E+01
C(3)	1.55E+02	1.10E+02	1.10E+02	2.19E+02	8.05E+01	1.21E+02	1.21E+02	6.04E+01
C(4)	3.27E+01	2.82E+01	6.64E+01	4.91E+01	4.00E+01	3.27E+01	2.64E+01	3.45E+01
C(5)	5.00E+01	3.60E+01	6.60E+01	5.20E+01	3.50E+01	4.20E+01	5.80E+01	5.30E+01
C(6)	2.80E+01	2.50E+01	4.30E+01	1.30E+01	2.30E+01	8.00E+00	1.70E+01	1.30E+01
C(7)	1.00E+01	2.00E+01	2.40E+01	3.40E+01	1.10E+01	1.20E+01	7.00E+00	1.30E+01
C(9)	2.80E+01	1.50E+01	1.00E+01	8.00E+00	1.40E+01	6.00E+00	8.00E+00	8.00E+00
C(10)	4.00E+00	6.00E+00	6.00E+00	1.00E+01	1.10E+01	4.00E+00	9.00E+00	6.00E+00
C(11)	1.10E+01	6.00E+00	1.30E+01	1.10E+01	2.00E+00	1.00E+00	3.00E+00	0.00E+00
C(12)	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
No sand	**d+e+**		**d+e-**		**d-e+**		**d-e-**	
Replicate	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
C(0)	4.03E+07	4.03E+07	1.24E+07	1.24E+07	3.82E+07	3.82E+07	1.05E+08	1.05E+08
C(1)	2.61E+07	9.43E+07	2.08E+07	1.48E+07	1.77E+07	2.85E+07	1.87E+07	3.38E+07
C(2)	7.56E+07	5.19E+06	2.52E+07	1.25E+07	3.04E+07	3.34E+07	2.41E+07	1.88E+07
C(3)	8.31E+06	6.44E+07	6.25E+06	1.66E+07	1.36E+07	8.32E+06	9.38E+06	6.24E+06
C(4)	2.32E+07	2.56E+07	1.54E+07	1.15E+07	9.60E+06	5.16E+06	6.87E+06	1.50E+07
C(5)	9.30E+06	1.68E+07	2.04E+07	1.15E+07	7.69E+06	1.57E+07	1.17E+07	2.71E+07
C(6)	3.09E+06	2.56E+07	1.62E+06	3.80E+06	2.10E+06	2.61E+06	3.34E+06	2.24E+06
C(7)	2.62E+06	1.90E+06	2.05E+06	2.17E+06	1.80E+06	3.06E+06	9.00E+05	4.20E+05
C(9)	1.61E+06	1.77E+05	2.36E+06	9.49E+05	2.09E+06	1.64E+06	2.14E+06	1.22E+06
C(10)	1.33E+06	1.11E+06	4.56E+06	2.19E+06	1.31E+06	9.44E+05	1.27E+06	3.42E+06
C(11)	1.22E+06	6.19E+04	1.35E+06	1.65E+05	3.71E+05	1.65E+05	2.48E+05	1.03E+04
C(12)	2.00E+05	8.67E+04	4.03E+01	8.77E+04	3.02E+01	3.42E+02	1.11E+02	6.04E+01

Table 16: *MS2 concentrations in PFU/ml for each time step as the average of both replicates*

Old sand	d+e+	d+e-	d-e+	d-e-
C(0)	4.03E+07	1.24E+07	3.82E+07	1.05E+08
C(1)	4.37E+07	2.99E+07	2.77E+07	2.56E+07
C(2)	9.38E+06	9.97E+06	9.43E+06	1.93E+07
C(3)	9.80E+06	9.12E+06	1.15E+07	1.64E+07
C(4)	8.83E+06	1.22E+07	1.21E+07	9.68E+06
C(5)	9.62E+06	7.65E+06	1.18E+07	1.43E+07
C(6)	2.12E+06	1.50E+06	6.73E+05	1.32E+07
C(7)	2.45E+06	2.11E+06	1.33E+06	2.70E+06
C(9)	2.41E+06	2.51E+06	2.22E+06	1.53E+06
C(10)	3.77E+06	2.54E+06	2.07E+06	2.10E+06
C(11)	1.83E+06	1.96E+06	1.62E+06	1.12E+06
C(12)	1.59E+06	7.12E+05	6.00E+05	9.03E+05
New sand	**d+e+**	**d+e-**	**d-e+**	**d-e-**
C(0)	4.03E+07	1.24E+07	3.82E+07	1.05E+08
C(1)	5.87E+03	1.03E+04	3.05E+02	1.73E+03
C(2)	1.79E+02	2.16E+02	1.36E+02	1.01E+02
C(3)	1.33E+02	1.64E+02	1.01E+02	9.06E+01
C(4)	3.05E+01	5.77E+01	3.64E+01	3.05E+01
C(5)	4.30E+01	5.90E+01	3.85E+01	5.55E+01
C(6)	2.65E+01	2.80E+01	1.55E+01	1.50E+01
C(7)	1.50E+01	2.90E+01	1.15E+01	1.00E+01
C(9)	2.15E+01	9.00E+00	1.00E+01	8.00E+00
C(10)	5.00E+00	8.00E+00	7.50E+00	7.50E+00
C(11)	8.50E+00	1.20E+01	1.50E+00	1.50E+00
C(12)	0.00E+00	0.00E+00	0.00E+00	0.00E+00
No sand	**d+e+**	**d+e-**	**d-e+**	**d-e-**
C(0)	4.03E+07	1.24E+07	3.82E+07	1.05E+08
C(1)	6.02E+07	1.78E+07	2.31E+07	2.62E+07
C(2)	4.04E+07	1.89E+07	3.19E+07	2.15E+07
C(3)	3.63E+07	1.14E+07	1.09E+07	7.81E+06
C(4)	2.44E+07	1.35E+07	7.38E+06	1.09E+07
C(5)	1.30E+07	1.60E+07	1.17E+07	1.94E+07
C(6)	1.43E+07	2.71E+06	2.36E+06	2.79E+06
C(7)	2.26E+06	2.11E+06	2.43E+06	6.60E+05
C(9)	8.93E+05	1.65E+06	1.86E+06	1.68E+06
C(10)	1.22E+06	3.37E+06	1.13E+06	2.35E+06
C(11)	6.40E+05	7.58E+05	2.68E+05	1.29E+05
C(12)	1.43E+05	4.39E+04	1.86E+02	8.56E+01

Table 17: *Sampling days*

Sampling date	Time after first sampling (days)	Sampling day
29.05.2015 13:15	0.00	0
30.05.2015 11:00	0.91	1
04.06.2015 10:32	5.89	6
07.06.2015 10:00	8.86	8
10.06.2015 09:48	11.86	12
13.06.2015 11:04	14.91	15
17.06.2015 10:00	18.87	19
20.06.2015 11:50	21.94	22
24.06.2015 09:55	25.86	26
27.06.2015 12:45	28.98	29
01.07.2015 14:13	33.04	33
06.08.2015 11:39	68.93	69

Table 18: *Removal rates λ in days^{-1}, computed for the „new sand"-experiments by the use of Equation 3 and the non-linear model parameters of Table 4.*

	d+e+	d+e-	d-e+	d-e-
λ(0.00)	1.51E+01	1.10E+01	2.65E+01	2.11E+01
λ(0.91)	5.75E+00	5.04E+00	5.05E+00	6.18E+00
λ(5.89)	2.89E-02	7.03E-02	5.56E-04	7.36E-03
λ(8.86)	1.22E-03	5.46E-03	2.39E-06	1.31E-04
λ(11.86)	5.06E-05	4.19E-04	1.00E-08	2.30E-06
λ(14.91)	1.97E-06	3.05E-05	3.75E-11	3.71E-08
λ(18.87)	2.94E-08	1.03E-06	2.69E-14	1.76E-10
λ(21.94)	1.12E-09	7.32E-08	9.67E-17	2.75E-12
λ(25.86)	1.73E-11	2.53E-09	7.41E-20	1.38E-14
λ(28.98)	6.31E-13	1.75E-10	2.47E-22	2.03E-16
λ(33.04)	8.41E-15	5.35E-12	1.46E-25	8.37E-19

Table 19: *Test on normality: p-values of the Anderson-Darling-test for all 22 data points tested (day 0 – day 33) and for the residuals of the linear model.*

	Data points	Residues (linear model)	Residues (non-linear model)
Old sand			
d+e+	0.0384	0.0668	0.7318
d+e-	0.1145	0.2306	0.9115
d-e+	0.1215	0.1684	0.5904
d-e-	0.0227	0.0437	0.5057
New sand			
d+e+	0.0003	0.0003	0.6522
d+e-	0.0003	0.0003	0.9603
d-e+	0.0003	0.0003	0.9889
d-e-	0.0003	0.0003	0.8505
No sand			
d+e+	0.1230	0.2556	0.9887
d+e-	0.4297	0.4671	0.9477
d-e+	0.1308	0.2270	0.9447
d-e-	0.0195	0.0332	0.9983

$p < 0.01$ means that the sample tested, is significantly differently distibuted than a normal distribution

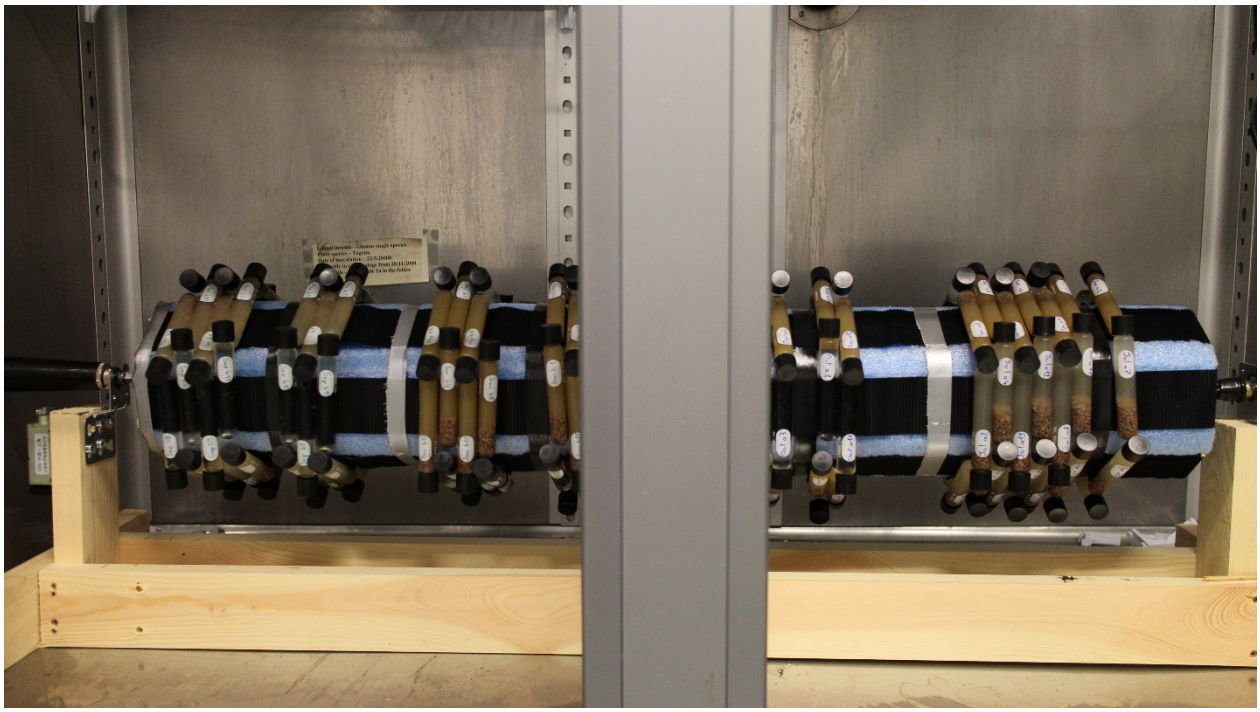


Figure 19: *Batch agitator with attached batch reactors. The photo was taken on the 20.06.2015 in the open Phytotron cabinet.*

T(1)	T(2)	T(3)	T(4)	T(5)	T(6)	T(7)	T(8)	T(9)	T(10)

Parameter	Result
Turbidity	0.28 FNU
Odor (20°C)	None
Odor (50°C)	None
Color	100 mg/l Pt
COD	13 mg/l
EC_{25}	41.4 mS/m
pH	8
Alkalinity	172 mg/l
Total hardness	11.6 °dH
Sodium	9.9 mg/l
Magnesium	6.1 mg/l
Aluminum	0.0064 mg/l
Calcium	73.1 mg/l
Iron	0.22 mg/l
Manganese	0.008 mg/l
Copper	< 0.02 mg/l
Uranium	9.5 µg/l
Ammonium	< 0.04 mg/l
Nitrite	0.026 mg/l
Fluoride	0.43 mg/l
Chloride	14 mg/l
Nitrite	4.9 mg/l
Sulfate	4.9 mg/l
TOC	46 mg/l
DOC	17.6 mg/l

Table 20*: Water sample Analysis of the raw water sampled at Tunåsen. (Sampling day: 15.04.2015. Analysis: 15.04.2015). Conducted by Uppsala Vatten och Avfall AB, Vattenlaboratoriet.*

Table 21: *Relative Standard Deviation*

Old sand										
d+e+	81.33%	113.44%	48.90%	1.72%	64.22%	44.65%	65.33%	47.02%	24.17%	66.53%
d+e-	68.14%	43.49%	31.02%	0.17%	59.61%	14.84%	46.66%	103.16%	64.08%	11.51%
d-e+	4.33%	115.14%	71.64%	2.25%	3.88%	32.84%	4.35%	7.40%	15.72%	13.02%
d-e-	31.46%	4.46%	77.36%	2.06%	119.09%	95.76%	23.10%	45.30%	5.14%	34.54%
New sand										
d+e+	12.00%	54.54%	23.98%	10.55%	23.02%	8.00%	47.14%	42.76%	28.28%	41.59%
d+e-	52.72%	55.77%	46.99%	21.16%	16.78%	75.76%	24.38%	15.71%	35.36%	11.79%
d-e+	47.23%	26.32%	28.28%	14.14%	12.86%	68.43%	6.15%	56.57%	66.00%	47.14%
d-e-	108.19%	14.27%	47.15%	19.00%	6.37%	18.86%	42.43%	0.00%	28.28%	141.42%
No sand										
d+e+	80.14%	123.25%	109.08%	7.02%	40.62%	110.99%	22.74%	113.34%	12.32%	127.74%
d+e-	24.05%	47.41%	64.13%	20.38%	39.40%	56.70%	4.03%	60.31%	49.55%	110.64%
d-e+	33.03%	6.65%	33.85%	42.50%	48.31%	15.34%	36.56%	17.13%	22.93%	54.39%
d-e-	40.46%	17.55%	28.46%	52.65%	56.22%	27.96%	51.41%	38.74%	65.01%	130.11%