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Chlorinated aliphatic hydrocarbons; an interdisciplinary study of degradation and distribution in complex environments

Åkesson, Sofia

2022

Document Version:

Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

Åkesson, S. (2022). *Chlorinated aliphatic hydrocarbons; an interdisciplinary study of degradation and distribution in complex environments*. [Doctoral Thesis (compilation), Department of Geology]. Lunds universitet, Media-Tryck .

Total number of authors:

1

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Chlorinated aliphatic hydrocarbons: an interdisciplinary study of degradation and distribution in complex environments

SOFIA ÅKESSON

QUATERNARY SCIENCES | DEPARTMENT OF GEOLOGY | LUND UNIVERSITY 2022



Chlorinated aliphatic hydrocarbons: an interdisciplinary study of degradation and distribution in complex environments

Sofia Åkesson



LUND
UNIVERSITY

Quaternary Sciences
Department of Geology

DOCTORAL DISSERTATION

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To be defended in Pangea, Geocentrum II, Sölvegatan 12

on the 18th of February 2022, at 13:15.

Faculty opponent

Associate Professor Mette M. Broholm
Technical University of Denmark

Funding information: This PhD project has been part of the research project MIRACHL, funded by FORMAS (ref. 2016-20099 & 2016-00808), Geological Survey of Sweden, SBUF (ref. 13336), ÅForsk (ref. 14-332), NCC, Sven Tyréns stiftelsen, and Lund University.

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Quaternary Sciences
Department of Geology
Faculty of Science

ISBN 978-91-87847-58-5 (print)


ISBN 978-91-87847-59-2 (pdf)

ISSN 0281-3033

Printed in Sweden by Media-Tryck, Lund University, Lund 2022



Media-Tryck is a Nordic Swan Ecolabel
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Read more about our environmental
work at www.mediatryck.lu.se

MADE IN SWEDEN 

Organization LUND UNIVERSITY Department of Geology Sölvegatan 12 SE-223 62 Lund Sweden Author: Sofia Åkesson	Document name DOCTORAL DISSERTATION	
	Date of issue: 2022-02-18	
	Sponsoring organization	
Title: Chlorinated aliphatic hydrocarbons: an interdisciplinary study of degradation and distribution in complex environments		
<p>Abstract</p> <p>In this thesis degradation of chlorinated solvents, e.g., tetrachloroethene (perchloroethylene [PCE]), has been examined, both natural occurring degradation and in situ remediation strategies. The projects included have focused on two field sites of former dry-cleaning facilities, which are contaminated with PCE in both the groundwater and the sediments. One site had only been exposed to natural degradation, whereas the other site has been targeted for enhanced <i>in situ</i> remediation, both abiotic and biotic. The aim of this thesis was to characterize the subsurface conditions and monitor changes at contaminated site with an interdisciplinary approach. The data included within the studies are contaminant concentrations, major and minor ions concentrations, Compound Specific Isotope Analysis (CSIA) of carbon, and DNA analysis describing microbial content. At the site with natural degradation geoelectrical resistivity and chargeability measurements were also performed. In addition, a laboratory study has been conducted to link microbial growth and activity to geoelectrical signals.</p> <p>The studies have shown that changes in geological setting with shifts in hydrogeological properties, i.e., transition units, proved to be important features associated with more efficient degradation. The transitions units showed larger variations in microbial communities, compared to the aquifer material. The microbial communities were similar when comparing samples from the sediment and the groundwater; however, more of the microbes associated with dechlorination were found in the sediment samples. Correlation between the amount of microbes and resistivity was possible, while the chargeability could only indicate to arise due to by-products. During monitoring of enhanced biodegradation PCE's metabolites were needed to be included when evaluating the CSIA data, but for the natural degradation it was not required.</p> <p>Since the location of transition units has been shown to be an important hydrogeological setting, both with regards to localization of the contaminant and as a preferred environment for the microbial communities, high quality data of the geological conditions are required from contaminated sites to capture changes in the settings. The sediment matrix should be investigated during in situ bioremediations to fully examine the microbial communities and to be able to evaluate the degradation effect. The interdisciplinary approach made it possible to challenge the limitation of commercially available analysis and state-of-the-art methods, and have contribute to more nuanced interpretations and evaluations of the complexities at contaminated sites.</p>		
Key words: groundwater, groundwater chemistry, contamination, chlorinated solvents, <i>in situ</i> remediation, biodegradation, PCR, DCIP, CSIA		
Classification system and/or index terms (if any)		
Supplementary bibliographical information	Language: English	
ISSN and key title: 0281-3033 LUNDQUA THESIS		ISBN 978-91-87847-58-5
Recipient's notes	Number of pages: 126	Price:
	Security classification	

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The science of hydrology would be relatively simple if water were unable to penetrate below the earth's surface.

Harold E. Thomas (1952)

Contents

LIST OF PAPERS	6	POPULAR SUMMARY	31
ACKNOWLEDGMENTS	7	SVENSK SAMMANFATTNING	32
1 INTRODUCTION	9	REFERENCES	33
2 SCOPE OF THESIS	11	PAPER I	41
3 BACKGROUND	11	PAPER II	59
3.1 Chlorinated solvents	11	PAPER III	83
3.1.1 Behavior in the subsurface	12	PAPER IV	105
3.1.2 Degradation	14	LUNDQUA THESIS	123
3.1.3 Remediation	15		
3.2 Motivation of used methods	16		
3.2.1 CSIA	16		
3.2.2 Microbial characterization	16		
3.2.3 Geoelectrical measurements	16		
3.3 Field sites	17		
3.3.1 Hagforstvädden	17		
3.3.2 Tvätteriet Alingsås	18		
4 METHODS	20		
4.1 Field data collection	20		
4.2 Microbiological analysis	20		
4.3 Quantification of biodegradation via CSIA	22		
4.4 Geoelectrical measurements and analysis	22		
5 RESULTS	22		
Paper I	22		
Paper II	23		
Paper III	24		
Paper IV	24		
List of contribution	25		
5.1 Additional findings outside publications	26		
6 DISCUSSION	27		
6.1 Synthesizing reflections	27		
6.2 Future Research	29		
7 CONCLUSIONS	30		

List of papers

This thesis is based on the following publications and manuscripts:

Paper I

Characterizing natural degradation of tetrachloroethene (PCE) using a multidisciplinary approach

Åkesson, S., Sparrenbom, C.J., Paul, C.J., Jansson, R. & Holmstrand, H. (2021)

Ambio, 5, p. 1074–1088.

DOI: 10.1007/s13280-020-01418-5

Paper II

Examining biofilm growth and metabolism of *Escherichia coli* using geophysical Induced Polarization measurements

Martin, T., Åkesson, S. & Paul, C.

Submitted to *Journal of Geophysical Research – Biogeosciences*

Paper III

Biochemical changes associated with two different *in situ* remediation strategies for tetrachloroethene

Åkesson, S., Frank, E., Sparrenbom, C.J., Zhang, Z., Holmstrand, H. & Paul, C.J.

Manuscript for *Science of The Total Environment*

Paper IV

Impact of spatial distribution of microbes in the subsurface on degradation of tetrachloroethene (PCE)

Åkesson, S., Benavides Höglund, N., Sparrenbom, C., Rosenqvist, T. & Paul, C.J

Manuscript for *Bioremediation Journal*

Acknowledgments

I am endlessly proud that you can read this thesis; for certain, nothing became as thought nor as planned, but throughout a bumpy journey, it is done. Since “no human is an island, entire of itself” (John Donne, 1623; Svante Björck, in all emails), I would like to express my gratitude to some specific persons.

I would like to start to thank my supervisors: Charlotte Sparrenbom for getting me into the project, for all support and guidance along the journey. Catherine Paul for introducing me to the microbial world, as well as putting up limitations and keeping track of them (and me). Tina Martin for always reading everything in detail and never missing anything, and for bringing in geophysics into the project. Henry Holmstrand for valuable guidance and feedback about the isotopes.

In addition, I would like to thank Beth Parker, for kick-starting my doctoral project and for getting me into the most worthwhile courses, even when I could not cross the ocean. Moreover, Daniel Conley, for your mentorship which has been highly appreciated.

I would also like to thank the research group for interesting meetings and challenging discussions across different disciplines. Especially, Aristeidis Nivorlis and Nikolas Brenavides Höglund for the shared doctoral studies and in addition Mats Svensson for keeping up the focus on the application of applied research. I am very thankful to all field assistants; Nikolas, Glenn Johansson, Robin Jansson, Jessica Jennerheim, and Love Lindberg Skutsjö; and for support with the microbial data; Ellinor Frank, Zixuan Zhung, and Tage Rosenquist.

To all colleagues at the Department of Geology, thank you for making it to a warm environment, for all interesting discussions of varying topics (including coffee’s “taste”) and I missed you while working from home for obvious reasons. It became clear that it is the academic milieu that turns academia into something valuable. An extra thanks to all fellow Ph.D. students, sorry, you are and have been too many to mention by name - thank you for everything! In addition, to the group of people who never becomes authors of papers, however no papers would ever get published without your help and services; the Department's technical and administrative crew - especially Åsa Wallin, Gert Pettersson, Git Ahlberg, and Britta Smångs (and sorry for the mess by the end, just on time for the vacation).

I am very grateful for the friendly embrace I received during my time at The Division of Applied Microbiology and that you have helped this geologist among genes, machines, and reagents. All my warmest gratitude to Linda Jansson, Anette Ahlberg, Yasmine Akelis, Christer Larsson, Daniel Brink, Nina Muratovska, Celina Tufvegren Borgström, Sandy Chan, and Kristjan Pullerits for doing the “little” extra.

Some colleagues become more than just someone at the same workplace: Martin Lund, Megan Allington, Anton Hansson, and Franzi Stamm - thank you for being there, it would have been impossible and boring without you.

Thanks to family and friends who have made it possible to recharge when needed, with some special mentions: Per, Helena, Kim, Ellen, Ulrika, Emmie, Jonna, Anne Cecilie and Jesper - thank you for being there and asking how things are going! The Cassells family and Bojsen family thank you for the social bubble alliance instead of turning completely crazy. An additional “thanks” to Ph.D. A and Ph.D. B for “informing” me about the process of writing a thesis, I think you have helped JJ more than me...

Jonas, Lisa, Ebbe and Fred you are simply the best, thank you for all joy and happiness!

My lovely parents, Ann and Thomas: thank you for your patience with my persistence, curiosity, and questions that never ended. Thank you for believing in me before day one and every single day after, and for being the roots of my tree into the storms and reminding me to enjoy the sunny days.

Johan, thank you for having done everything at home and for every day reminding me to be brilliant. Thank you for standing steady, cheering, and loving me when I do not understand but need it the most – you are brilliant, and my supreme delight!

Thanks to all of you who made the journey possible and the hard work worth it!

1 Introduction

Groundwater is the subsurface water that completely saturates the pore spaces and fractures in the ground. Groundwater is formed when water infiltrates through the ground via gravitation as a part of the hydrologic cycle (Fig. 1). The hydrologic cycle describes the transport of global water, which has a constant mass, and is transported between reservoirs and phases by solar energy and gravitation. The vast majority of the globe's water is distributed in the ocean (Tab. 1), while less than 1% is groundwater (Hiscock & Bense, 2014). Commonly water is associated with drinking water, hygiene, food preparation, and irrigation; the water used in production of e.g. manufactured articles, and food production, is often invisible and referred to as virtual water. In addition, water constitutes an important habitat that contributes to biological diversity (Irfan & Alatawi, 2019).

Drinking water most often originates from shallow groundwater, lakes, and rivers (Tab. 1) (Hiscock & Bense, 2014); desalination of ocean water is possible, although it incurs significant costs for energy and maintenance of the treatment plant's equipment (Cooley *et al.*, 2006; Guo *et al.*, 2012). Groundwater supplies more than half of the global population with drinking water (WWAP, 2015), and 43% of the irrigation water comes from groundwater sources (Siebert *et al.*, 2010; WWAP, 2015). Globally 2.5 billion people are exclusively dependent on groundwater to cover their basic daily water needs (WWAP, 2015). Therefore, groundwater is a crucial natural resource for society, and annually it is the largest extracted volume of raw material compared to all others (Margat & van der Gun, 2013). According to World Health Organization (WHO, 2019), by 2025, 50% of the world's population will be living in areas that will suffer from water scarcity, for at least one month per year. The prognosis for 2050, based on increasing population and climate change, is that this group could increase to 4.8-5.7 billion people (WWAP, 2018; Burek *et al.*, 2016). Rockström *et al.* (2009) have identified ten "planetary boundaries" for humanity, where water withdrawal is one. Although this boundary has not yet been not crossed, water withdrawal is now more than six times higher compared to pre-industrial use.

Groundwater quality is also of great importance whether being used as drinking water, for hygiene, or irrigation, as stated in the UN Sustainable Development Goals. Goal 6: Clean water and sanitation, covers the importance of safe water and the need for improving water quality through target 6.3. "By 2030, improve water quality by

reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally" (UN, 2021). The groundwater quality can be poor due to substances in elevated concentrations above background levels, which are then considered contaminants, and if they constitute a risk for human health, they are considered pollutants (Chapman, 2006). These substances can have a natural origin e.g. from the bedrock, such as arsenic, fluoride and iodine (Wen *et al.*, 2013), or from an anthropogenic source, e.g. pharmaceuticals, pesticides, industrial components (Lapworth *et al.*, 2012) and micro plastic (Panno *et al.*, 2019). Natural substances can also be introduced by anthropogenic actions, e.g. seawater intrusion in coastal aquifers because of excessive groundwater withdrawal or sea level rise due to climate change (e.g. Barlow & Reichard, 2009; Ketabchi *et al.*, 2016). Anthropogenic contaminants can be introduced to the environment through areal and aerial discharge, e.g. pesticides and fertilizers in irrigation, gas emissions from transport and industry, or from a point source such as a leaking pipe system or an accidental spill. The two latter have a more distinct location and are commonly referred to as contaminated sites, and are common in a global perspective. Potentially contaminated sites in the European Union (including the United Kingdom) are estimated at 2.8 million sites; however, only 24% were inventoried (EEA, 2019). Contaminated sites substances pose a risk to reach the groundwater, consequently affecting safe drinking water supplies.

Chlorinated solvents are common as contaminants because of their versatile use in a range of areas (Pankow & Cherry, 1996). Two common chlorinated solvents are tetrachloroethene (perchloroethylene [PCE]) and trichloroethene (TCE), which have been and are used mainly as solvents in dry cleaning and for degreasing in metal industry (Pankow & Cherry, 1996). The disadvantages of these components are their toxic properties, including its degradation products. PCE and TCE are degradable, and it is of importance during remediation to clean up the most hazardous metabolic products. The remediation is possible to perform in situ, e.g. via thermal treatment (Triplett Kingston *et al.*, 2014), abiotic or biotic degradation, either via reduction or oxidation processes, or when biotically also via co-metabolic processes (Wiedemeier *et al.*, 1999; Stroo & Ward, 2010).

To investigate the degradation process, multiple methods can be applied; beyond the contaminant compounds concentrations, the presence of microorganisms are of importance (see section 3.1.2). During biodegradation, fractionation occurs between ^{13}C and ^{12}C , which is possible to investigate through compound specific isotope analysis (Hunkeler *et al.*, 2008). To obtain an overview of the ground it is possible to measure the subsurface geoelectrical properties, which are also affected by the process-

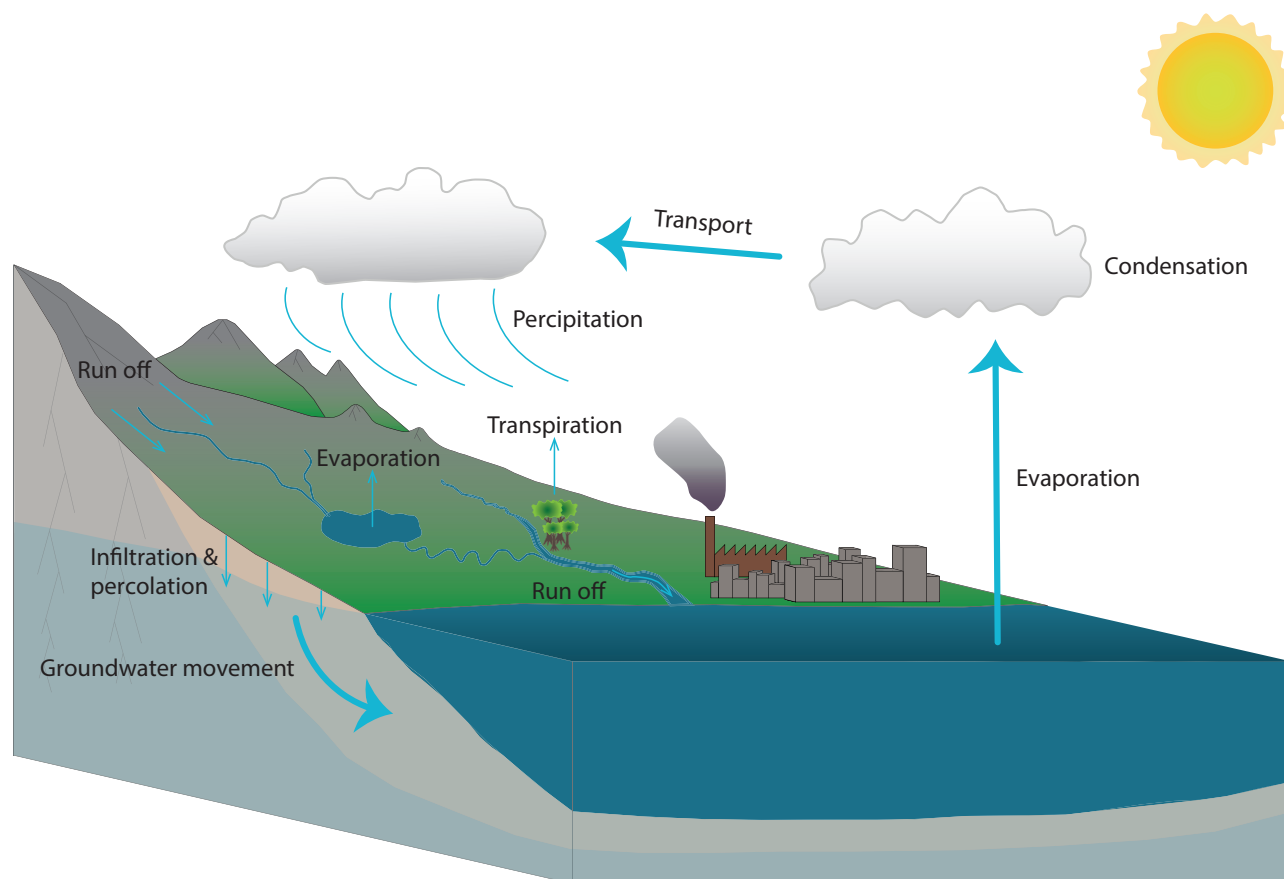


Figure 1. The hydraulic cycle describes the circulation of natural water due to energy fluxes in the system. When the sun heats the Earth's surface, water evaporates into the atmosphere as well as transpires from vegetation. Up in the atmosphere it condenses and clouds form. Via wind transport, the clouds reach land and the water falls as rain, snow or hail (precipitation). Most evaporation occurs from the ocean (85%), however, only 80% of all rain falls over the ocean, which leads to imbalance (Bogren *et al.*, 2006). To balance the system, gravitationally driven transport brings the water back to the ocean, either by surface runoff, in rivers and streams, or by infiltration of water to the ground, i.e. groundwater formation and transport therein.

es within the subsurface such as biological processes (e.g. Atekwana & Slater, 2009) and groundwater chemical content. Geoelectrical measurements has previous been applied on contaminated sites and can contribute to delimit plume extent and indicate degradation of PCE (see section 3.2.2). In the studies included in this thesis, these methods have been used and analyzed through commercially available methods and state-of-the-art geoelectrical measurements in a new combination. Two field sites have been studied; the first where only natural attenuation has been monitored, and the second where monitoring has been performed at a field site with active *in situ* remediation through two different strategies, a combination of abiotic and biotic reduction, and biotic reduction only.

Table 1. Water distribution on Earth, with estimated numbers from Hiscock & Bense (2014). Marked in blue, are those that are possible to use for drinking water supply within reasonable cost.

Location	%	Volume (x10 ⁶ km ³)
Ocean water	97.25	1370
Ice caps and glaciers	2.06	29
Deep groundwater (>750 m)	0.38	5.3
Shallow groundwater (<750m)	0.30	4.2
Lakes	0.01	0.125
Soil moisture	0.005	0.065
Atmosphere*	0.001	0.013
Rivers	0.0001	0.0017
Biosphere	0.00004	0.0006
Total		1408.7

* Given as liquid equivalent of water vapor

2 Scope of thesis

The general aim of this thesis is to investigate subsurface conditions and monitor changes of the groundwater, with a focus on PCE and its metabolites, during both natural and enhanced degradation, with regards to concentration and distribution. The multidisciplinary approach aims to obtain more relevant data and aspects of the physical and biogeochemical characterization of the complex subsurface conditions. One field site was investigated regarding contaminants within groundwater, and a second site regarding contaminants in both groundwater and unconsolidated sediments. The intention of the study of natural degradation was to understand the mechanisms and interpret the data in an undisturbed system, and then apply this knowledge to increase the understanding of the impact of remediation methods. In addition, a laboratory study is included that focus on how biological cells and their metabolic processes affects geophysical signals.

This thesis's specific objectives are the following:

- O1.** To validate ongoing degradation by natural processes and investigate the value of using the combination of all methods.
- O2.** To study how the presence of bacteria growing as biofilm and bacterial activity can influence geoelectrical signals.
- O3.** To study two different remediation strategies by commercially available methods to evaluate the dechlorination, and, if possible, conclude the most suitable approach given the site-specific conditions.
- O4.** To study how the hydrogeological setting influence the diversity of the microbial community and the ability of the community to degrade chlorinated ethenes.

The aims correlate to the following hypotheses:

- H1.** Natural degradation via microbial activity leads to changes in groundwater chemistry and microbial cell density, which will be possible to detect with single resistivity and induced polarization measurements.
- H2.** Changes in geoelectrical measurements can be used to measure the growth of microbial biofilm, which causes clogging of the pores and subsequent changes in the flow of electric current.
- H3.** Abiotic remediation strategies will be faster and

more efficient than biotic remediation strategies, as the microorganisms need more time to establish than physical reduction media.

H4. The sediment grain size will have an impact on where microbial growth is most abundant. Heterogeneities present, especially in the transition zones, provides the most favorable conditions with larger surface areas within the finer material for microbes to attach to, coupled to enhanced transport of nutrients within the coarser material.

3 Background

3.1 Chlorinated solvents

Chlorinated solvents have been used, and some are still in use, in industry as solvents where water-containing products cannot be used. PCE is used as dry-cleaning fluid, and TCE as degreaser in metal industry and previously as dry-cleaning fluid (Pankow & Cherry, 1996). The production of PCE started in Germany in the 19th century, and the components became popular to use during the World War II with an increasing usage the following 30 years (Pankow & Cherry, 1996; Doherty, 2000). In Sweden, PCE was introduced in the 1940s and TCE in 1930s, with some national production (Englöv *et al.*, 2007). PCE found in sediments and groundwater have an anthropogenic origin, while metabolites originate from degraded PCE (e.g., Vogel & McCarty, 1985). However, TCE and vinyl chloride are possible to find as primary contaminants. TCE from old dry-cleaners and metal industry, and vinyl chloride can be found from polyvinyl chloride (PVC) plastics production (Hartmans *et al.*, 1985).

In this thesis, chlorinated solvents are limited to chlorinated aliphatic hydrocarbons (CAH), sometimes referred to as chlorinated ethenes (CE). The components are straight and unsaturated hydrocarbons, containing two carbon molecules with a carbon-carbon double bond and have chloride ions substituted for one or several hydrogen ions (Fig. 2). The primary compound is PCE with the formula C_2Cl_4 . This is dechlorinated to TCE with one chloride ion replaced by a hydrogen ion and further systematically losing a chloride ion (Fig. 2), to dichloroethene (DCE), vinyl chloride, and ethene (Pankow & Cherry, 1996; Wiedemeier *et al.*, 1999). DCE can be found in three isomers, where *cis*-1,2-DCE (*cis*-DCE) is the predominant (e.g., Parsons & Lage, 1985).

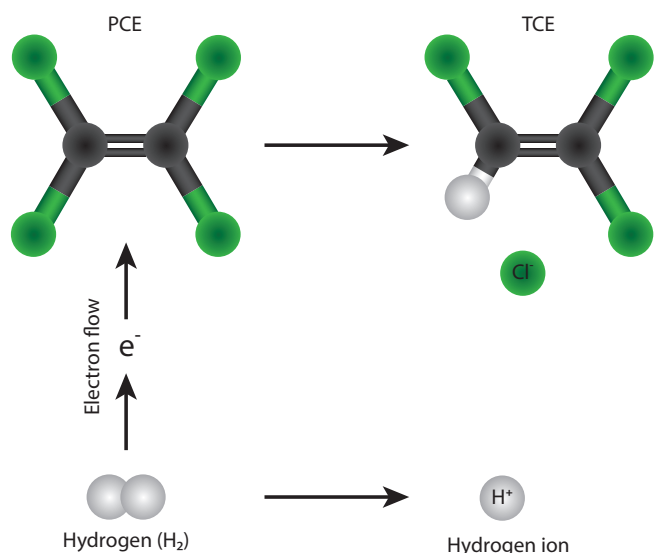


Figure 2. Transport of electrons and its function in a degradation step through hydrogenolysis.

The CAHs are hydrophobic with a relatively low solubility and higher density than water (1.2-1.7 g/cm³); therefore, they are so-called dense non-aqueous phase liquids, DNAPLs. The combination of properties, including low viscosity (Tab. 2), has an immediate effect on distribution and transport in the subsurface, as described in section 3.1.1. Prominent is the CAHs' ability to appear as "free-phase" where residuals of pure compound can be found, and dissolve in very low concentrations (µg/L) (Pankow & Cherry, 1996). Free-phase can be expected when the substance's concentration is 1-5% of its effective solubility in water (SGE, 2011). The CAH can be present in groundwater also as a gas, due to their high volatility (given as vapor pressure, Tab. 2). Commonly the gas phase is present in the unsaturated zone in the subsurface, while vinyl chloride can be found as a gas in the groundwater due to its low boiling point. CAH are difficult to recognize in the field by human senses, as it is odorless and tasteless (Pankow & Cherry, 1996). If the concentration increases, a smell of sweetness can be noticed. The products are most often colorless; however, the color may be effected e.g. by organic substances dissolved in the system

(Fig. 3), or on purpose by a color dye (e.g. Sudan IV).

CAHs are hazardous and toxic substances. Both vinyl chloride and TCE are classified in Group 1 as "carcinogenic to human" (IARC, 2012; IARC, 2014). While PCE is classified in Group 2A as "probably carcinogenic to humans" and there is "sufficient evidence" from animal testing (IARC, 2014). Exposure to vinyl chloride, increases the risk for two types of liver cancer, a rare form (*angiosarcoma* of the liver) and the most common form (*hepatocellular carcinoma*), brain and lung cancers, lymphoma, and leukemia (IARC, 2012). TCE can cause kidney cancer, and positive associations have been found between exposure to TCE and non-Hodgkin lymphoma, and liver cancer. Positive associations have been observed for PCE and cancer of the bladder (IARC, 2014). Exposure to PCE for females can have adverse effects on the reproduction system and pregnancy outcomes with spontaneous abortion, besides birth defects like child with cleft lip and cleft palate (Burdorf *et al.*, 2006). Males exposed can experience negative effects on sperm motility and morphology (Eskenazi *et al.*, 1991). Exposures to the contaminant are via inhalation or drinking water. Due to these risks, guidelines values for drinking water quality have been established in many countries, as well as for indoor air quality. Guideline values for drinking water are given in Tab. 3 for Sweden, EU, Denmark, Canada and WHO.

3.1.1 Behavior in the subsurface

The physical properties of CAHs have a great impact on the compounds' behavior in the subsurface. The groundwater table works as a border of behaviors. Above, in the unsaturated zone CAHs can vaporize due to their high vapor pressure and low solubility, especially vinyl chloride (Barbee, 1994). The vapor compounds either migrate to the atmosphere or may enter into buildings (Barbee, 1994). As the groundwater table fluctuate, compounds in the upper part of the groundwater may "return" to the unsaturated zone.

Table 2. Physical properties of compounds of interest with water as a reference (Pankow & Cherry, 1996; a Schwarzenbach *et al.*, 2003; b Riddick *et al.*, 1985; c Braker *et al.*, 1980; d Daubert *et al.*, 1989; e Chen *et al.*, 2012; f Haynes *et al.*, 2014-2015; g Swindells *et al.*, 1952).

Compounds	Density at 25°C	Solubility	Absolute Viscosity	Vapor pressure	Henry's Law Constant	Boiling point
	g/cm ³	mg/L	cP	mm/Hg	atm·m ³ /mole	°C
PCE	1.63	200	0.90	18.9	1.74*10 ⁻²	121.4
TCE	1.46	1100	0.57	75	9.37*10 ⁻³	86.7
1,1-DCE	1.22	3350	0.36	603	2.55*10 ⁻²	31.9
<i>cis</i> -1,2-DCE	1.28	3500	0.48	205	3.74*10 ⁻³	60
<i>trans</i> -1,2-DCE	1.26	6300	0.40	315	9.16*10 ⁻³	48
vinyl chlorid	0.91a	2700b	0.01c	2980d	2.56*10 ⁻²	-13.8f
water	0.99	n.a.	1.00g	-	-	99.98



Figure 3. Photos from the field work, seeing CAH in free-phase at the first field site (Hagfors), where CAH for unknown reasons is colored dark. A) The tube turned upside down, with free-phase product at the bottom, sediment and dissolved contaminants. B) A “bubble” of PCE marked with the circle and dissolved halo. C) “Bubbles” of free-phase sinking in the bottles (circles) towards the bottom marked with a line.

Table 3. Guideline values for drinking water quality from a selection of countries and organizations. n.v. = no value for guidance is established. For 1,1-DCE and *trans*-1,2-DCE no established guideline value exists (a Swedish Food Agency, 2017; b EU, 1998; c Miljøstyrelsen, 2020; d Health Canada, 2020; e WHO, 2017).

	PCE	TCE	<i>cis</i> -DCE	VC
	µg/L	µg/L	µg/L	µg/L
Sweden (a)	10*		3.0	0.5
EU (b)	10*		3.0	0.5
Denmark (c)	3*		1.0	0.5**
Canada (d)	10	5.0	n.v.	2
WHO (e)	40	20	50	0.3

* The sum of PCE and TCE

** In tap water

The behavior of CAH below the groundwater table depends on the compounds' concentrations and the geological setting. In high concentrations, CAHs are pure liquids (free-phase) and then behave as DNAPLs, which can deeply penetrate the subsurface until they reach an impermeable or low permeability unit (Pankow & Cherry, 1996). In liquid phase the low tension to water allows CAHs enter small fractures and pores, as exist in e.g. clay and sedimentary bedrock (O'Hara *et al.*, 2000; Pankow & Cherry, 1996). However, CAHs are miscible and can be dissolved, hence form a plume. They do no longer behave as DNAPLs, and instead, their behavior becomes similar to the water (Chapman & Parker, 2005). The mobility of CAHs increases with increasing ratio between density and viscosity (Pankow & Cherry, 1996).

In unconsolidated sediments, DNAPLs sink through sediment to a confining unit (Fig. 4) or less permeable layers e.g. crystalline bedrock, where it pools. When DNAPLs reach a clay layer they can crack the mineral structure leading to a significant increase of the unit's hy-

draulic conductivity and hence a rapid breakthrough and migration (Barbee, 1994). The compounds can also get stacked in pores (Fig. 4-1a), occur as bubbles (Fig. 4-1b), or encapsulate groundwater or air (Fig. 4-1c) (Pankow & Cherry, 1996). Minor heterogeneities in the geological setting cause variations in permeability and capillary characteristics that have a particular impact on the compounds movement (Poulsen & Kueper, 1992), greater than on groundwater flow. Field site studies has shown that DNAPLs formed many layers due to minor heterogeneities in what was expected to be a homogenous aquifer (Brewster *et al.*, 1995).

Predominant for sedimentary bedrock (Fig. 4-2) is its dual porosity, both fractures and pores, where the permeability commonly is higher in the fractures. Hence most migration of free-phase DNAPLs occurs in fractures (Pankow & Cherry, 1996). In the fractures, the DNAPLs can dissolve or resume as free-phase (Fig. 4-2a). They can enter the pore system by either advection or diffusion and can occur in both free-phase and in dissolved form (Pankow & Cherry, 1996) (Fig. 4-2b). When the concentrations in the fractures decrease, compounds diffused into the matrix can diffuse back to the fractures as the concentration gradient shifts (Parker *et al.*, 2012). This back diffusion can occur up to several years and decades later, even after the pollution has “disappeared” from the fractures (Parker *et al.*, 2012).

In crystalline bedrock (Fig. 4-3), all migration occurs in the interconnected fractures. The transport is controlled by fracture orientation, also allowing spreading along horizontal fractures. The high density, low solubility in water and low viscosity, allows deep penetration into bedrock fractures (Pankow & Cherry, 1996).

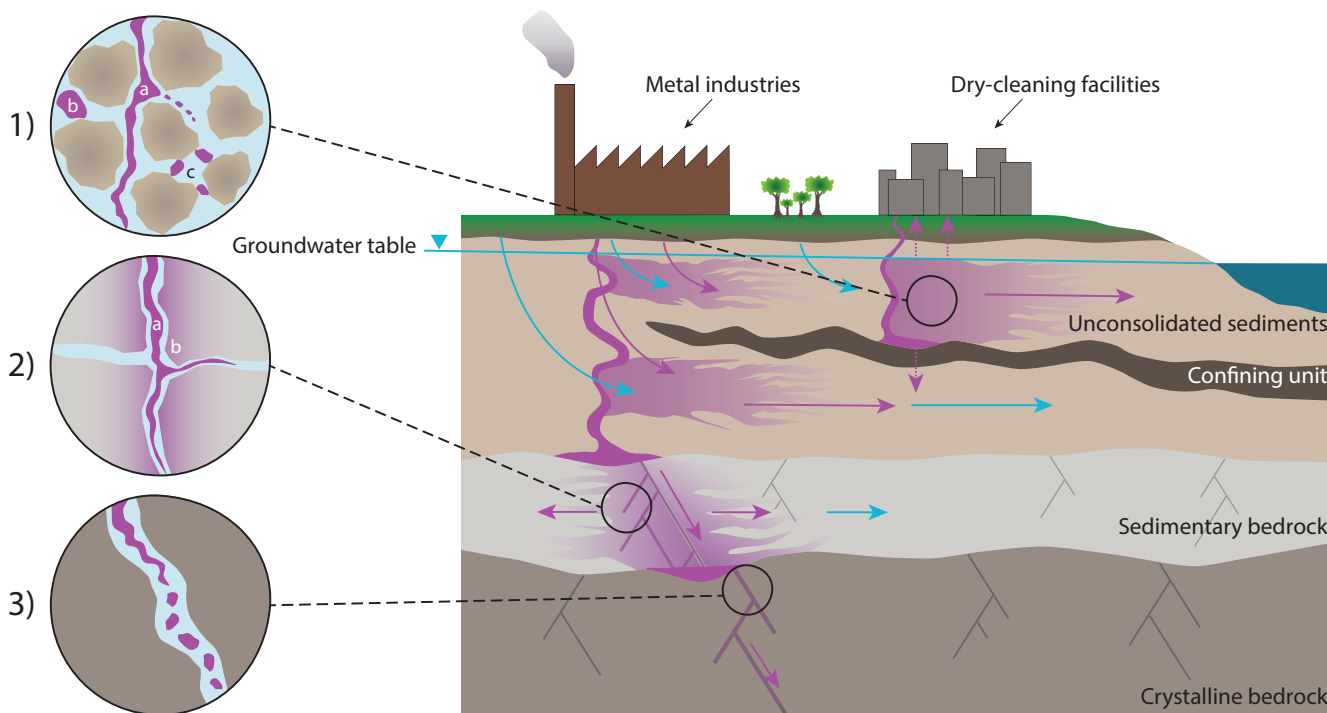


Figure 4. Two theoretical spills of CAH in hypothetical geological setting show the behavior in the subsurface. Most CAHs are denser than water and hydrophobic (DNAPLs), which has a great impact on their transport and behavior in the subsurface. They tend to stay in free-phase, and sink as deep as possible. However, they can dissolve in water and then move via groundwater flow (diffusion and advection). 1) Unconsolidated sediments, a. sinks through, b. as reservoir “bubbles” in the groundwater, c. stacked in pores encapsulated by groundwater (or air if present). 2) Sedimentary bedrock, primary DNAPLs move through the fracture system; movement via the pore system is possible and/or diffusion out off and into the pores. 3) In crystalline bedrock, the migration occurs through the active fracture system. Adapted from: Pankow & Cherry (1996) and Parker *et al.* (2012).

3.1.2 Degradation

The degradation of PCE and its metabolites can occur both via abiotic and biotic processes, via reduction or oxidation and when biotically also as a co-metabolic process (Wiedemeier *et al.*, 1999; Stroo & Ward, 2010). This thesis has exclusively focused on degradation via reductive dechlorination as suggested to be the predominant process to degrade CAHs (Wiedemeier *et al.*, 1999) and the following description excludes other processes.

Both abiotic and biotic processes can occur in natural systems; however, the biotic process is faster than the abiotic (Dong *et al.*, 2009; Tobiszewski & Namieśnik, 2012). The degradation follows specific pathways and depends on possible reactions (Vogel *et al.*, 1987). Most common mechanisms are hydrogenolysis and reductive abiotic β -elimination (Fig. 5) (Arnold & Roberts, 2000; Gillham *et al.*, 2010). The biotic process occurs through hydrogenolysis, which is the most frequent studied pathway. The abiotic process is more efficient during iron and sulfate reducing conditions in the aquifer, leading to high concentrations of solid Fe(II) and S(-II), which suggest that these minerals contribute to the abiotic reductive dechlorination (Dong *et al.*, 2009). Both degradation products in a β -elimination, dichloroacetylene and chlo-

roacetylene, are highly unstable and further degradation occurs rapidly (Gillham *et al.*, 2010). Naturally occurring electron donors are needed in both pathways.

Reductive dechlorination via hydrogenolysis occurs with the CAH as the electron acceptor and the chloride ion as the electron donor (Holliger & Schumacher, 1994) (Fig. 5). The electron is donated from hydrogen that is produced through fermentation of organic compounds by microbes in the subsurface (Wiedemeier *et al.*, 1999). The reaction is thermodynamically favorable and exergonic. From PCE to DCE, the available free energy (ΔG°) is -189 kJ/mol of hydrogen. The energy is utilized by microbes and is coupled to the ATP synthesis (Holliger *et al.*, 1999). The possible supply of hydrogen is a limiting factor for degradation to occur. Other required condition for reductive dechlorination is that it is anaerobic, and that the redox potential is low; i.e. oxygen (O_2) and nitrate (NO_3^-) have to be depleted, as they are higher energy acceptors than CAHs and will inhibit the process (Wiedemeier *et al.*, 1999). Additionally, to prevent competition of the available energy sulfate should be reduced in the aquifer (Wiedemeier *et al.*, 1999; US EPA, 1998). It is common that degradation stalls at the *cis*-DCE or vinyl chloride step (Bradley, 2000; Stroo & Ward, 2010) due to lack of electron donors in the aquifer (Blázquez-

Pallí *et al.*, 2019) or absents of appropriate microorganisms who can further progress the dechlorination.

Bacteria are needed for the biotic reductive dechlorination process, where different species perform separate or several degradations steps. The only bacteria known to completely dechlorinate PCE to ethene is *Dehalococcoides mccartyi* (Maymó-Gatell *et al.*, 2001; He *et al.*, 2003a) and include strain 195 (Maymó-Gatell *et al.*, 1999), or strain BTF08 (Pöritz *et al.*, 2013). Other *Dehalococcoides* spp. are known to participate in different steps in the dechlorination (Sung *et al.*, 2006a; Stroo *et al.*, 2013). To avoid *cis*-DCE or VC stall and complete the dechlorination, the species should include e.g. strain BAV1 (He *et al.*, 2003b), strain VS (Cupples *et al.*, 2003), or strain GT (Sung *et al.*, 2006a). Vitamin B₁₂ has been shown to be a limiting factor for *D. mccartyi* to complete dechlorination and they cannot synthesize it themselves (He *et al.*, 2007; Yan *et al.*, 2021). However, *D. maccartyi* was more efficient in performing dechlorination while living in a consortium that included bacteria producing B₁₂, compared to adding B₁₂ (He *et al.*, 2007). In addition, other microorganisms known to take part in the degradation, with dechlorination often stalled at *cis*-DCE, are *Geobacter lovleyi* (Sung *et al.*, 2006b), *Desulfotobacterium* sp. (Miller *et al.*, 1997), *Desulfotobacterium hafniense* (Gerritse *et al.*, 1999; Suyma *et al.*, 2001), and *Sulfurospirillum multivorans* (Stroo *et al.*, 2013).

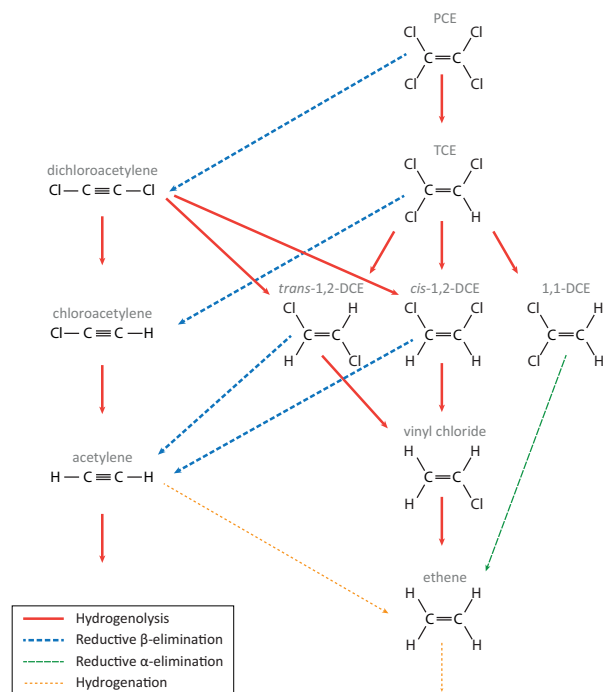


Figure 5. Possible degradations pathways for reductive conditions. Red lines indicate hydrogenolysis and blue lines β-elimination. The green and yellow lines are considered as negligible (Arnold & Roberts, 2000; Gillham *et al.*, 2010).

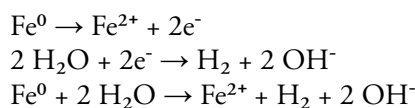
3.1.3 Remediation

Natural degradation is a slow process, and the anticipated time may be exceeded (Wiedemeier *et al.*, 1999), therefore active remediation might be considered. “Dig and dump” have historically been a trusted approach and more used than enhanced *in situ* remediation actions. During the 1990s, the number of publications rapidly increased (Stroo *et al.*, 2013), and since 2014 *in situ* remediations have been emphasized by the Swedish government as being more sustainable (S EPA, 2014). Enhanced *in situ* reductive dechlorination remediation is often performed by direct push injections. Possible approaches are to use a reducing substance e.g. Zero-Valent Iron (ZVI), or to stimulate existing biological processes and if needed, augmentation of organisms (i.e. bioaugmentation). In this project, all three approaches have been studied.

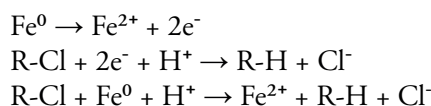
Before designing an enhanced biotic dehalogenation, an initial investigation and evaluation of the biotic system is needed to optimize the remediation. Biostimulation requires naturally occurring dehalogenating bacteria to be present in order to be stimulated. The aim with the injected substances is to change the conditions, e.g. drive towards more reducing conditions, and/or provide the system with organic carbon that can be utilized as additional electron donors. If dehalogenating bacteria are absent or in too low concentrations, bioaugmentation is an alternative. There are available products with mixed cultures known to complete dechlorination of PCE to ethene, e.g. KB1[®], and SDC-9[™] (Stroo *et al.*, 2013).

ZVI is a strong reducing agent and can thermodynamically reduce most CAHs, with a standard reduction potential of -0.44V (Cwiertny & Scherer, 2010). Introduction of ZVI (Fe⁰) in CAH contaminated water results in two redox reactions; firstly a reduction of the water and oxidation of the iron (Eq. 1), and secondly a reduction of the CAH (R-Cl) and an oxidation of the iron (Eq. 2) (Gillham *et al.*, 2010).

Equation 1.



Equation 2.



Degradation of PCE with ZVI may lead to degradation through β-elimination, which could explain why lower chlorinated ethenes are not reported when ZVI is used as the reduction agent (Arnold & Roberts, 2000; Gillham *et al.*, 2010).

3.2 Motivation of used methods

To investigate natural and enhanced degradation beyond the study of conventional concentrations of CAH and groundwater chemistry, the following methods have been used: Compound Specific Isotope Analysis (CSIA), microbial investigations using quantitative Polymerase Chain Reaction (PCR) and community profiling with amplicon gene sequencing; and, geoelectrical measurements.

3.2.1 CSIA

When PCE and its metabolites degrade, it leads to a fractionation of stable carbon isotopes. The bond between ^{12}C -Cl is weaker compared to the ^{13}C -Cl bond, resulting in a faster reaction when the weaker bond is involved. This leads to an enrichment of ^{13}C in the remaining compound and a depletion in the metabolite (Hunkeler *et al.*, 1999). The effect is largest when biodegradation occurs in contrast to abiotic non-degrading processes (Hunkeler *et al.*, 2008). By applying CSIA, other non-degrading processes that change the concentrations, e.g., dispersion and advection can be evaluated, which is complicated with only CAH concentrations. When the $\delta^{13}\text{C}$ is $\geq -20\text{‰}$, biodegradation is proved to occur (Kuntze *et al.*, 2019), and an enriched by $>2\text{‰}$ is considered a significant change (Hunkeler *et al.*, 2008). Dual element isotope analysis, i.e., using both $^{13}\text{C}/^{12}\text{C}$ and $^{37}\text{Cl}/^{35}\text{Cl}$, are found in the literature (e.g. Shoukar-Stash *et al.*, 2003; Cretnik *et al.*, 2013) giving further detailed information on degradation pathways (Cretnik *et al.*, 2013), however this is not used within this project. The CSIA analysis typically requires more sample material than conventional analyses, sometimes hundreds of $\mu\text{g}/\text{L}$ for reproducible $\delta^{13}\text{C}$ values (Palau *et al.*, 2007). The results are expressed, to ensure comparability and accuracy, relative to the international standard Vienna PeeDee Belemnite, with a known $^{13}\text{C}/^{12}\text{C}$ ratio (Eq. 3) (Gonfiantini *et al.*, 1995). The results are given as parts per mil (Hunkeler *et al.*, 2008).

Equation 3.

$$\delta^{13}\text{C}(\text{‰}) = \left(\frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}} \right) \times 1000$$

3.2.2 Microbial characterization

Since microorganisms carry out biotic degradation, knowledge of the microbial communities and the presence of specific species or genera, e.g., *Dehalococcoides*, are of interest to study. To evaluate this from the field sites, DNA-based methods can be used, e.g., PCR and amplicon sequencing. DNA-based approaches do not require culturing in laboratory, which can be difficult as microbes commonly lives in consortium in natural settings (e.g., He *et al.*, 2007). Instead, DNA-based methods assess the abundance and identity of the microbes in their natural setting. PCR assesses specific known targets, while amplicon sequencing gives an overview of the relative abundance of different microbes, from genus level and higher (i.e., phylum, and family). Both approaches use the unique combination of nucleotides in the DNA molecules, which describes the DNA molecule; hence, the specific characteristics of these sequences can reveal detailed information including the identity of the microbes or activities of the microbes (Berg *et al.*, 2006). To study the DNA, the 16S rRNA gene is of interest as it is highly conserved through evolution within the phylum (Woses & Fox, 1977).

In this thesis, only DNA sequences that identify the type or identity of the bacteria have been used and their activity has been inferred from what is known from literature about the genus or phyla that have been identified. In addition, as amplicon sequencing describes the entire microbial community and does not only report on targeted organisms (e.g., *D. mccartyi*) it was thought that this approach could provide new insights into any cooperative biological processes participating in the degradation, since few organisms are able to fully dechlorinate these compounds (Stroo *et al.*, 2013; Dolinová *et al.*, 2017).

3.2.3 Geoelectrical measurements

Geoelectrical measurements are used to capture the electrical properties of a subsurface or a material. By applying an electrical current, the resulting voltage (potential) difference can be measured and information about the resistivity respectively, the resistivity distribution of the subsurface can be gathered. As a field investigation, it is a nonintrusive tool to obtain an overview of the spatial electrical properties due to the physical and biogeochemical properties dependent on the subsurface conditions. Previous studies have shown that the geoelectrical measurement methods can locate CAHs in the subsurface (e.g. Ajo-Franklin *et al.*, 2006; Cardarelli & Di Filippo, 2009; Power *et al.*, 2014), that it is possible to visualize the results in 3D (Johansson *et al.*, 2015), and to monitor changes with time as a fourth dimension (Åkesson, 2015; Sparrenbom *et al.*, 2017; Nivorlis *et al.*,

2019). From studies in biochemistry, it is shown that microbial communities may influence the aquifer regarding both chemical and physical properties due to growth and metabolism (Atekwana *et al.*, 2004; Prodan *et al.*, 2004; Flores Orozco *et al.*, 2011; Flores Orozco *et al.*, 2012). In addition, bacterial cells may become polarized and are commonly negatively charged, which affects the electrical response due to the development of an electrical double layer (Abdel Aal *et al.*, 2006; Atekwana & Slater, 2009; Kessouri *et al.*, 2019). Growth of biomass may also lead to clogging of pores, hence the flow paths for liquids and the electrical current may be affected by this (Brovelli *et al.*, 2009). Geoelectrical studies of landfills have shown the possibility to monitor gas migration, which is a co-product in the degradation process by bacteria (Rosqvist *et al.*, 2011; Auken *et al.*, 2014). To understand the influence of bacteria, their by-products and the formation of biofilm on the geoelectrical properties under the investigated conditions, detailed laboratory studies were also performed (Paper II).

In this project, two different types of measurements have been performed. In field, the Direct Current resistivity and time-domain Induced Polarization tomography (DCIP) were used and, in the laboratory, the frequency-domain Spectral induced polarization (SIP) method was used. Both methods measure the material dependent parameter, electrical resistivity ρ , that quantifies how strongly the given material resists the flow of an electrical current, (unit Ωm). The induced polarization (IP) identifies the electrical chargeability m (unit mV/V or msec) in time-domain (Sharma, 1997), or as phase shift φ (unit: mrad or $^\circ$) in frequency-domain. In time-domain, a rectangular current signal is used whereas in frequency-domain sinus waves are applied. By using a variety of different frequencies in frequency-domain, the spectral behaviour of the resistivity and phase signal can be observed. From these spectra additional information about e.g., mineral grain size and concentration (Pelton *et al.*, 1978), permeability and porosity (e.g., Börner *et al.*, 1996, Kemna *et al.*, 2012) can be obtained.

In general, geoelectrical data is obtained through a quadruple of electrodes, two for current injection and two for measuring the potential response. With modern DCIP measurements, a multi-electrode set up can be used where several electrodes are located in a line, with the instrument having an embedded relay switch to controls the electrodes included in the quadruple via a pre-programmed protocol. By running the measurements and varying the quadruple, a pseudo section of the measured and calculated apparent resistivities (ρ_a) is obtained in both horizontal and vertical directions. The calculation of the “true resistivity” (ρ) is done by an inversion process where a model is found that can explain the measured data at the best fit due to successive improvements of a start model.

3.3 Field sites

Two field sites have been investigated within this project, Hagforstvädden and Tvätteriet Alingsås. Both sites are located in Sweden (Fig. 6) and have previously been dry cleaning facilities where PCE was used as the cleaning agent, and today remain contaminated. The Hagfors site was studied in the context of natural attenuation and the Alingsås site for in situ remediations. The geology differs between the two locations; where the Hagfors site has coarser material at the top of the subsurface overlying a silty till aquitard, while the Alingsås site has a thicker clay layer on top which holds the contamination and an underlying sandy aquifer. At both sites contamination has been found in all types of unconsolidated sediments.



Figure 6. Locations of field sites in Sweden are marked in capital letter.

3.2.1 Hagforstvädden

The dry-cleaning activity in Hagfors started in 1970 and served the Swedish Armed Forces the first decade. It operated as dry-cleaning facility until 1993 for the county and private companies. The facility took care of more than 1000 kg laundry/day (Nilsen, 2013), but the PCE volume used is undocumented, as well as any knowledge about potential volume of lost into nature. Nilsen (2013) made an estimation of the latter by estimating the amount of laundry, returned products, deposited fluff with absorbed PCE and indoor air measurements, ending up with a likely loss of 1000 tons PCE during the whole operation. The estimation states that 5% of the lost PCE has reached the ground, which means 50 tons, equivalent to 83,100 L.

Dominating the area is the large variation in elevation, due to both a construction for a railway and a naturally occurring creek with a meandering stream, Örbäcken. Fill material with an unknown content has been used to

form a large plateau for the railway tracks. The tracks have been removed; however, the remaining fill material likely includes metal since disturbance in DCIP measurements have been observed (Jönsson & Wiberg, 2017). Several boreholes have been drilled with installation of monitoring wells to investigate the contamination and the geology (Nilsen, 2013; Larsson *et al.*, 2017). This study focused at the point bar that is located close to the first outflow area of the plume to the stream (Fig. 7). The area's contamination origin is confirmed to be from the secondary source zone, close to well B19, which was formed due to leakage from a stormwater pipe that starts at the primary source. (Nilsen, 2013; Larsson *et al.*, 2017). The site's geology is very complex regarding unconsolidated deposits, from both glacial and postglacial periods. The natural sediments are divided into three units; the bedrock is overlaid with a less permeable silt dominated till. This is followed by a permeable transition unit of sand and gravel, before the top unit, dominated by sand and silt, which has a higher permeability than the till unit but less than the transition unit (Larsson *et al.*, 2017). The groundwater has the highest pressure at the bottom of the till, less pressure at the top of the till and the lowest in the sand layer. The depth of unconsolidated sediments varies between 15-25 m in the area, with the largest depths under the plateau (Larsson *et al.*, 2017).

One fieldwork at the point bar was conducted in April 2017, where five DCIP lines were measured as well as water samples taken from nine filter screens including one representing the source zone (B19) and one as a natural background (B29). The groundwater was analyzed for chemistry, CSIA, and microbes (Paper I).

3.2.2 Tvätteriet Alingsås

The operation started 1963 at the Tvätteriet Alingsås and is ongoing, but the use of PCE stopped in 2014 (pers. com. Charlotta Olsson, Tvätteriet Alingsås). An accident in the 1960-1970s resulted to a spill estimated to a volume of up to 200L of PCE located close to well B2 (Cedhagen, 2002; Haglund & Larsson, 2010) (Fig. 8).

The ground surface is dominated by asphalt with an underlying fill material, approx. 0.5m thick. Below the fill, the natural geology consists of a thick layer of heterogeneous clay with interbedded coarser material. The unit has a thickness of approx. 2-3 m in the southern part of the estate and increases towards the north (approx. 5-6 m). The clay layer is deposited on a layer of sand, situated on top of the crystalline bedrock (Branzén *et al.*, 2016; Balzarini & Van Herreweghe, 2017; Davidsson *et al.*, 2020; Ahlberg, 2017). Most PCE is trapped in the clay layer, however there is a plume migrating towards a recipient to NW. The concentrations of contaminants in the clay could either be explained by: 1) the clay has pre-

vented a migration of DNAPLs into the sand, or 2) the higher hydraulic conductivity in the sand layer results in an outwash of available PCE.

The site has been targeted twice for *in situ* pilot remediation tests, in 2012-2014 and 2017. The first test was located in Area C (Fig. 8), perpendicular to the plume migration, and was a biostimulation followed by a bioaugmentation due to the low impact of the biostimulation (Branzén *et al.*, 2016). The second test took place in Area A and Area B to evaluate most effective strategy. Area A was injected with Zero-Valent Iron (ZVI), granular activated carbon and microbes, and Area B was treated with a combination of biostimulation and bioaugmentation (Ølund, 2017).

Baseline measurements for groundwater chemistry, CSIA and microbial data were conducted prior to the second pilot test remediation, followed by annual measurements in October. In parallel, a control program was established by the Geological Survey of Sweden to monitor the chemical changes.

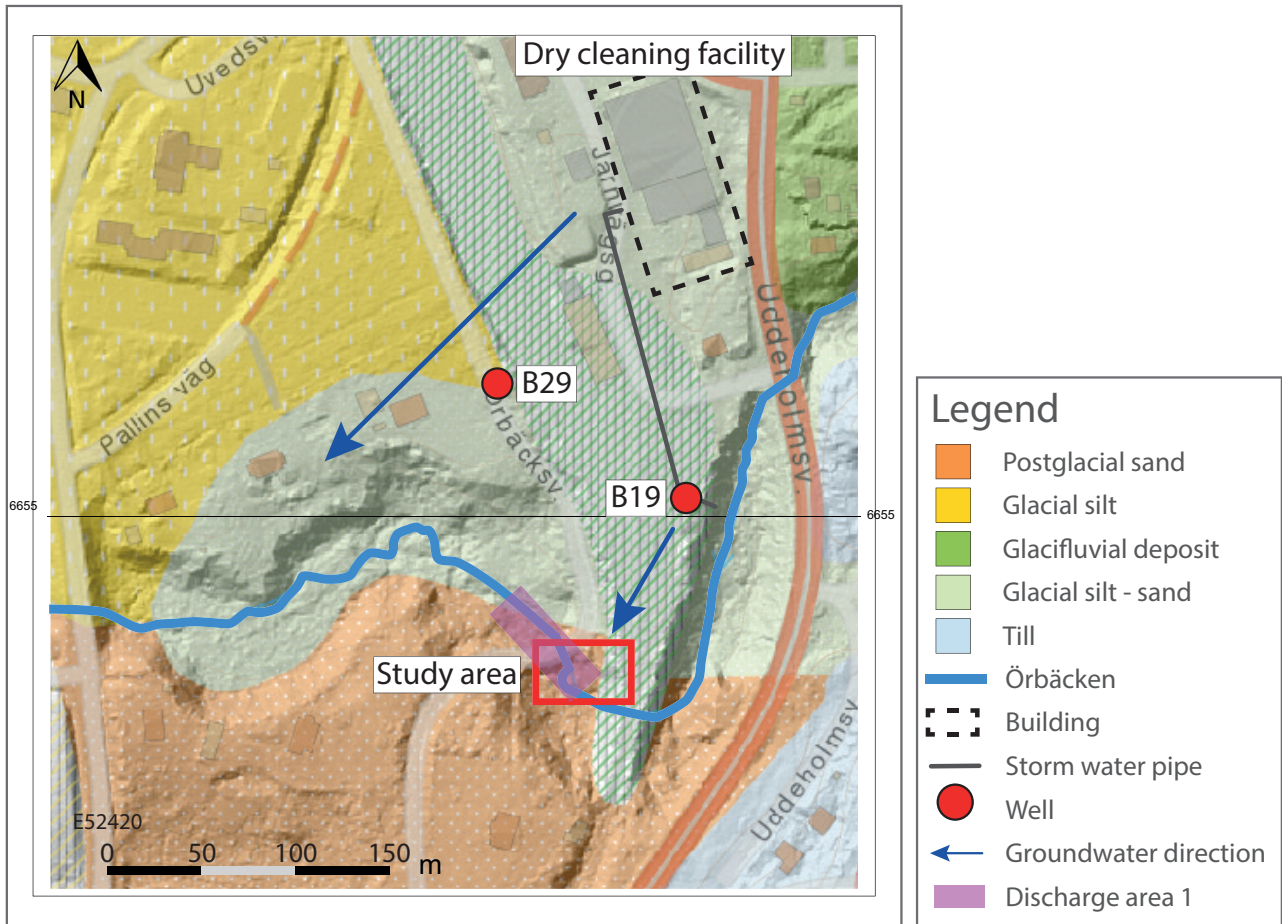


Figure 7. Map of unconsolidated sediment at the Hagfors site with hill shading to visualize the elevation in the area. The checked pattern refers to fill material, and the color indicates the underlying natural material. Modified from ©Geological Survey of Sweden (Åkesson *et al.*, 2021).

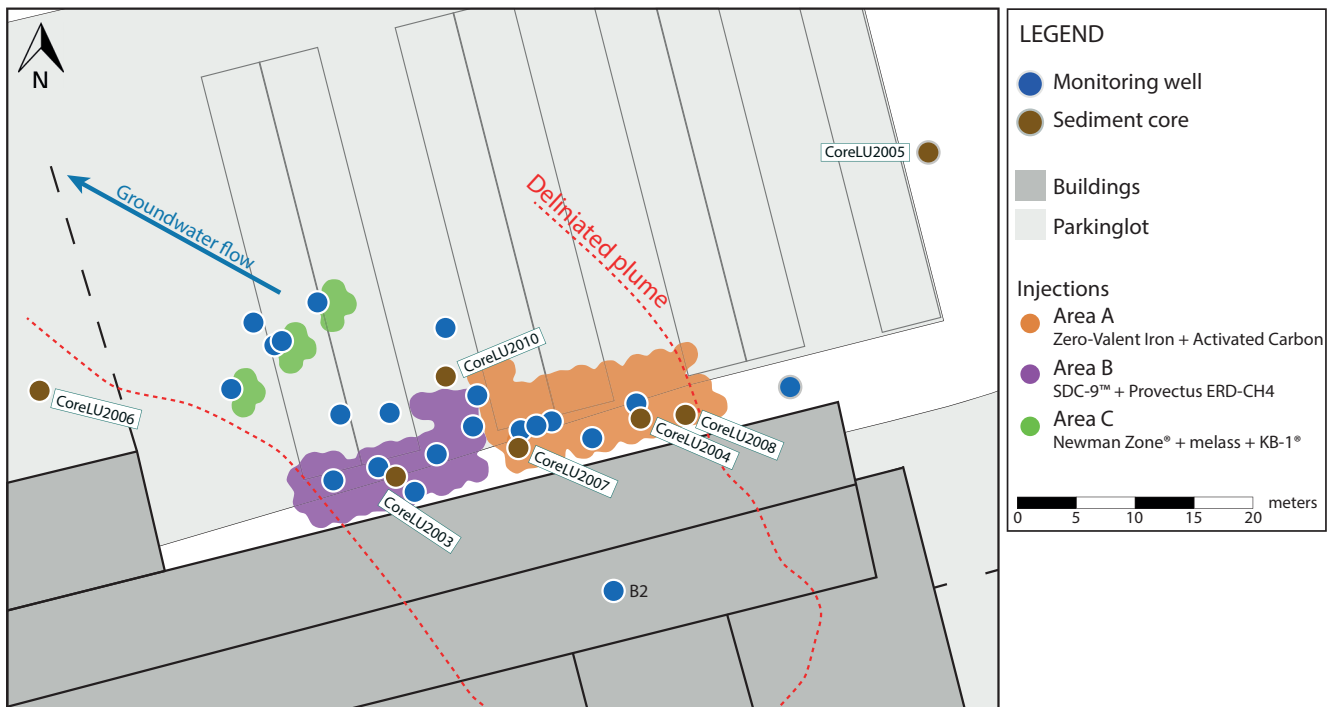


Figure 8. An overview map of Alingsåstväterriet, which shows the location of monitoring wells and sediment core locations, the areas where injections have been made, and the delineation of the plume via MIP soundings in 2017 (dashed red line).

4 Methods

4.1 Field data collection

To collect groundwater samples, an Eijkelkap Peristaltic Pump was used for low flow sampling aiming to acquire representative data from the aquifer, and to avoid cavitation and minimize degassing (Papers I and III). The monitoring wells were pre-purged in combination with the monitoring of physical properties (e.g., redox potential, pH, dissolved oxygen, and electrical conductivity) until stable readings were achieved (Fig. 9A). The groundwater samples were then taken in the following order: CSIA, water chemistry, and microbiological samples. The CSIA samples were taken in 1L amber glass bottles with PTFE lined caps. The water was allowed to overflow followed by adding 10 mL of hydrochloric acid (19%) to the bottle for preservation (Hunkeler *et al.*, 2008). The laboratory Isodetect GmbH, Leipzig/Germany, performed the CSIA analysis (section 4.3). The groundwater samples for chemical analysis were collected in bottles provided by the laboratory SYNLAB AB, Sweden, that performed the analysis. The microbial samples were collected by filtering water through a 0.22 μm Isopore filter until it clogged, with suction from the Peristaltic Pump (Fig. 9B-E). The filter was placed in a Petri glass dish on dry ice for instant freezing, and kept frozen (-20°C or colder) until DNA extraction.

Sediment material was collected for Paper IV from cores obtained with Sonic drilling technique. The undisturbed cores were lithologically logged in field, photographed, and sampled for e.g. contaminants, grain size analysis and microbial community. The samples for contaminant analyzes were taken in amber glass bottles provided by the laboratory SYNLAB AB (Sweden), and microbial samples in glass tubes and initially put on dry ice and thereafter kept frozen (-20°C) until DNA extraction. The lithological grain size description from the field agreed well with the grain size analysis results received from SYNLAB B.V (Netherlands). As the field descriptions were more detailed, it was decided to use these as better data for further interpretations.

4.2 Microbiological analysis

The microbiological samples have been investigated through quantitative PCR (qPCR), or amplicon sequencing of the 16S rRNA gene. DNA data from qPCR are included in Papers I and II, while amplicon sequencing data are included in Papers III and IV. The change of analysis method was decided after problems with the PCR process of the data in Paper III, likely due to inhibition. Samples analyzed in Papers III and IV are from the same site but different matrices (groundwater and unconsolidated sediment), and by using the amplicon sequencing approach for both of these samples sets, the downstream data analysis was identical, and included the possibility to compare the bacterial communities existing in the two matrices.

The DNA was extracted from the archived filter papers or sediment, with the FastDNA™ Spin Kit for soil (MP Biomedicals, USA) according to manufacturer's instruction, except the bead beating time for the sediment samples (4x according to the protocol, following Albersten *et al.*, [2015]). The extraction kit has been shown to be beneficial for extraction also from materials other than soil, such as drinking water and eDNA (e.g. Lührig *et al.*, 2015; Eichmiller *et al.*, 2016) and is the suggested kit for DNA extraction from challenging matrices (Albersten *et al.*, 2015).

To obtain specific molecules of DNA in concentrations that are possible to study it needs to be amplified through PCR. Instead of the complete DNA, a selected part is amplified, referred to as the target DNA. In this thesis, the 16S rRNA gene was chosen as the target for quantifying the total amount of bacteria (Papers I and II), specifically targeting *D. mccartyi* (Paper I) and as the amplicon target for Papers III and IV. The 16S gene encodes a ribosomal subunit important for protein synthesis and, due to this central function, it is ubiquitous among bacteria. PCR primers targeting different sections of the gene that are specific for certain species, and conversely capture all species as universal primers, are well described. To search for total amount of bacteria description by Nadkarni *et al.* (2002) was followed. To search specifically for *D. mccartyi* (Paper I) primers specific for this species 16S rRNA gene (He *et al.*, 2003a) as well as a linearized control plasmid, containing gene pBAV1 were used (provided by Frank Löffler, University of Tennessee).

The qPCR was performed in the thermal cycler Roche LightCycler 2.0. The amplification of the DNA target occurs by during 25-45 cycles with three major steps:

- 1) Denaturation of the double stranded DNA into two strands by heating the sample ($94-96^{\circ}\text{C}$).
- 2) Then two primers (one for each strand, and specific for the desired target) anneals to the strand where the



Figure 9. Photos from fieldwork in Alingsås. A) The set-up for the pre-purge process in combination with monitoring of physical properties. B) The sampling set-up during microbial sampling. C) Filtering water from MW4 in 2018, grey colored. D) MW4 with filtered water, rust colored likely due to oxidation of iron coming from Area A. E) Filtering water from Area B in 2018, a thick and dark liquid.

target DNA starts at each strand (3' ends). The temperature is lowered to c. 45-60°C, depending on the primer.

3) The last step is the extension of the DNA (at a temperature of 72°C), where the free nucleotides (dNTPs) make double stranded DNA with help of the polymerase that attaches to the original single stranded DNA, (Berg *et al.*, 2006).

The results can either be evaluated by electrophoresis using an agarose gel to visualize the DNA and determine if the product is of the expected size, relative to the positive control (conventional PCR, Berg *et al.*, 2006), or

quantified by using qPCR with a light producing dye and comparing to a parallel experiment using known concentrations of the target DNA. In these studies the control DNA used was either a dilution serial of isolated genomic DNA from *Escherichia coli* (DSM1116) to quantify total number of bacteria (Papers I and II), or pBAV1 in a dilution series for quantification of gene copies in *D. mccartyi* (Paper I). The concentrations of the positive controls were measured using a Qubit Fluorescence 3.0 (ThermoFischer). The advantage of the parallel runs is to control the reactions' progress and verify that they run correctly, as well as to allow for absolute gene copy quan-

tification by comparing the results from the control dilutions to those of the queried samples. MilliQ water used as a negative control.

The 16S rRNA gene has defined regions for sequencing, and for the amplicon sequencing experiments, the region V3-V4 was used. Brandt and Albertsen (2018) recommended the region, since it offers a broad overview of the microbial community. The sequencing is performed on the PCR products, or amplicons, after DNA is extracted from the samples and the target region of the 16S rRNA gene is amplified through PCR using specific primers followed by an additional PCR amplification to add sequencing adapters. The resulting amplicons are DNA sequenced, the data is trimmed and quality filtered, and then divided into clusters based on the similarity of their DNA sequences (either Operational Taxonomic Units [OTUs] or Amplicon Sequence Variant [ASVs]). The number of sequences in each group are then compared to each other and between samples as a percentage of the total sequences. This is known as relative abundance and all sequences from one sample represent 100%. In this way, the changing proportions of different types of bacteria in the community can be assessed. In addition to comparing the proportions, the DNA sequence from each cluster is compared to a reference database to correlate taxonomy and identify which group of organisms the sequences may have originated from. DNASense ApS, (Denmark) performed the sequencing and some of the analysis.

4.3 Quantification of biodegradation via CSIA

The CSIA analysis included in Paper I and Paper III were performed by Isodetect GmbH (Germany), and follows the US EPA guidelines (Hunkeler *et al.*, 2008). The CSIA analyses were performed using a hyphenated special instrument for gas-chromatography isotope-ratio mass spectrometry (GC-IRMS). The process starts with the separation of individual compounds using gas chromatography. A high-temperature reactor (1030-1050°C) converts the molecules quantitatively to CO₂, with H₂O produced due to combustion and being removed through a Nafion membrane (Merritt *et al.*, 1995; Hunkeler *et al.*, 2008). Then, the produced CO₂ pulse that was derived from the target molecule is introduced into the mass spectrometer and ionized. In the Isotope Ratio Mass Spectrometer, the isotopologue ions (¹²CO₂ and ¹³CO₂) are separated via different mass-to-charge ratio in space. This allows the simultaneous measurement of the ions with fixed Faraday cups. The natural abundance level of the stable isotopes requires this simultaneous measurement to achieve high precision in CSIA (Hunkeler *et al.*, 2008).

4.4 Geoelectrical measurements and analysis

The DCIP data (Paper I) were acquired by using an ABEM Terrameter LS2 instrument which works in time-domain and injects current for a specific pulse length. The measured profiles length were 20 m with a small electrode spacing ($a = 0.5$ m) to focus on high spatial resolution of the shallow subsurface. Separated current and potential cables were used to minimize electromagnetic disturbances and to optimize the data quality (Dahlin & Leroux, 2012). All electrode positions were determined using a Global Navigation Satellite System (GNSS). Afterwards, the field data was processed with the software Aarhus Workbench (v. 5.5.0.0). The inversion of the data was done with the constant phase parametrization after Fian-daca *et al.* (2012).

The IP data for Paper II was measured in frequency-domain at frequencies between 1 mHz and 20 kHz. The used measurement device was a PSIP instrument (Ontash & Ermac, 2018), and as sample holder a cylindrical 4-point measuring cell was used with stainless steel plate current electrodes at the outer sides and silver wire ring electrodes to measure the potential at the middle parts (Fig. 11; see also Kruschwitz, 2008).

5 Results

This thesis is based on the four appended papers, and the following are summaries of each paper. The contribution of the authors to each paper is listed in Tab. 4 below.

Paper I: Characterizing natural degradation of tetrachloroethene (PCE) using a multidisciplinary approach

Åkesson, S., Sparrenbom, C.J., Paul, C.J., Jansson, R. & Holmstrand, H. (2021)

Ambio, 5(50), p. 1074–1088.

In this paper, natural attenuation of PCE was investigated at the Hagfors site, in the area where the second contaminant plume enters the surface water recipient. The investigation included the following methods: groundwater chemistry, CSIA, quantification of bacteria through

quantitative PCR, and DCIP measurements. The aims of the study were to confirm biodegradation by natural processes, and to evaluate the use of the combination of methods for monitoring degradation in situ.

All chemical analysis from all the groundwater samples taken within this study showed metabolites of PCE. Larger concentration of TCE than of PCE was found in an area where also the most positive $\delta^{13}\text{C}$ value and the large amounts of bacteria were found, *D. mccartyi* bacteria were detected, and where DCIP signals showed anomalies. These findings together could only be explained as being a result of ongoing biodegradation. This area was located within a transition unit, i.e., the boundary between finer and coarser grained material, where the finer grained material has a low hydraulic conductivity compared to the coarser grained high-flow unit. The explanation is that the low permeable unit allowed minor pooling on top of it, diffusion into the unit, and a large surface area for the attachment and growth of a microbial consortium (Fig. 10). The higher hydraulic conductivity in the coarser grained unit could supply the consortia with essential nutrients. This together suggests that this type of hydrogeological setting is preferable for biodegradation. These findings could improve the design of biotic in situ remediation and monitoring thereof. With this combination of measurement techniques, a deeper understanding of the processes at this complex site was gained, including a more nuanced and robust interpretation.

Paper II: Examining biofilm growth and metabolism of *Escherichia coli* using geophysical Induced Polarization measurements

Martin, T., Åkesson, S. & Paul, C.

Submitted to *Journal of Geophysical Research – Biogeosciences*

In this paper, the impact on spectral induced polarization (SIP) signals from growth of *E. coli* in biofilm, and possible metabolites, were investigated in a controlled laboratory experiment. In the first step, three test set-ups were conducted i) sand without any bacteria present (Ottawa sand wetted with salinated Milli-Q water), ii) sand with nutrients for bacteria that might exist in the sand (Ottawa sand and Luria Bertani [LB] media), iii) and sand with nutrients and inoculated with *E. coli* bacteria (Ottawa sand, LB media, and *E. coli*). These were observed over a period of 21 days. In a fourth test series, the trial with the *E. coli* were repeated, but focussed on the first 8 days to verify the measurements. An additional test, with the double amount of the initial inoculation of bacteria and a higher shaking velocity to stimulate biofilm development, was performed to evaluate these specific factors on the SIP results.

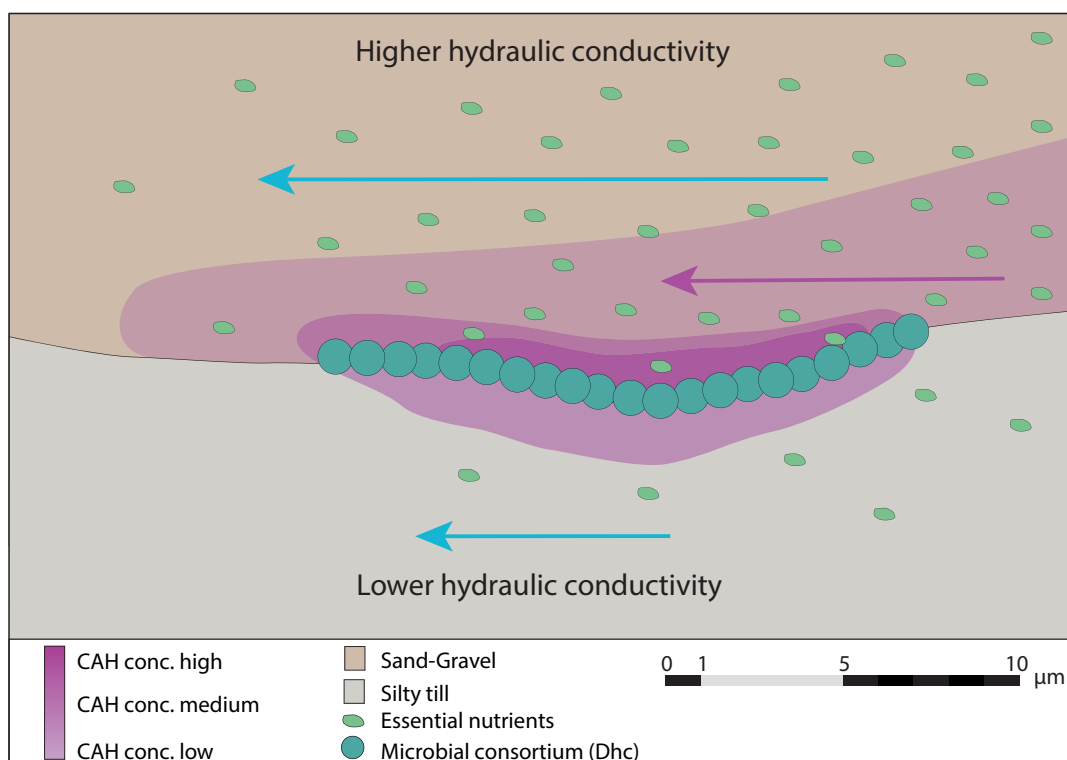


Figure 10. The conclusion from Paper I was that the geology has an impact on the degradation. The transition from higher to lower hydraulic conductivity is the optimal environment. In the case of the Hagfors site, the unit with lower hydraulic conductivity is a silty till that, due to its large grain surface area, is preferable for the bacterial consortium to attach to and form a biofilm. The contamination may pool up on top of the silty till or diffuse into the unit. The unit above, here sand and gravel, can be beneficial for bacterial growth by providing nutrients, both CAHs in the plume and other essential substances.

The samples were measured with SIP to acquire the geo-electrical signal response both for the resistivity and the phase shift, and then analyzed for correlation with the growth of bacteria and its activity. The concentration of bacterial cells was estimated in the liquid phase (filtered) via plate counts and quantified from the sand via qPCR. Scanning electron microscopy (SEM) was used to confirm presence and growth of bacteria as biofilm attached to the sand. In addition, high performance liquid chromatography (HPLC) was applied on selected samples to detect bacterial metabolites.

The study showed that the presence of bacterial growth influences the SIP signal. The bacterial density had a significant impact on the resistivity, which decreased when the bacterial density increased. Compared to the control samples with only water and LB media, the resistivity for the bacterial samples was usually lower. The phase shift did not show any significant trend or clear pattern. Only very small effects on the phase shift were observed, possibly due to high salinity that could have masked the polarization effect. Improvements in the experimental design could also produce higher resolution data quality. However, the maximum phase shift for one of the bacterial samples correlated to two unidentified small, charged molecules found via the HPLC analysis. This supports the observation that biological processes, such as the generation of charged metabolites, can influence the SIP measurements.

Paper III: Biochemical changes associated with two different *in situ* remediation strategies for tetrachloroethene

Åkesson, S., Frank, E., Sparrenbom, C.J., Zhang, Z., Holmstrand, H. & Paul, C.J.

Manuscript for *Science of The Total Environment*

In this paper, the Alingsås site was monitored for two years during which two *in situ* pilot test remediations were applied. The strategy for one of the two pilots, Area A, was a combination of abiotic and biotic enhanced dechlorination, and the strategy for the second, Area B, was a combination of biostimulation and bioaugmentation. The focus of the paper was to describe and understand the microbial and chemical changes within the groundwater due to the remediation actions.

At three occasions, samples for groundwater chemistry, CSIA, and microbial community analyses (16S rRNA gene amplicon sequencing) were taken; before the remediations started, and one and two years after. In addition, groundwater chemistry was sampled every third month. The aim of the study was to apply and combine commercially available methods, and if possible, conclude which remediation strategy was most suitable for the site-specific conditions.

The results showed decreasing concentrations of PCE in the groundwater in both treated areas. *Cis*-DCE was the metabolite analyzed in highest concentrations, although vinyl chloride and ethene were also detected, indicating that complete dechlorination had also occurred. The degradation occurred rapidly (months), which had an impact on the study of isotope fractionation of PCE via CSIA data. This led to the inclusion of metabolites within the CSIA data evaluation, and this showed more biodegradation in Area B, compared to Area A. The microbial communities in both Area A and Area B showed shifts related to the injections, and this shift remained for at least the two years. The class Clostridia became the most relatively abundant class after injections in both areas. Microbes producing methane increased in relative abundance, while Gammaproteobacteria decreased. The groundwater conditions in the two areas ranged from favorable to optimal for biodegradation regarding e.g., pH, redox potential, sulfate, dissolved organic carbon; however, the groundwater was nutrient poor in nitrogen and phosphorus. Because of the untreated source zone, located upstream, observations were made of inflow of PCE into the treated areas. The only observed impact on the source zone from the injections was a change of microbiological class composition.

The differences in results from the two remediation strategies could not be fully evaluated as the CAH concentrations differed between the areas before the remediation started. The areas were hydrogeologically connected, which caused mixture of the injected substances between the areas. More knowledge is needed about which degradation pathway is the most likely when combining abiotic and biotic remediation strategies. The abiotic degradation could not be followed with the commercial analyses via β -elimination as these components were not included in these analyses.

Paper IV: Impact of spatial distribution of microbes in the subsurface on degradation of tetrachloroethene (PCE)

Åkesson, S., Benavides Höglund, N., Sparrenbom, C., Rosenqvist, T. & Paul, C.J.

Manuscript for *Bioremediation Journal*

In this paper, the Alingsås site was investigated in 2020, with the focus on microbial community differences within the unconsolidated sediment. The aim was to study how the hydrogeological setting influences the microbial community, and to compare the sediment microbial communities with the findings from groundwater in Paper III.

Seven sediment cores were taken and were geologically described and documented in the field. Samples were taken for microbial community analysis (16S rRNA amplicon sequencing), and chemical analyses for PCE, TCE,

List of contribution

Table 4. The work contribution within the four attached papers is listed below, and the names are given in the order of contribution, names in italic are not co-authors.

	Paper I	Paper II	Paper III	Paper IV
Study design	S. Åkesson C. Sparrenbom R. Jansson	T. Martin C. Paul	S. Åkesson C. Sparrenbom C. Paul	S. Åkesson C. Sparrenbom C. Paul
Field work	S. Åkesson R. Jansson (DCIP) <i>N.B. Höglund</i>	n.a.	S. Åkesson Field assistants: <i>G. Johansson (2017-2019)</i> <i>R. Jansson (2017)</i> <i>H.T. Olander (2017)</i> <i>N. Benavides Höglund (2018)</i> <i>L. Lindberg Skutsjö (2019)</i> <i>J. Jennerheim (2019)</i>	N. Benavides Höglund <i>J. Jennerheim</i>
Laborator analysis	S. Åkesson	T. Martin S. Åkesson (qPCR) C. Paul	S. Åkesson <i>S. De</i>	S. Åkesson
Data analysis	S. Åkesson R. Jansson	T. Martin C. Paul S. Åkesson	S. Åkesson E. Frank Z. Zhang C. Sparrenbom	S. Åkesson T. Rosenqvist
Data interpretation and discussion	S. Åkesson R. Jansson C. Sparrenbom C. Paul	T. Martin C. Paul S. Åkesson	S. Åkesson C. Sparrenbom E. Frank Z. Zhang C. Paul H. Holmstrand	S. Åkesson C. Sparrenbom C. Paul N. Benavides Höglund T. Rosenqvist
Manuscript	S. Åkesson C. Sparrenbom C. Paul H. Holmstrand R. Jansson	T. Martin C. Paul S. Åkesson	S. Åkesson E. Frank C. Sparrenbom C. Paul H. Holmstrand	S. Åkesson C. Sparrenbom C. Paul N. Benavides Höglund
Comment on manuscript	All authors <i>T. Martin</i> <i>T. Dahlin</i>	All authors <i>C. Sparrenbom</i>	All authors <i>M. Lund</i>	All authors

cis-DCE, iron and total organic carbon (TOC). The microbiology results were compared between the hydrogeological units. The sediment chemistry results from 2020 were compared to the sediment sampling performed in 2017 before the pilot *in situ* remediations.

The variation of the total microbial community was larger in the transition unit (between the aquitard and aquifer material), compared to the aquifer. When comparing at taxonomic class level this observation was not detected, which suggests that the variation was at a lower taxonomic level, such as genus or species. The comparison between sediment and groundwater samples showed that Clostridia and Gammaproteobacteria were dominant in both ma-

trices, although they were relatively more abundant in the samples from the sediment. Dehalococcoidia showed a higher relative abundance in the sediment matrix than in groundwater, while Deltaproteobacteria showed the opposite. These observations show that the two matrices do not completely reflect each other, which suggests that both matrices need to be investigated to fully understand and monitor the microbial community in the subsurface. The CAH concentrations had decreased in the sediment matrix from 2017 to 2020. The highest concentration of PCE was found outside the plume delineation made in 2017, before the remediation injections.

5.1 Additional findings outside publications

5.1.1 Sample collection – bottle vs vial

At the Alingsås site, duplicate groundwater samples were taken in September 2018 to compare two sampling methodologies, i.e., groundwater collection in amber glass bottles (2x50 mL) and amber EPA vials (2x40 mL). Both containers included conservation liquid, where the bottles had H₂SO₄ and vials had NaHSO₄, no information about the cap’s material has been given. The aim was to evaluate if the two ways of sample collection gives comparable results and can be used together in the same studies. For the samples in bottles, SYNLAB AB performed the analysis, and for the samples in vials ALS AB. Both laboratories are accredited for the analysis.

Most of the duplicate results showed similar PCE concentrations via both techniques, or within the reported uncertainty, 20% for bottles and 40% for vials. However, this was not the case for the samples taken from well B2, close to the source zone. The PCE concentration was lower in the sample collected in a bottle (1600 µg/L) than in a vial (8580 µg/L). During the two-year monitoring, the analyzed concentrations collected via bottles always showed lower concentrations than those taken in vials (Fig. 11). The samples taken via bottles showed concentrations below 2000 µg/L, while all the samples taken in vials showed concentrations above 8580 µg/L. The vial samples all showed concentrations >5% of the solubility in water, while the bottled samples showed concentrations around or below 1% of the solubility.

The groundwater sample from well B2 taken in October 2017 was collected in a bottle and had a concentration of 42 µg/L according to SYNLAB AB’s analysis. From the same sampling occasion, CSIA samples were collected in 1L amber glass bottles and conserved with hydrochloric acid. Prior to the CSIA analysis, the concentration was verified by the Isodetect GmbH laboratory. They showed that the B2 sample in the 1L bottle had a PCE concentration of >6000 µg/L, compared to 42 µg/L.

The PCE concentrations in groundwater samples from well MW5 (taken in 2017-2019) are shown as a comparison, since the second highest concentrations were observed from this well (Fig. 11). The samples from September 2018 shows similar concentrations, while the other sampling occasions show varying concentrations. The concentrations have been around or below 1% of the solubility.

As the PCE can occur in free-phase, the groundwater sample could include droplets of the component. The larger

the sampling volume is, the more likely it is to “catch” a droplet. This would lead to a higher concentration being detected during an analysis. However, the larger the sampling container volume, the smaller the impact of a single free-phase droplet will be on the total concentration. The two bottle samples from October 2017 had large differences in volume (100mL versus 1L), while the vials and the concentration bottles volumes (i.e., 80 compared to 100mL) do not have such large differences. If the likelihood of catching a free-phase droplet during sampling is the explanation, then the concentration results would logically show a more random pattern from the same well, and results with occasionally higher concentrations within the bottle samples would be expected. A sampling bias with regards to equipment or personnel is unlikely from the sampling occasion in September 2018 as equipment and field personnel were the same and the samples were taken right after each other.

These observations suggest that vials are better for collecting samples in areas of where the free-phase of DNAPL is likely to occur, e.g., the source zone. When the concentrations were lower, ≤1% of the solubility in water, no distinct pattern between the two methodologies was observed.

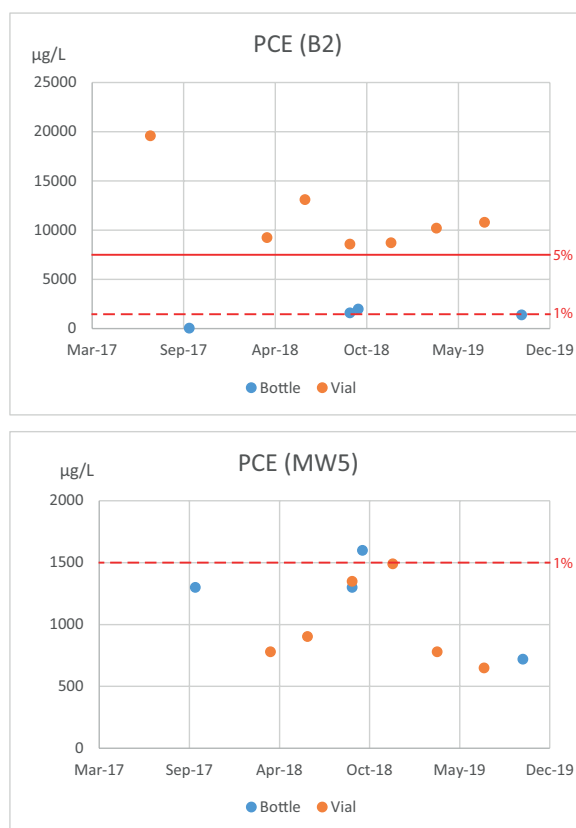


Figure 11. Samples have been collected in both bottles and vials. The PCE concentrations from B2 (source) were lower from the samples collected in bottles compared to those in vials. Free-phase of DNAPLs was expected in the source zone where B2 is located. For the samples taken from well MW5, no distinct pattern of higher concentrations within the samples via vials was found. The solid red line shows 5% solubility of PCE in water and the dashed lines show 1% solubility in water.

6 Discussion

6.1 Synthesizing reflections

The studies in this thesis investigated issues related to contaminated sites, with different temporal and spatial perspectives. The multiple investigation methods have given new insights into the topic, and the approach has led to more nuanced and reliable interpretations for each of the methods used.

By investigate two field sites, which have had both natural degradation and enhanced reductive dechlorination by *in situ* remediation, different perspectives of the variations in degradation over time have been obtained. The largest temporal effect was found with the CSIA method. For natural degradation, sufficient information was found from the study isotope fractionation of PCE only. During enhanced dechlorination, the PCE concentrations decreased too rapidly, and the metabolites needed to be included within the evaluation of biodegradation (Papers I & III). The focus only on the ^{13}C isotopes in PCE during enhanced dechlorination could likely have been improved by more frequent sampling, especially soon after injections, to be able to evaluate the biodegradation. However, as the overall aim with *in situ* remediation is complete dechlorination, to only study the isotope fractionation of PCE will eventually be limited. This finding indicates that the metabolites should be included, to be able to follow the complete dechlorination process, as described by Aeppli *et al.* (2010) and Kuntze *et al.* (2020).

How the spills of CAH occurred is another temporal difference between the two sites which could have had an impact on the CSIA results. In Hagfors, multiple spills occurred spanning many years, while at the Alingsås site, only one documented spill has occurred. It is reasonable to assume that the initial isotope composition for different spills of PCE has varied at Hagfors over a long-term period of PCE use. From other studies, the primary carbon isotope ratios have been observed to range between -22‰ and -35‰ (Thullner *et al.*, 2012). During the period of spills at the Hagfors site, variations in biodegradation could also have occurred during transport within the subsurface. However, in Alingsås, as a single spill incident at a specific time, different initial isotope composition and different residence times do not exist, and therefore the CSIA data becomes easier to evaluate. The variations

observed at the first sampling occasion in Alingsås is most likely a reflection of variation of natural degradation in the subsurface.

The fact that different geological settings cause different geoelectric signal responses is well established (e.g., Palacky, 1987), and the main principal when applied as a nonintrusive field instigation method (e.g., Danielsen *et al.*, 2007; Sparrenbom *et al.*, 2017; Maurya *et al.*, 2018; Gómez *et al.*, 2019). Resistivity signals have also been associated with biological processes and chemicals, e.g., tracking gas emissions from landfills (Rosqvist *et al.*, 2011; Auken *et al.*, 2014), and to investigate the extent of contaminant plumes (Johansson *et al.*, 2015). For the combination of biological and geoelectrical investigations, most work has been focused on by-products of microbial processes, as they have a significant larger impact on the signal than microbial presence and activity (Kessouri *et al.*, 2019). Some studies have assigned higher resistivity as caused by bacterial activity or growth (e.g., Davis *et al.*, 2006; Atekwana & Slater, 2009). The laboratory study in Paper II contributed to an improved understanding of how geoelectrical signals are affected by microbial growth and metabolic activities. In Paper I, the resistivity of a specific area (α anomaly) was interpreted as partly microbial activity, and partly as due to the geological shift, and the induced polarization signals (γ anomaly) as a result of ongoing biodegradation. The resistivity findings in Paper II are partly consistent with the interpretation of the anomaly in Paper I, and the induced polarization data from the two papers indicate that similar results are caused by by-products. The findings in the field were more complex, of course, and therefore could have several explanations, compared to the results obtained in the controlled laboratory experiment.

Since the transition units, or geological boundaries, have been shown as productive hydrogeological settings for the establishment of microbial communities, and hence the locations where greater degradation occurs (Papers I and IV; Sale *et al.*, 2013; Puigserver *et al.*, 2016), more studies on this are needed. It would be interesting to combine an investigation of a geological shift, including differences in hydraulic conductivity and microbial growth, with geoelectrical measurements in a controlled laboratory experiment. Since both the geology and the microbial features have an impact on the geoelectrical signals, and the geological shift has an impact on the microbial growth. First testing the geological shift in a laboratory setting only, and then subsequently letting microbes grow in the sediment material and retesting. This would provide a more nuanced understanding of the relative impact of both of these factors on the geoelectric signatures. Such experimental data would increase the understanding and improve the interpretation of the signals since it is nearly impossible to distinguish the various separate contributions to the geoelectrical signals when investigating a field

site.

To obtain a broader perspective of the microbial community present at the Alingsås site, DNA amplicon sequencing was applied. The advantages of amplicon sequencing data, compared to qPCR, are the detailed data obtained which can include a broad range of taxonomies, as there is no need to search for specific genes. It is relatively fast, compared to qPCR, and commercially available. However, the amplicon sequencing only reports the relative abundance while the qPCR is quantitative and reports the amount of microbes in the samples, which can be of interest when evaluating biodegradation. The laboratory delivers the data in an application, from which some analysis of the community can be performed or downloaded. However, notable is that the statistical analysis in Paper III required expert knowledge in bioinformatics, and this was not commercially available.

The finding that the transition zone is important for degradation in Paper I, inspired the investigation of the sediment matrix for Paper IV. The geology between the two sites varies. At the Alingsås site, the aquifer is confined, with several layers of finer and coarser sediment resulting in a thicker transition zone. At the Hagfors site, it is an unconfined aquifer with a coarser bed overlaying the silty till aquitard. Although the geology differs between the sites, and the aquitard is differently located, the same conclusion was made; the interface is an important environment for the microbes, hence for biodegradation to occur. The transition unit in Alingsås is similar to the setting studied by Puigserver *et al.* (2016), who also came to the conclusion that biodegradation occurred preferentially in the transition zone.

The Papers III and IV, investigated the microbial content from two matrices, groundwater and sediment, at the taxonomic class level. The findings between the two matrices were similar, although Clostridia and Gammaproteobacteria were more dominant in relative abundance in the sediment matrix. The class Dehalococcoidia, which *D. mccartyi* belongs to, was relatively more abundant in the sediment samples than in the groundwater samples. This could be an indication that the microbial community naturally lives in a biofilm, as reported from laboratory experiments of membrane biofilm (Chung *et al.*, 2007) and supports the finding that *D. mccartyi* performs better in a consortium, possibly due to limitations in vitamin B₁₂ (He *et al.*, 2007). *D. mccartyi* has been reported in bioflocs, either separately or with other species (Delgado *et al.*, 2017). The formation of biofilm is associated with surfaces (e.g., Donlan, 2002; Palmer *et al.*, 2007), and as the data from the two matrices varied, this suggests that the sediment matrix needs to be investigated to accurately evaluate which microbes are present. This is consistent with findings from undisturbed settings, which has shown that microbial content in groundwater samples do not cover the bacterial density or activity in the subsur-

face alone (Alfreider *et al.*, 1997), and that the biota in biofilm attached to a surface can distinctively differ from the surrounding water (Liu *et al.*, 2017; Pullerits *et al.*, 2020). In a controlled experiment, it was shown that the majority, approx. 99%, of the total biomass was attached to the material (Lehman *et al.*, 2001). The variation in total microbial community between the transition unit and aquifer (Paper IV), was not observed when studying the taxonomic level of class, which suggest that it occurs at a lower taxonomic level. The region V3-V4 was selected due to its broad overview of the microbial community (Brandt & Albertsen, 2018). However, it has also been reported to underestimate lower taxonomic levels (Bukin *et al.*, 2019), and deeper data evaluation of the result was therefore not possible.

The extracted samples from the sediment matrix at the Alingsås site had in general low microbial content. This seems remarkable, as 55 L of liquid containing high concentrations of microbes has been injected at the site, including optimizing the environment by lowering the redox conditions and providing carbon sources to assist the microbes in establishing in the area (Ølund, 2017). In addition, biodegradation was observed, however PCE was still present 3 years after the injections, which indicated that nutrients for the microbes still remained. One possible explanation is that the extraction of DNA from the sediment was inhibited. This can occur due to compounds present in the samples, such as organic content or iron (Schrader *et al.*, 2015; Kuffel *et al.*, 2020). The sediment samples were extracted twice, using the same extraction kit but performed at different laboratories, with some optimization the second time and variations were observed between the two extractions. This could indicate that something in the extraction was impacted. All samples used for Paper IV were from the same extraction, and thereby it is assumed that the impact of the extraction technique is similar among the samples.

One aspect that is missing at the Alingsås site is that the plume extent has not been delineated down gradient. The well located furthest (MW5) from the source zone is within approx. 40 m. Other field studies have followed plumes for hundreds meters to kilometers scale, e.g. Chapman *et al.* (2007), Hunkeler *et al.* (2011), Filippini *et al.* (2016), and obtained a higher resolution of the plume, the degradation and the impact of the geology. In the study by Hunkeler *et al.* (2011) more vinyl chloride was found at the front of the plume, including higher concentrations of methane and detection of *Dehalococcoides*. However, the part of the plume containing PCE had not been transported the full distance (Hunkeler *et al.*, 2011). The same site was later studied, and the subsurface conditions and degradation mechanisms were possible to divide into zones and monitor the zones' changes over time (Murray *et al.*, 2019). In both studies' data from different depths were acquired, which increased

the resolution further (Hunkeler *et al.*, 2011; Murray *et al.*, 2019). At the Alingsås site, high concentrations of PCE were analyzed from the MW5 well, which indicates that the plume extends further out than what is covered within the monitored area and yet the edge effect of the plume has not been investigated. The plumes at the Hagfors site, as a contrast, has been better delineated (Larsson *et al.*, 2017), although only a smaller part of the plume was included in this thesis.

A general recommendation for remediations at the Alingsås site is not possible as the initial conditions varied too much and the fact that there was mixing of injection fluids between the two areas (observed by the iron concentrations). In Paper IV, it appeared that parts of the plume had moved during or after the injections. This is potentially due to the increased hydraulic pressures induced during the injections. From an environmental point of view and for society, it was positive that both remediation strategies had an impact on the contaminants, and the concentrations have decreased through dechlorination in both areas. However, repeated investigations of the plume extension should be considered. The CSIA data indicated that the main reason of decreasing concentrations was not biodegradation in Area A. Since the concentrations decreased, the PCE either was abiotically degraded, or dispersed down gradient. It was however, not possible to conclude which case could explain the findings, as the metabolites included in the β -elimination were not studied, and thus the abiotic degradation could not be clearly described.

6.2 Future research

Among the published, controlled studies on biodegradation, many have been conducted on single species, or even on a certain strain (e.g., He *et al.*, 2003a; He *et al.*, 2005; Sung *et al.*, 2006a, Sung *et al.*, 2006b). More studies are needed to understand the complex microbial communities that degrade CAHs. It is known that *D. mccartyi* perform the degradation better if living in a community (He *et al.*, 2007), and many injection products for *in situ* remediation include more than only *D. mccartyi* (Stroo *et al.*, 2013). This strongly indicates that the community is important and there is a need to better understand the cooperation between the microbes within the community. From other fields working with microbial services the microbial performance seems more robust and reliable if they can cooperate within a biofilm. This microbial knowledge is widely exploited in the biological process steps found in wastewater, activated sludge, (e.g., Saunders *et al.*, 2016) and drinking water treatment plants, biological filters (e.g., Moll *et al.*, 1998; Chan, 2018).

The step between laboratory experiments and field scale

tests or field investigations is still large. The latter would benefit from larger and more complex controlled tests, to improve the understanding of real field situations, including laboratory or pilot scale constructions of the transition zone. For example, as previously mentioned, a shift in the geoelectric signal may be due to several different possible causes that are present, such as geological changes, chemical and biological processes. Complex controlled tests will improve understanding of the signals and minimize the required validation. For microbial studies, the step from laboratory experiments to the field investigations are also too large. The complexity in the subsurface conditions at field site, e.g., geological heterogeneities, redox potential, transport of groundwater and contaminant plume, etc., will be challenging to mimic in the laboratory. Therefore, more *in situ* studies are recommended, of both natural and stimulated biodegradation, to improve the understanding of biogeochemistry.

Several results from the studies in this thesis have shown that the transition unit is important. The findings suggest that the geology has a fundamental impact on the field sites, and steers both where the contaminants are located and where the microbes establish. Even minor variations in the geology should be documented during investigations of contaminated sites and included in the evaluation.

Suggestions for the environmental sector

At contaminated sites, monitoring wells are commonly located with regards to surface physical boundaries and obstacles. An improvement would be to locate the monitoring wells based on, or at least considering, the hydrogeology of the site. For example, multiple parallel lines of monitoring wells perpendicular to the plume should be considered as standard (e.g., Rivett *et al.*, 2001; Hunkeler *et al.*, 2004; Chapman *et al.*, 2007). These transects would improve both temporal and spatial monitoring of the plume. By also having monitoring wells outside the delineated plume, any subsequent spread of the plume can be identified as it occurs. And they can also provide background values or negative control samples. At the sites studied in this work, all the monitoring wells (with some few exceptions) have one-meter filter screens. To obtain better resolution of the variations in the subsurface, multi-level wells should be considered, as they allow several shorter filter screens at different depths in the same location (e.g. Parker *et al.*, 2006). The spatial distribution of the plume can then be obtained and a higher understanding of the dynamic in the system as shown by Hunkeler *et al.* (2011) and Murray *et al.* (2019).

From a Swedish national perspective, there is limited available literature and guidance in Swedish about degradation via the β -elimination pathway, which leads to lack of awareness of possible or likely metabolites. As author-

ities request more *in situ* remediations (S EPA, 2014), possibly abiotic remediation by injections of Zero-Valent Iron (ZVI) will be more frequently used, including more monitoring and evaluation thereof. The majority of monitoring and evaluation of remediations are performed by consultants, who send the chemical samples to external laboratories for analysis. To ensure accurate monitoring and evaluation of these types of remediations, there is a need for the complete environmental sector (e.g., problem owners, authorities, consultants, entrepreneurs) to require the inclusion of metabolites produced via β -elimination from the commercial laboratories. As long as TCE is the only first step metabolite of PCE considered, evaluation of the degradation will be incomplete, as abiotic degradation would not be fully evaluated.

7 Conclusions

The focus of this thesis was to investigate the subsurface conditions and monitor changes at sites contaminated with PCE and its metabolites. Based on the previously mentioned aims and hypotheses, it is possible to draw the following conclusions:

1. The occurrence of natural degradation at the Hagfors site was verified. It was demonstrated by chemical analysis, microbial investigation and CSIA analysis, which were spatial consistent, and the same area was identified with measurements by DCIP tomography.

Corroboration of hypothesis H1.

2. The controlled laboratory experiments showed that microbial growth and activity had an effect on the SIP signals. The increased density of bacteria in biofilm caused a decrease in resistivity. While the phase variations were found within a very small range, and the maximum phase shift coincident with the presence of small charged molecules.

Hypothesis H2 could partly be corroborated.

3. The two strategies for enhanced reductive dechlorination had positive effects on the contamination at the Alingsås site. It was not possible to evaluate the most efficient and best-suited method for the site-specific conditions due to groundwater mixing between the two injected areas, which are hydrogeologically interconnected.

Thus, hypothesis H3 could not be corroborated.

4. The sediment matrix at the Alingsås site had a larger variation in the microbial community within the

transition unit than in the aquifer. The variation was at a lower taxonomic level than class. The communities in the groundwater showed similarities between the two matrices although they did not completely reflect each other. This suggests that the sediment matrix should also be studied.

Corroboration of hypothesis H4.

The studies have also shown that the metabolites of PCE should be included when applying CSIA analysis in areas where *in situ* biostimulation or bioaugmentation have been performed. For groundwater samples taken in areas where free-phase is suspected or has been previously detected, sample collection with vials is recommended.

In addition, it is possible to conclude that the heterogeneities between two hydrogeologically different units are of importance regarding biodegradation. The heterogeneities in the geological settings are fundamental as they control i) where the contamination is located and how it is transported within the subsurface, and ii) the preferred environment for establishment of the microbial communities, both during natural and enhanced degradation.

The interdisciplinary studies in this thesis have applied several investigation methods and challenged the limitations of the commercially available analysis and state-of-the-art methods to obtain knowledge of the complexity at contaminated sites and degradation of PCE. The findings could be used to further optimize remediations of CAH contaminations by using sustainable remediation strategies for the groundwater and protecting these valuable resources.

Popular summary

Water is essential for life. A large portion of the water we as a society use comes from groundwater, such as drinking water, water for hygiene, food production and cooking. Groundwater is affected by the surrounding geology, the atmospheric composition through which rainwater moves and the geological layers through which rainwater infiltrates to become groundwater. If contaminants are present in the ground during the infiltration process, the groundwater will be contaminated, and can then pose a risk for human health or the environment, possibly limiting the use of it. The contaminants can either be natural or caused by human actions.

In this thesis, two contaminated sites in Sweden have been investigated. Historically, there have been dry cleaners at the investigated sites, where the chlorinated solvent that were used as dry-cleaning fluid leaked out and contaminated both the groundwater and the sediment. The washing fluid substance, tetrachloroethene, does not easily dissolve in water and has higher density than water. In addition, it is slippery due to its low viscosity. These three traits allow it to move differently than water within the subsurface, with deep penetration into the ground as a result. It is possible to find tetrachloroethene as pure liquid in groundwater. Tetrachloroethene and some of its degradation products can cause cancer, it is therefore important to clean the sites. The degradation can occur naturally through biological processes, which is commonly slow. However, it is possible to increase the rate of the degradation, either by chemical or biological processes.

At the first site studied, only naturally occurring biological processes have degraded the contaminants. At the second site, an enhanced remediation has been performed, through both chemical and biological processes, by adding substrates and microbes to the ground. The second site has been investigated for both the groundwater and the loose geological material, e.g., clay and sand. The idea was to compare these two sites and study if there were any differences or similarities between natural degradation and remediation, and monitoring of the latter.

To collect data to describe and evaluate the sites and processes, multiple methods have been used. The groundwater chemistry has been analyzed as the core method. Studies of what kind of microbes present in the ground have been performed. During biological degradation of tetrachloroethene, different variations of the substance, isotopes, have various likeliness to be degraded; therefore, the variants of the substance have been analyzed. In ad-

dition, the ground's electrical properties have been measured at the first site. Beyond the two sites, a laboratory experiment has been performed, where the impact of microbial growth and activities on the electrical properties were studied.

In the study of natural degradation at the first site, all used methods showed that most degradation occurred just where the geology shifted from coarser to finer grains. The electrical signals could therefore not confidentially be interpreted, as both the geological shift and microbial growth and activities were possible explanations. Through the laboratory experiment, it was shown that the microbial growth made it more difficult to transport an electricity current through the material.

At the site where enhanced remediation was performed, the degradation was rapid and more efficient than at the site with natural degradation. Both the tested strategies of remediation showed reduced concentrations of the contaminants efficiently. The ambition was to conclude if chemical or biological processes was the best strategy for this site. However, this was not possible as the two injection fluids were mixed since they were closely located and combined in the ground, and the area dominated by chemical processes was mixed with biological processes. It was possible to see a shift in the microbial content due to the injections, which lasted two years after. From the study of the site's geological material, a larger variation of the microbial communities was found in the area of the shift between finer and coarser grains. Since the microbes prefer to live together with other species, this seems positive for the "right" microbes to live and degrade the contaminants. When comparing the microbes found in the groundwater and the ones found in the geological material, the results had many similarities but some of the most important microbes for degradation of tetrachloroethene had a greater abundance in the geological material.

The conclusion of these studies is that geological changes are important for the biodegradation process. The contaminant transport is dependent on the geological setting, and the microbes seems to prefer the area where the geology is shifting. This could be due to that the finer grains can be used for attachment and colonialization, whereas the coarse-grained material provides a better groundwater flow for transport of nutrients to the microbes. Therefore, smaller geological shifts should be remembered in studies of contaminated sites and especially if microbes may play a role in the degradation. By combining the different measurement methods, it was possible to make better interpretations of the processes and the ground composition.

Svensk sammanfattning

Vatten är livsavgörande. En stor del av det vattnet som används i samhället kommer från grundvatten, t.ex. dricksvatten, vatten för hygien, och i matproduktion samt matlagning. Grundvattnet påverkas av de miljöerna genom vilket det passerar, d.v.s. luftens innehåll då vattnet faller som regn och de geologiska lagren som det infiltreras genom. Om det finns föroreningar i marken vid infiltrationen kan grundvattnet bli förorenat och kan då bli en risk för människors hälsa och miljön. Det finns både naturliga föroreningar och de som kommer från mänskliga aktiviteter.

I denna avhandling har två förorenade platser i Sverige blivit undersökta, Hagforstvätten och Tvätteriet i Alingsås där det tidigare har funnits kemtvättar. Av olika anledningar har tetrakloretylen, ett klorerat lösningsmedel som använts som tvättvätska läckt ut i naturen. Idag är platserna förorenade, med påverkan på både grundvattnet och de lösa avlagringarna. Tetrakloretylen är svårösligt i vatten och tyngre än vatten, samtidigt är det "glatt". Tillsammans gör dessa egenskaper att det rör sig annorlunda än vatten i marken och att det kan hittas som egen fas. Tetrakloretylen och vissa av dess nedbrytningsprodukter är cancerframkallande, vilket gör att platser förorenade med dessa ämnen är viktiga att åtgärda. Nedbrytningen av föroreningen kan ske naturligt via biologiska processer men sker oftast långsamt, dock är det möjligt att påskynda nedbrytningen genom kemiska och biologiska saneringsmetoder.

I Hagfors har endast naturlig nedbrytning av föroreningen via biologiska processer skett. I Alingsås har förstärkt nedbrytning skett, både via kemiska och biologiska saneringsmetoder. Detta har skett genom tillförsel av ämnen, bl.a. organiskt material och järn, och mikrober till marken. I Alingsås har både grundvattnet och de lösa geologiska avlagringarna undersökts. Idéen med denna avhandling var att kunna undersöka likheter och olikheter mellan de två platserna och deras olika processer, samt att i Alingsås följa hur saneringen fungerade.

För att beskriva och utvärdera nedbrytningen så har flera olika metoder använts. Den kemiska sammansättningen och föroreningshalten i grundvattnet, samt föroreningshalten i sediment har analyserats. Vilka mikrober som finns i grundvattnet och marken har undersökts för att kunna konstatera om biologisk nedbrytning kan ske. Vid biologisk nedbrytning av tetrakloretylen har olika varianter av molekylen varierande benägenhet att bli nedbrutna, därför har dessa varianter, isotoper, analyserats.

Markens elektriska egenskaper mättes i Hagfors, samt i försök i laboratorium för att se hur mikrobiell tillväxt påverkar hur en elektisk ström kan transporteras.

Vid undersökningen av naturlig nedbrytning visade alla undersökningsmetoder att mest nedbrytning skedde i ett område där geologin ändrade sig från grövre till finare material. Då både en förändring i geologin och mikrobiell tillväxt kan ge upphov till samma förändring i elektrisk signal, gick det inte att avgöra vilken förklaring som faktiskt var korrekt. Genom experimenten i laboratoriet visade det att mikrobiell tillväxt gör det lättare för en ström att transporteras genom materialet, troligen då tomrummen fylls upp med mikrober som leder strömmen.

På platsen i Alingsås, med förstärkt nedbrytning, skedde nedbrytningen snabbt och mer effektivt än vid naturlig nedbrytning. Båda de testade saneringsmetoderna, kemisk och biologisk, som använts för att öka nedbrytningen fungerade. Målsättningen var att avgöra vilken av metoderna som var bäst lämpad för platsen, dock gick det inte att avgöra. De två områdena ligger nära varandra och visade sig vara sammanlänkade i maken vilket ledde till att ämnena som tillsattes blandades. Dessutom hade det ena området högre halter av föroreningen än det andra området. Det var möjligt att studera en förändring av vilka mikrober som fanns på platsen till följd av de tillförda medlen som varade åtminstone två år. Vid undersökningen av markens material så visade det sig att det fanns en större variation av mikrober i området där geologin ändrades från finare till grövre material. Eftersom mikrober föredrar att leva tillsammans med andra arter framför att leva art för art så verkar detta lovande att "rätt" mikrober finns i övergångsområdet och som kan bryta ned föroreningen. När resultatet av mikroberna jämfördes mellan grundvattnet och det geologiska materialet så fanns många likheter, dock fanns det mer av de viktiga mikroberna i avseende att bryta ned tetrakloretylen i proverna från det geologiska materialet.

Slutsatserna från dessa undersökningar är att geologisk uppbyggnad och skillnader i hydrogeologiska egenskaper är viktiga för de biologiska processerna. Det är välkänt att föroreningsens transport påverkas av de geologiska materialen, samtidigt som det verkar vara fördelaktigt för mikrober att leva i områden med olika geologiska lager. Troligen beror detta på att det finare materialet kan användas för att fästa och kolonisera sig på, samtidigt som det grövre materialet har ett högre vattenflöde och kan transportera näringsämnen till mikroberna. Därför behöver även mindre geologiska förändringar innefattas i studier av förorenade platser, speciellt om mikrober är delaktiga i nedbrytningen av föroreningen. Genom att kombinera de metoder som använts i dessa undersökningar blev det möjligt att göra bättre tolkningar av nedbrytnings- och flödesprocesserna i marken samt markens sammansättning.

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CHLORINATED ALIPHATIC HYDROCARBONS:
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ISSN 0281-3033
ISBN 978-91-87847-58-5