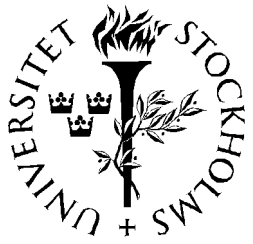
Application of two dimensional compound specific carbon-chlorine isotope analyses for degradation monitoring and assessment of organic pollutants in contaminated soil and groundwater

Charline Wiegert



Doctoral Thesis

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Have no fear of perfection,
you'll never reach it.

Salvador Dali

ABSTRACT

Nearly 250,000 sites with past and present potentially polluting activities need urgent remediation within Europe. Major pollutants include organochlorines (OCls), e.g. chlorinated ethenes (CEs) and hexachlorocyclohexanes (HCHs), mainly used as industrial solvents and pesticides, respectively. Due to improper handling and disposal, OCls contaminants are present in the soil or groundwater surrounding sites, where they have been produced or used. CEs and HCHs can undergo degradation by microorganisms indigenous to the soil or groundwater. Therefore natural attenuation (NA), relying on the in situ biodegradation of pollutants, is considered as a cost effective remediation strategy, yet it requires accurate monitoring methods. Compound specific isotope analysis (CSIA) is a powerful tool to provide information on the extent of degradation and, when combining two isotope systems (2D-CSIA), such as carbon ($\delta^{13}C$) and chlorine ($\delta^{37}Cl$), on reaction mechanisms.

The diagnostic reaction-specific isotope enrichment factors (ε_C and ε_{Cl}) were determined in laboratory experiments for the anaerobic degradation of PCE, TCE (**Paper II**) and α-HCH (**Paper III**) by mixed bacterial cultures enriched from CEs and HCHs contaminated sites, respectively. The related mechanism-specific $\varepsilon_{Cl}/\varepsilon_C$ ratios were calculated as 0.35 ± 0.11 (PCE), 0.37 ± 0.11 (TCE) and 0.52 ± 0.23 (α-HCH). These values are smaller than previously reported values for pure cultures. This is explained by the microbial community composition changes observed during degradation of PCE and α-HCH, which also reflect the variability of the microbial community at the field level. Furthermore, $\varepsilon_{Cl}/\varepsilon_C$ ratio might be bacteria specific.

These values allowed the estimation of the extent of contaminant degradation at the respective study sites (**Paper III** and **IV**). Application of both isotope systems ($\delta^{13}C$ and $\delta^{37}Cl$) led to comparable estimates. However the choice of representative ε values is crucial for an accurate assessment.

These studies show that CSIA is useful to quantify in situ degradation of OCls contaminants and identify reaction pathways, by combining $\delta^{13}C$ and $\delta^{37}Cl$.

SAMMANFATTNING

Nästan 250.000 områden med tidigare och nuvarande potentiellt förorenande verksamheter behöver akut sanering inom Europa. De största föroreningarna inkluderar organochlorines (OCls), t.ex. klorerade etener (CEs) och hexaklorcyklohexaner (HCHs) som huvudsakligen har använts som industriella lösningsmedel respektive bekämpningsmedel. På grund av felaktig hantering och destruktion, förorenar OCls mark och/eller grundvatten i närområden kring lokaler för produktion och användning. CEs och HCHs kan genomgå naturlig nedbrytning via mikroorganismer i mark och grundvatten. Därför anses naturlig *in situ* nedbrytning (NA – Natural Attenuation), ha stor patential som kostnadseffektiv saneringsstrategi, förutsatt att precisa övervakningsmetoder utvecklas för att följa nedbrytningsförloppet. Ämnesspecifik isotopanalys (CSIA - Compound Specific Isotope Analysis) är ett kraftfullt verktyg för att tillhandahålla information om omfattningen av nedbrytning och, om två isotopsystem kombineras (2D-CSIA med exempelvis $\delta^{13}C$ och $\delta^{37}Cl$), för att identifiera reaktionsmekanismer.

De diagnostiska reaktions-specifika isotopanrikningsfaktorerna (ε_C och ε_{Cl}) bestämdes i laboratorie-experiment för anaerob nedbrytning av PCE, TCE (**Artikel II**) och α-HCH (**Artikel III**) med bakteriekulturer, som anrikats från förorenade områden med höga halter av CEs respektive α-HCH. De tillhörande mekanismspecifika $\varepsilon_{Cl}/\varepsilon_C$ kvoterna beräknades till 0.35 ± 0.11 (PCE), 0.37 ± 0.11 (TCE) och 0.52 ± 0.23 (α-HCH). Dessa värden är lägre än tidigare rapporterade värden för rena kulturer. Detta förklaras av de förändringar i den mikrobiella sammansättningen som observerats under nedbrytning av PCE och α-HCH, vilket också återspeglar variationen i den mikrobiella sammansättningen på fältnivå. Förhållandet $\varepsilon_{Cl}/\varepsilon_C$ förhållandet är i sannolikt i viss mån bakteriespecifikt.

Dessa värden (ε_C och ε_{Cl}) användes för att uppskatta omfattningen av föroreningarnas nedbrytningsgrad respektive undersökningsområden (**Artiklar III** och **IV**). Tillämpning av två olika isotopsystem ($\delta^{13}C$ och $\delta^{37}Cl$) ledde till jämförbara resultat. Valet av representativa ε värden befanns dock vara kritiskt för en korrekt bedömning.

Dessa studier visar att CSIA är lämpligt för att kvantifiera *in situ* nedbrytning av förorenande OCls och identifiera reaktionsprocesser, i synnerhet om $\delta^{13}C$ och $\delta^{37}Cl$ kombineras.

RÉSUMÉ

En Europe, près de 250 000 sites ayant présenté ou présentant des activités potentiellement polluantes ont un besoin urgent de décontamination. Les principaux polluants incluent les composés organochlorés (OCls), tels que le perchloroéthylène (PCE), le trichloroéthylène (TCE), et les hexachlorocyclohexanes (HCHs), principalement utilisés comme solvants industriels et pesticides, respectivement. En raison de manutention inappropriée, stockage et rejet inadaptés, les contaminants organochlorés sont présents dans les sols ou les eaux souterraines aux alentours des sites, où ils ont été produits ou utilisés. PCE, TCE et HCH peuvent être dégradés par des micro-organismes indigènes du sol ou des eaux souterraines. Par conséquent l'atténuation naturelle (NA), qui repose sur la biodégradation *in situ* de polluants, est considérée comme une stratégie d'assainissement rentable, qui exige toutefois des méthodes d'évaluation précises. L'analyse des rapports isotopiques des composés spécifiques (CSIA – Compound Specific Isotope Analysis) est un outil puissant pour fournir des informations sur l'étendue de la dégradation et, en combinant deux systèmes isotopiques (2D- CSIA), tels que le carbone ($\delta^{13}C$) et le chlore ($\delta^{37}Cl$), sur les mécanismes réactionnels.

Les facteurs d'enrichissement isotopiques qui sont spécifiques d'une réaction (ε_C et ε_{Cl}) ont été déterminés en laboratoire pour la dégradation anaérobie du PCE, TCE (**Article II**) et α-HCH (**Article III**) par des cultures bactériennes mixtes enrichies de sites contaminés par PCE, TCE et HCHs, respectivement. Le calcul des ratios $\varepsilon_{Cl}/\varepsilon_C$ qui sont spécifiques à un mécanisme a conduit à 0.35 ± 0.11 (PCE), 0.37 ± 0.11 (TCE) et 0.52 ± 0.23 (α -HCH). Ces valeurs sont inférieures aux valeurs précédemment reportées pour des cultures pures. Cela s'explique par les modifications de la composition de la communauté microbienne observées lors de la dégradation de PCE et α-HCH, et qui reflètent également la variabilité de la communauté microbienne sur le terrain. En outre, le ratio $\varepsilon_{Cl}/\varepsilon_C$ pourrait être spécifique à chaque bactérie.

Ces valeurs ont permis d'estimer le taux de dégradation des contaminants sur les deux sites respectivement étudiés (**Articles III** et **IV**). L'utilisation des deux isotopes ($\delta^{13}C$ et $\delta^{37}Cl$) a conduit à des estimations comparables. Cependant, le choix de valeurs représentatives de ε est essentiel pour une évaluation précise.

Ces études montrent que CSIA est utile pour quantifier la dégradation *in situ* de contaminants organochlorés et pour identifier les réactions de dégradation, si $\delta^{13}C$ et $\delta^{37}Cl$ sont combinés.

ABBREVIATIONS

CEs	Chloroethenes
CSIA	Compound Specific Isotope Analysis
cDCE	cis-Dichloroethene
CI	Confidence Interval
DCE	Dichloroethene
DNAPLs	Dense Non-Aqueous Phase Liquids
$\delta^{13}C$	Stable carbon isotope signature
$\delta^{37}Cl$	Stable chlorine isotope signature
ε_i	Isotope enrichment factor of element i
f_i	remaining fraction of compound i
GCqMS	Gas Chromatograph quadrupole Mass Spectrometer
HCHs	Hexachlorocyclohexanes
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
IRMS	Isotope Ratio Mass Spectrometry
KIE	Kinetic Isotope Effect
MNA	Monitored Natural Attenuation
NA	Natural Attenuation
OCls	Organochlorines
OTU	Operational Taxonomic Unit
POPs	Persistent Organic Pollutants
PCE	Tetrachloroethene, Perchloroethene
SMOC	Standard Mean Ocean Chloride
TCE	Trichloroethene
TIMS	Thermal Ionization Mass Spectrometer
VC	Vinyl Chloride
VPDB	Vienna Pee Dee Belemnite

TABLE OF CONTENTS

LIST OF PAPERS

Paper I

Dual Carbon−Chlorine Stable Isotope Investigation of Sources and Fate of Chlorinated Ethenes in Contaminated Groundwater.

Wiegert, C.; Aeppli, C.; Knowles, T.; Holmstrand, H.; Evershed, R.; Pancost, R.D.; Macháčková, J. and Gustafsson, Ö. (2012) *Environmental Science & Technology*, **46** (20), 10918-10925

Paper II

Carbon and Chlorine Isotope Fractionation During Microbial Degradation of Tetra- and Trichloroethene.

Wiegert, C.; Mandalakis, M.; Knowles, T.; Polymenakou, P.; Aeppli, C.; Macháčková, J.; Holmstrand, H.; Evershed, R.P.; Pancost, R.D. and Gustafsson, Ö. (2013) *Environmental Science & Technology*, **47** (12), 6449-6456.

Paper III

Carbon and Chlorine Stable Isotope Fractionation during Anaerobic Degradation of α-Hexachlorocyclohexane by a Mixed Culture Enriched from a Contaminated Site.

Wiegert, C.; Mandalakis, M.; Knowles, T.; Hovorková, I.; Polymenakou, P.; Aeppli, C.; Holmstrand, H.; Evershed, R.P.; Pancost, R.D.; Klánová, J. and Gustafsson, Ö.

Submitted to *Environmental Science & Technology*.

Paper IV

Carbon Stable Isotope Investigation of Hexachlorocyclohexanes in Field Contaminated Soils.

Wiegert, C.; Aeppli, C.; Knowles, T.; Hovorková, I.; Holmstrand, H.; Evershed, R.P.; Pancost, R.D.; Klánová, J. and Gustafsson, Ö.

Manuscript.

STATEMENT

I, Charline Wiegert, contributed to the papers as follow:

Paper I

The sampling was planned and performed by others. I carried out the samples' extractions and stable chlorine isotope analysis ($\delta^{37}Cl$). Data interpretation was made in close collaboration with co-authors. I had the main role in the writing of the article.

Paper II

The soil sampling was planned and performed by others. The microbial degradation experiments and microbial characterization were carried out by others. I performed the samples' extractions and $\delta^{37}Cl$ measurements. I took the lead role in writing the article.

Paper III

The soil sampling was planned and performed by others. The microbial degradation experiments and microbial characterization were carried out by others. I was responsible for adapting the $\delta^{37}Cl$ method to hexachlorocyclohexanes (HCHs) and performed the analyses. I took the lead role in writing the article.

Paper IV

The sampling was planned and performed by others. I carried out the $\delta^{37}Cl$ analyses and took the lead role in writing the article.

THESIS OBJECTIVES

This PhD thesis was embedded within the EU – FP7 research project isoSoil "Contaminant-specific isotope analyses as sharp environmental-forensics tools for site characterization, monitoring and source apportionment of pollutants in soil", coordinated by ITM at Stockholm University. The overarching objective of the thesis was thus to provide a new and complementary approach to firmly establish the analytical scope of compound-specific isotope analysis (CSIA), and therefore to facilitate more precise and reliable site-specific characterization of soil and groundwater contamination.

The specific objectives of this thesis were:

1. To apply a newly developed CSIA method for $\delta^{37}Cl$ determination of some prioritized organochlorinated pollutants, i.e. chloroethenes (CEs) and hexachlorocyclohexanes (HCHs) (**all papers**)

2. To determine ε_C and ε_{Cl} for the anaerobic degradation of tetrachloroethene (PCE) and trichloroethene (TCE; **Paper II**) and α-HCH (**Paper III**) by mixed cultures enriched from contaminated sites, and subsequently calculate the mechanism specific $\varepsilon_{Cl}/\varepsilon_C$ and $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratios.

3. To investigate the degradation patterns, i.e. extent and mechanism, of CEs (**Paper I**) and HCHs (**Paper IV**) at the studied field sites, by combining carbon and chlorine isotopes measurements, applying the determined ε values and using microbial characterization.

1. INTRODUCTION

1.1 Soil contamination and remediation

1.1.1 Soil contamination: brief history and status

Centuries of human activity have affected the environment, and particularly our soil health. Since the first mining works, chemical wastes have been openly dumped onto the soil, under the belief that soil could self-replenish. Soil contamination globally increased with the Industrial Revolution, and more largely with the 20th century's technical developments, including the spreading of pesticides and fertilizers, the use of fossil fuels, the intensification of industrial production, and the rising population growth. Awareness of soil and groundwater contamination, however, started in the late 1970s, when huge environmental and human health scandals shook policy makers. The *Love Canal disaster* in the USA and the *Lekkerkerk's* case in the Netherlands are still notorious examples of contaminated sites. In both cases, residential areas were built on former chemical waste disposal areas, causing disease and health threat among the inhabitants (Swartjes, 2011).

In the last decades, the number of potentially polluted sites reached six or seven digits in most developed countries, because awareness of their existence raised (Swartjes, 2011). The European Environmental Agency (EEA) estimated the number of sites with past and present potentially polluting activities at nearly 3 million within Europe (EEA, 2007). Among them, about 250,000 need remediation. The US Environmental Protection Agency (US EPA) reports a similar situation in the USA with 294,000 hazardous waste sites (US EPA, 2004). The EEA also evaluated the contribution of industrial production, as well as waste treatment, disposal and storage as sources to soil contamination at more than 50% within the European Union.

The soil is now recognized as essential for supporting life on Earth (Jeffrey et al., 2010). Therefore, recent understanding of the soil ecosystem services and public awareness make soil remediation one of nowadays largest challenges.

1.1.2 Remediation strategies

A remediation plan depends on each site, its contamination history, geographical features, geochemical settings, etc. Thus careful monitoring and risk assessment is needed to choose the most appropriate remediation method. For this purpose, the EEA urged for standardized investigation and data collection methods (EEA, 2010).

Remediation technologies are of two types ex and in situ. Ex situ technologies require excavation or extraction of the contaminated zone. These include bioremediation, chemical treatment, incineration, mechanical soil aeration, neutralisation, open burn/open detonation, physical separation,

phytoremediation, soil vapour extraction, soil washing, solidification/stabilization, solvent extraction, thermal desorption, and vitrification. In addition, pump and treat is commonly applied for groundwater cleanup (US EPA, 2007).

In situ technologies are bioremediation, chemical treatment, electrical separation, flushing, multi-phase extraction, mechanical soil aeration, neutralization, phytoremediation, soil vapour extraction, solidification/stabilization, thermal treatment and vitrification (US EPA, 2007).

However, most of the conventional cleanup technologies are time consuming and expensive and often lead to incomplete decontamination. Since the 1990s, natural attenuation (NA) has gained enormous popularity, because it relies on in situ biodegradation of the contaminants, without human intervention (Bombach et al., 2010).

1.1.3 Natural attenuation (NA) as remediation strategy

NA refers to the reduction of mass, toxicity, mobility, volume, and/or concentration of contaminants in soil or groundwater using naturally occurring processes in soil (US EPA 1999). These processes can be physical, chemical or biological and include biodegradation, dispersion, dilution, sorption, volatilization, radioactive decay and chemical or biological stabilization, transformation, or destruction of contaminants (Swartjes, 2011). Biodegradation is one of the most important processes, since microorganisms transform the pollutants thereby decreasing their mass load. Therefore in situ biodegradation has been the focus of numerous studies over the past decades (Wiedemeier et al., 1999).

In order to accept NA as an effective remediation strategy, several issues must be addressed, including the occurrence, efficiency and timeframe of biodegradation, so that the contaminant removal occurs in a reasonable time scale (Bombach et al., 2010). Thus monitoring is necessary to demonstrate that NA works in a sustainable manner and the term Monitored Natural Attenuation (MNA) is used. Various approaches have therefore been developed to assess NA at contaminated field sites, and more specifically to accurately qualify and quantify the degradation processes. Methods include hydrogeochemical methodologies, e.g. geochemical approaches, tracer tests, metabolite analysis, as well as microbial or molecular methods (Bombach et al., 2010).

The common concentration based assessments are often hampered by other processes, such as dilution and dispersion, which prevent from establishing a reliable mass balance. Therefore several methodologies are often combined to answer the questions and identify the lines of evidence that can prove NA. However direct quantification of the extent of degradation and identification of the underlying pathways is often not feasible via these methods solely.

1.2 Organochlorines – OCls

1.2.1 Generalities

Chlorinated organic compounds, or organochlorines (OCls) are molecules with a carbon skeleton and at least one covalently bonded chlorine atom. OCls include for example the chloroethanes, the chloroethenes (CEs), dichloro-diphenyl-trichloroethane (DDT), chlorophenols, polychlorinated biphenyls (PCBs), hexachlorocyclohexanes (HCHs). They have been industrially produced since the 1920s, and were used for different purposes, such as degreasing and dry cleaning solvents, pesticides, electrical insulators, etc. However some of them, such as tetra- and trichloroethene (PCE and TCE, respectively) are also naturally produced (Gribble, 1998).

This wide class of compounds exhibits different physico-chemical properties as well as toxicity. Some OCls are highly toxic towards the environment and humans. DDT, widely used as pesticide in the 1940s, is a famous example of a toxic OCl, with a tendency for bioaccumulation and long-range transport (Simonich and Hites, 1995).

This thesis focused on two types of OCls, i.e. CEs and HCHs.

1.2.2 Chlorinated ethenes, chloroethenes - CEs

Tetrachloroethene (PCE) and trichloroethene (TCE) have been among the most widely used chlorinated solvents since the 1940s, mainly as dry cleaning and degreasing agents, respectively (Doherty, 2000). The use of PCE in dry cleaning has been registered under REACH in 2010.

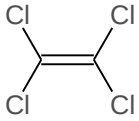
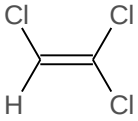
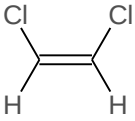
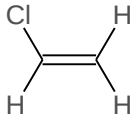
They enter the environment mostly by evaporating into the air during use. Improper handling and disposal also lead to leaks and spills and made these solvents major environmental contaminant in soil and groundwater worldwide (ATSDR, 1997a). Because they are denser than water and have relatively low water solubility, they refer as Dense Non-Aqueous Phase Liquids (DNAPLs). Their organic carbon partition coefficients indicate a relatively high mobility through the soil matrix (see Table 1 for the physico-chemical properties). As a consequence these compounds can leak to the saturated zone, where they can accumulate and persist over decades. As they reach the water table, they are transported along with the groundwater flow, posing a threat to drinking water resources. Growing concern about contamination of PCE and TCE arose since they were suspected carcinogenic (NRC, 1980) and shown, from the 1980s, to be sequentially biodegraded, under anaerobic conditions, to the lesser chlorinated and more toxic compounds dichloroethenes (DCE) and vinyl chloride (VC), and eventually further to the non-toxic ethene (Bouwer et al., 1981; Freedman and Gossett, 1989; Vogel et al., 1987).

Although the results of studies on the carcinogenic effect of PCE and TCE on humans are contradictory (Jollow et al., 2009; Mattes et al., 2010), their environmental threat is widely recognized, mainly because of the toxicity and tendency to accumulate of cis-DCE (cDCE) and VC, and therefore

motivates their remediation. As a result, they are regulated contaminants in drinking-water. The US EPA has set a maximum contaminant level (MCL) of 5μg·L^{-1} (U.S. EPA, 2009) for both PCE and TCE, while the World Health Organization (WHO) guideline values are 40 μg·L^{-1} for a drinking water contribution of 10% for PCE and 20 μg·L^{-1} for a 50% drinking water contribution for TCE (WHO, 2011). In Europe, a directive value of 10 μg·L^{-1} for the sum of concentrations of PCE and TCE was established by the European Commission Council (EU council, 1998).

The sequential reaction, known as reductive dechlorination, is an important process for NA of these compounds and has been the focus of numerous studies (Figure 1; see Bradley, 2003 and Wiedemeier et al., 1999 for reviews). The process involves bacteria using the CEs as electron acceptors and generally H_2 as electron donor, to support their growth (Häggblom, 2003; Maymó-Gatell et al., 1995; U.S. EPA, 1996; Zinder and Gossett, 1995). Several dehalorespiring microorganisms, in pure, mixed, as well as enriched cultures, such as bacteria from the genera *Dehaloccocoides* (Cichocka et al., 2010; Cupples, 2008; Duhamel et al., 2002; Sung et al., 2006), *Dehalobacter* (Holliger et al., 1993 and 1998), *Desulfonomonile* (Cole et al., 1995; Fathepure et al., 1987), *Desulfitobacterium* (Gerritse et al., 1996), *Desulfuromonas* (Krumholz et al., 1996; Krumholz, 1997; Sung et al., 2003), *Enterobacter* (Sharma and McCarty, 1996) and *Sulfurospirillum* (Neumann et al., 1996) have been shown to reduce PCE and TCE (DiStefano et al., 1991; Fetzner, 1998; Häggblom, 2003) but to date, *Dehaloccocoides ethenogenes* strain 195 is the only known isolate to be able to dechlorinate PCE all the way to ethene, although the last step is not coupled to growth (Futagami et al., 2008; Magnuson et al., 1998; Maymó-Gatell et al., 1997; Maymó-Gatell et al., 1999). Hence, and since the oxidizing potential of chlorinated ethenes decreases with the number of chlorine, complete reductive dechlorination is often the result of cometabolism (Flynn et al., 2000; He et al., 2003; Löffler et al., 2000; Maillard et al., 2011; Rosner et al., 1997; Smidt et al., 2000). Therefore, the availability of dehalorespiring bacteria, hydrogen, or other electron donors, and proper redox conditions are crucial parameters (Bradley, 2003; U.S. EPA, 1996; Wiedemeier et al., 1999), and accumulation of cDCE and VC is commonly observed at field sites (Ballapragada et al., 1997; Bradley, 2000; Semprini, 1995; Tandoi et al., 1994). However, in contrast to PCE and TCE, both cDCE and VC can be biotically oxidized to CO_2 under aerobic as well as anaerobic conditions (Bradley, 2003; Mattes et al., 2010). Nonetheless, aerobic cometabolic degradation of PCE and TCE has been shown (Ryoo et al., 2000). Moreover, the latter can undergo abiotic reductive reactions by iron bearing soil minerals (Lee and Batchelor, 2002a and b).

Table 1. Physico-chemical properties of CEs. Molecular mass (M_w), density (ρ), melting (T_m) and boiling (T_b) temperatures, water solubility (S_w), vapor pressure (V_p), air-water (K_{aw}), octanol-water (K_{ow}) and organic carbon (K_{oc}) partition coefficients. All data were retrieved from Schwarzenbach et al. (2003), except the K_{oc} values are from the ATSDR toxicological profiles respective to each compound (ATSDR 1996, 1997a, 1997b, 2006). The guideline values for drinking water are from WHO (2011).

Abbreviation	**PCE**	**TCE**	**cDCE**	**VC**
Name	Tetrachloroethene (Perchloroethene)	Trichloroethene	cis-1,2-Dichloroethene	Vinyl Chloride
Formula	C_2Cl_4	C_2HCl_3	$C_2H_2Cl_2$	C_2H_3Cl
Structure				
CAS number	127-18-4	79-01-06	156-59-2	75-01-04
M_w [g·mol^{-1}]	165.83	131.39	96.94	62.50
ρ [g·mL^{-1}]	1.62	1.46	1.27	0.91
T_m [°C]	−22	−73	−81	−154
T_b [°C]	121	87	60	−14
V_P [Pa]	2.5E+03	1.0E+04	2.8E+04	3.5E+05
S_w [mg·L^{-1}]	135	1090	5080	2790
K_{aw}	0.76	0.40	0.16	1.12
K_{ow}	759	263	72	19
K_{oc}	158-501	107-457	49	98
WHO [µg·L^{-1}]	40	20	50	0.3

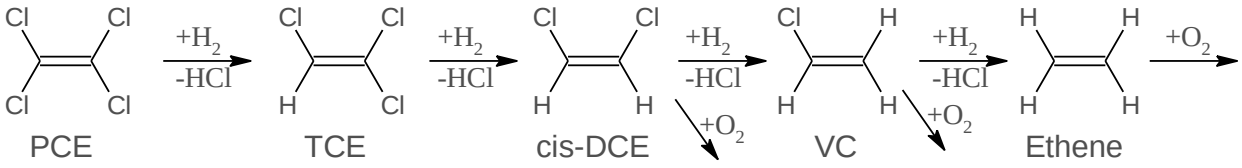


Figure 1. Scheme for the reductive dechlorination of PCE to ethene. PCE and TCE undergo anaerobic degradation, while cDCE, VC and ethene can be further oxidized to CO_2 (see main text).

1.2.3 Hexachorocyclohexanes – HCHs

HCHs are widely known OCls, which were mainly used as pesticides but also as pharmaceutical products since the 1940s. They were first synthesized by Faraday in 1823, but their insecticidal properties were only discovered in 1943 (López et al., 2011). HCHs present eight different isomers, named α to θ (Figure 2). Preparation of HCHs yields a mixture of the five major stable isomers, the composition of which varies depending on the technical process, and consists of 60-70% α-HCH, 6-10% β-HCH, 10-12% γ-HCH and 6-10% δ-HCH, 3-4% ε-HCH and residues of the three minor isomers (Li et al., 2011). This mixture, known as technical HCH, was first commercialized as a cheap pesticide, although the insecticidal properties were attributed to the γ isomer only. In the 1950s, the toxicity and persistence of some isomers was discovered. γ-HCH was therefore isolated and purified to 99%, and marketed as the well-known pesticide Lindane (Willett et al., 1998).

The environmental persistence, bioaccumulation and toxicity effect of the HCHs isomers were then demonstrated, and the use of technical HCH was banned or restricted in many developed countries from the 1970s, followed by developing countries in the 1980s (Li, 1999). Lindane also became highly scrutinized and its persistence, bioaccumulation in the food chain, toxicity, including neurological, reproductive, immunological, and suspected carcinogenic effects were further established (ATSDR, 2005). As a result, γ-HCH production and use has been prohibited in many countries in the last few decades (Breivik et al., 1999; Hauzenberger, 2004). In 2004, the Stockholm Convention added the HCHs in the list of persistent organic pollutants (POPs) and banned the production of α-, β- and γ-HCH in 2009 (UNEP, 2009). Their production and agricultural use is now banned in the 179 countries that are parties to the Convention, although pharmaceutical use of γ-HCH, e.g. to control head lice and scabies, is allowed until 2015.

Extensive production and use of HCHs has led to two major types of environmental pollution, i.e. point source and diffuse contamination (Bhatt et al., 2009; Lal et al., 2010). As a consequence of over 60 years of lindane production, mixtures of the other isomers, mainly enriched in α- and β-HCH, were dumped around production sites. Each ton of lindane producing 8 to 12 tons of wastes, a total amount of 4 to 7 million tons have been generated worldwide (Vijgen et al., 2011). In addition to open-air stockpiling, improper management and illegal disposal still cause high levels of contamination around former HCHs production sites, calling for remediation of these sites, including the last operating lindane production facility in India. Furthermore, dispersion from stockpiles through e.g. wind or leaching to groundwater, and direct spreading as pesticide led to the propagation of lower concentrations of HCHs. HCHs are persistent in all environmental compartments, i.e. air, water, sediment and soil, as well as in food commodities, fish, mammals and human blood (Lal et al., 2010).

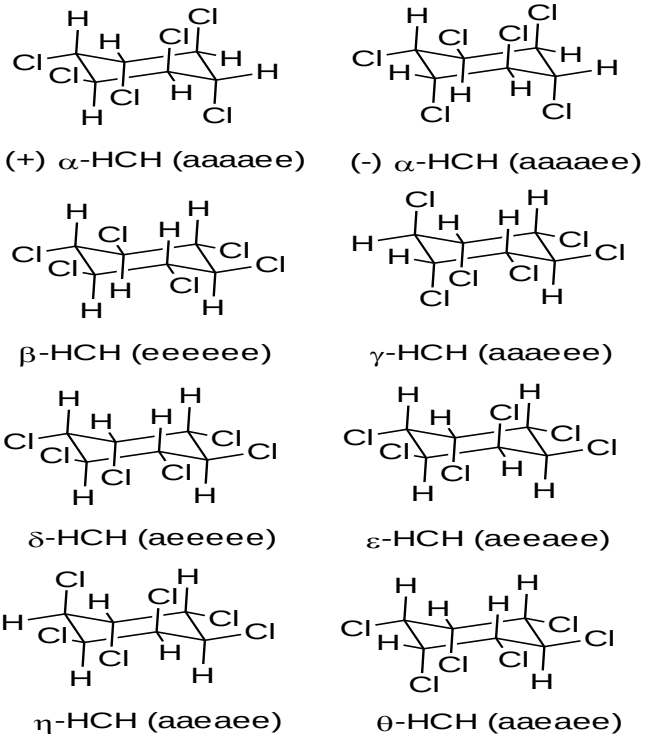


Figure 2. Structure of the HCHs isomers, including the two α enantiomers. They differ by the position of the chlorine atom in the molecule, i.e. axial (a) vs. equatorial (e), which confer them different reactivity (see main text).

Table 2. Physico-chemical properties of the four major HCHs isomers. Molecular mass (M_w), density (ρ), melting (T_m) and boiling (T_b) temperatures, water solubility (S_w), vapor pressure (V_p), octanol-water (K_{ow}) and organic carbon (K_{oc}) partition coefficients. All data are from ATSDR (1997b) except [a]vapor pressures (Li et al., 2011) and [b]data from the US EPA Technical factsheet on lindane.

Isomer	α-HCH	β-HCH	γ-HCH	δ-HCH
Formula	$C_6H_6Cl_6$	$C_6H_6Cl_6$	$C_6H_6Cl_6$	$C_6H_6Cl_6$
CAS number	319-84-6	319-85-7	58-89-9	319-86-8
M_w [g·mol^{-1}]	290.83	290.83	290.83	290.83
ρ [g·mL^{-1}]	1.87	1.89	1.89	
T_m [°C]	159	314	112	141
T_b [°C]	288 at 101 kPa	60 at 67 Pa	323.4 at 101 kPa	60 at 48 Pa
V_P [Pa][a]	4.4E-02	4.3E-05	3.5E-03	2.0E-03
S_w [mg·L^{-1}]	69.5 at 28°C		7.3 at 25°C[b]	
Henry's law constant	6.9E-06	4.5E-07	3.5E-06	2.1E-07
K_{ow}	6310	6026	5248	13804
K_{oc}	3715	3715	1000-3715	6310

Due to their structural differences, i.e. the axial vs. equatorial orientation of the chlorine atoms (Figure 2), the isomers have different physical and chemical properties as well as reactivity (Table 2; Willett et al., 1998). Mostly the α (two axial chlorines) and γ (three axial chlorines) isomers are more unstable and therefore more reactive than the β (only equatorial chlorines) and δ (one axial chlorine) isomers (Li et al., 2011). As a result, each isomer exhibits different occurrence and fate in the environment (Walker et al., 1999; Willett et al., 1998). For example, the α and γ isomers predominate in air and seawater because they have higher volatilities and Henry's law constants than β- and δ-HCH (Wania and MacKay, 1996). Consequently, α- and γ-HCH have been transported to remote areas, such as the Arctic, Antarctic and Pacific ocean (Bhatt et al., 2009; Lal et al., 2010). HCHs are generally considered relatively hydrophobic compounds. If released in groundwater or surface water, they will partition to soils and sediments (Li et al., 2011). This is also reflected by their relatively low polarity and high organic carbon partition coefficient (K_{oc}; Table 2). Since β-HCH exhibits lower vapor pressure, higher Henry's law constant and octanol water partition coefficient(K_{ow}), it is often found in soil (Li et al., 2011).

Once in the soils, HCHs can volatilize to the atmosphere or undergo degradation. Abiotic degradation of α-, β-, γ- and δ-HCH has been reported, and is generally more favorable under basic conditions (Bhatt et al., 2009). Microbial degradation under both aerobic and anaerobic conditions, as well as by pure and mixed cultures, has also been demonstrated and occurs with degradation rates increasing in the order α > γ > δ > β (Bhatt et al., 2009; Li et al., 2011; Willett et al., 1998). *Clostridium sp.* and *Pseudomonas sp.* are commonly known for anaerobic and aerobic degradation, respectively, of the four isomers (Bhatt et al., 2009). Bioremediation has recently been suggested as a strategy for decontamination of HCHs polluted site, although the studies pointed out the need for efficient monitoring methods (Alvarez et al., 2012; Lal et al., 2010; Phillips et al., 2006).

1.3 Compound Specific Isotope Analysis - CSIA

1.3.1 Isotopes basics

The discovery of radioactivity by Henry Becquerel and Marie and Pierre Curie at the end of the 19th century led to the description of radioactive decay and subsequently to the fact that a same element can exhibit different molecular weights. The technical advances and discovery of the neutron confirmed the existence of isotopes, so called from the greek "isos", equal, and "topos", place. Isotopes of an element have the same number of electrons and protons, but different number of neutrons, leading to a different mass. Joseph John Thomson, a British physicist, was first to identify the electron by measuring the charge-to-mass ratio (*q/m*) of the cathode rays. After modification of his apparatus, he determined the *q/m*

ratio of heavier particles, confirming the existence of isotopes. This modified instrument was the first prototype of mass spectrometer (MS) (Criss, 1999).

Elements have commonly one major stable isotope, often with the greatest natural abundance, and can have one or more other stable isotopes. For most of the elements, the lightest isotope is also the most abundant. Heavier isotopes tend to form shorter and more stable bonds, to occupy smaller volumes and to diffuse more slowly than lighter isotopes. As a result, environmental processes, such as degradation, evaporation, gas-phase diffusion, have slightly different rates for each isotope. This so called kinetic isotope effect (KIE) is measurable by comparing the isotope ratio of elements involved in the process (Aelion, 2010).

These ratios are reported as the ratio of the abundance of the heavy (*h*) to the light (*l*) isotope of the element E:

$$R = \frac{^{h}E}{^{l}E} \qquad \text{(eq. 1)}$$

The stable isotope composition of a sample (smp) is usually measured by mass spectrometry towards a standard of known isotopic composition and reported in the delta (δ) notation, given in per mil (‰), as:

$$\delta^{h}E = \left(\frac{R_{smp}}{R_{ref}} - 1\right) \cdot 1000\ [‰] \qquad \text{(eq. 2)}$$

As a result, positive δ values express an enrichment of the heavier isotope in a sample relative to the reference, whereas negative δ values reflect a depletion.

1.3.2 Stable carbon isotopes

Carbon has two stable isotopes ^{12}C and ^{13}C, with a mean global isotope ratio of

$$R = \frac{^{13}C}{^{12}C} = 0.0011237.$$

This reflects a ^{13}C natural abundance of about 1%.

Stable carbon isotopes analysis is widely used in environmental studies. The most common technique for this purpose is isotope ratio mass spectrometry (IRMS), either coupled to a gas chromatograph (GC-IRMS) or to an elemental analyser (EA-IRMS; Hofstetter and Berg, 2010).

The international standard used for carbon is Vienna Pee Dee Belemite (VPDB)

1.3.3 Stable chlorine isotopes

Chlorine has two stable isotopes ^{35}Cl and ^{37}Cl, with a natural abundance of about 76 and 24%, respectively. Standard mean ocean chloride (SMOC) is the commonly used standard reference to report Cl isotope composition of materials. SMOC is defined from seawater from the North Atlantic, which is considered homogenous.

Stable chlorine isotopes were first measured by off-line methods such as thermal ionization mass spectrometry (TIMS; Magenheim et al., 1994; Xiao and Zhang, 1992) as cesium chloride (CsCl), and was successfully adapted to the measurement of DDT (Holmstrand et al., 2004). Holt et al. (1997) developed a method for $\delta^{37}Cl$ analysis of chlorinated volatile organic compounds, measuring methyl chloride (CH_3Cl) on a dual-inlet triple collector isotope ratio mass spectrometer (DI-IRMS). On-line methods were then investigated. Shouakar-Stash et al. (2006) directly coupled a GC to an IRMS with continuous flow (CF-IRMS) to determine $\delta^{37}Cl$ values of pure phase and aqueous CEs. At the same time, Van Acker et al. (2006) coupled a GC to a high-resolution multiple collector inductively coupled plasma mass spectrometer (MC-ICP-MS) to measure $\delta^{37}Cl$ values of pure phase PCE and TCE.

Sakaguchi-Söder et al. (2007) first determined $\delta^{37}Cl$ values of CEs during both pure phase and chemical processes, using a standard benchtop quadrupole GC/MS system. Since then, several GC/MS based methods have been developed for $\delta^{37}Cl$ determination and allowed for a wider application of this technique in environmental studies (Aeppli et al., 2010b; Bernstein et al., 2011; Jin et al., 2011).

1.4 CSIA, OCls and NA

1.4.1 Quantification of isotope fractionation during transformation processes

The recent technical developments in CSIA now allow the determination of both $\delta^{13}C$ and $\delta^{37}Cl$ in organic compounds, such as OCls, at environmentally relevant concentrations. As mentioned in the previous section, the organic compounds undergo transformation processes in the environment. In the case of chemical or biological transformations, such as degradation reactions, bonds are broken in the molecule. Since the heavy and light isotopes exhibit different reaction rates (*k*), this kind of process can have a large effect on the isotopic composition of the target compound. As a consequence, the breaking of a chemical bond during e.g. degradation gives rise to a kinetic isotope effect for the element E (KIE_E) involved in the bond breaking:

$$KIE_E = \frac{{}^{l}k}{{}^{h}k} = \frac{1}{\alpha_E} \qquad \text{(eq. 3)}$$

Commonly, molecules containing light isotopes react faster than molecules containing heavy isotopes, giving rise to a measurable fractionation factor (α_E), often reported as the isotope enrichment factor (ε_E) in the per mil scale:

$$\varepsilon_E = (\alpha_E - 1) \cdot 1000\ [‰] \qquad \text{(eq. 4)}$$

This implies that during the course of contaminant degradation, the remaining fraction (*f*) of the primary contaminant (substrate) becomes enriched in the heavier isotope whereas the products may

become depleted in heavier isotopes. This is expressed by a Rayleigh-type equation, initially derived by Lord Rayleigh to describe fractional distillation of mixed liquids (Schmidt et al., 2004):

$$R = R_0 \cdot f^{\alpha_E - 1} = R_0 \cdot f^{\varepsilon_E / 1000} \qquad \text{(eq. 5)}$$

Where R corresponds to the isotopic composition of the substrate at a certain time of the degradation, and R_0 its initial composition. This equation is commonly used in its linearized from:

$$\ln\left(\frac{R}{R_0}\right) = (\alpha_E - 1) \cdot \ln f = \frac{\varepsilon_E}{1000} \cdot \ln f \qquad \text{(eq. 6)}$$

Introduction of eq. 2 yields:

$$\ln\left[\frac{(\delta^h E + 1000)}{(\delta^h E_0 + 1000)}\right] = (\alpha_E - 1) \cdot \ln f = \frac{\varepsilon_E}{1000} \cdot \ln f \qquad \text{(eq. 7)}$$

1.4.2 Quantification of the extent of degradation using 1D-CSIA

The isotope enrichment factor (ε_E) of a degradation reaction can be determined in laboratory experiments with pure or mixed cultures (Elsner et al., 2005). As a result, by measuring the isotopic composition of a contaminant at the source of contamination and further downstream in a contaminated aquifer, the extent of degradation (*B*) can be calculated as:

$$B = 1 - f = 1 - \left[\frac{1000 + \delta^h E}{1000 + \delta^h E_0}\right]^{1000/\varepsilon_E} \qquad \text{(eq. 8)}$$

This approach has been used at numerous field sites using $\delta^{13}C$ (see Elsner, 2010 and Thullner et al., 2012 for reviews). However, the use of other isotopic systems has so far been hampered by the lack of effective techniques. Sturchio and co-workers were the first to demonstrate that $\delta^{37}Cl$ could be used to evaluate ongoing reductive dechlorination of TCE (Sturchio et al., 1998).

1.4.3 Identification of reaction pathways using 2D CSIA

Van Warmerdam et al. (1995) measured the $\delta^{13}C$ and $\delta^{37}Cl$ of chlorinated solvents and demonstrated that the combination of the two isotopic systems (2D-CSIA) could be used to distinguish contaminant sources. A recent model-based work on the carbon-chlorine isotopic system established its applicability to assess reaction pathways and mechanisms at both laboratory and field scales (Hunkeler and Van Breukelen, 2009). Combining both $\delta^{13}C$ and $\delta^{37}Cl$ may, for example, distinguish between the two possible degradation pathways of cDCE and VC, e.g. oxidation and reductive dechlorination (Figure 1), each leading to different $\varepsilon_{Cl}/\varepsilon_C$ ratios.

This 2D approach has further been investigated and the calculation of an apparent kinetic effect ($AKIE_E$) has been proposed to obtain a mechanism-diagnostic measure, by removing the influence of non-reactive positions and intramolecular competition of isotopes involved in the bond breaking (Elsner and Hunkeler, 2008):

$$AKIE_E = \frac{1}{1+z_E \cdot \frac{n_E}{x_E} \cdot \varepsilon_E} \qquad \text{(eq. 9)}$$

Where n_E is the total number of atoms of the element E in the compound, x_E the number of atoms of the element E at the reactive position and z_E the number of atoms of the element E in identical reacting positions. Then the calculation of the $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratio produces a direct mechanism-specific diagnostic (Abe et al., 2009).

2D-CSIA has mainly been demonstrated for benzene, toluene and methyl *tert*-butyl ether (MTBE), using $\delta^{13}C$ and $\delta^{2}H$ (Fischer et al., 2007). Applications of 2D-CSIA using $\delta^{13}C$ and $\delta^{37}Cl$ for chlorinated compounds were so far investigated for cDCE, VC and polychlorinated ethanes, but neither for PCE, TCE nor HCHs (Elsner, 2010).

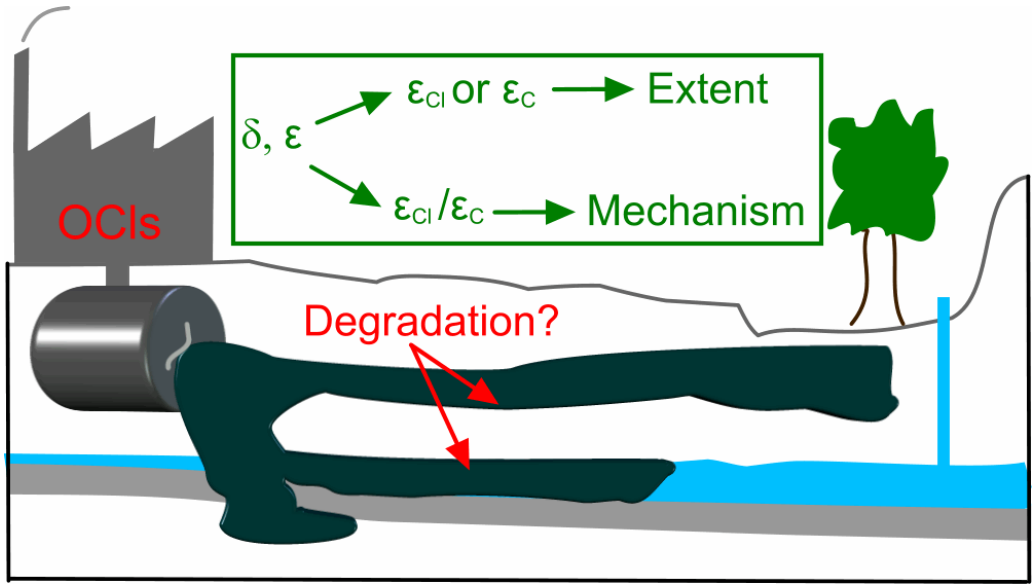


Figure 3. The big simplified picture: organochlorines (OCls) pollutants such as chloroethenes (CEs) and hexachlorocyclohexanes (HCHs) can be degraded by indigenous bacteria in the groundwater and/or soil matrices. Compound specific isotope analysis (CSIA) allows for direct quantification of the degradation (1D-CSIA), as well as identification of the related reaction mechanisms (2D-CSIA), in order to evaluate the feasibility of natural attenuation (NA) as remediation strategy for these compounds at contaminated sites.

2. METHODS

2.1 Sites description

In order to investigate the feasibility of CSIA for CEs and HCHs, two sites with a history of contamination were selected within the isoSoil project. Details about the sites, the soil and groundwater sampling, the degradation experiments and the $\delta^{13}C$- and $\delta^{37}Cl$-CSIA can be found in the respective papers. Therefore only brief descriptions are provided in this section.

2.1.1 SAP, a CEs contaminated site in Czech Republic (Papers I and II)

The North Bohemia Carcass Disposal Plant (SAP) Mimoň belongs to the largest and most intensive CEs´ contaminations of soil and groundwater in the Czech Republic. The factory is situated on the bank of a small river (Ploučnice), in the river valley. PCE was used, from 1963 to 1988, for fat extraction from processed material, e.g. animal carcasses, slaughterhouse waste, food production waste, to obtain final meat-bone powder and clean fat for further use, e.g. chemical and cosmetics production, pet food production, fertilizers. The factory now uses an extraction technology based on thermo-mechanical procedure without extracting agents for the treatment of processed raw material.

The PCE consumption was evaluated at about 160-200 tons per year; total consumption was estimated at 4,250 tons. Frequent operational leakages caused a large CEs plume in a sandstone aquifer. The pollution spread from the factory according to the main groundwater flow direction. The spreading was increased by pumping at waterworks wells, downstream from the source of pollution. Contamination of drinking water from waterworks was the first sign of contamination, detected in 1988.

The total amount of leaked PCE was evaluated to range from 149 to 246 tons (95% confidence interval, CI). In 1997, the highest pollution level was present in the 2-20 m layer in an area of about 10 ha, in Quaternary sediments and weathered Cretaceous sandstones. The contamination was drained to the neighbouring river. During 11 years, from 1997 to 2008, intensive pump-and-treat together with air sparging and venting treatment was applied at the site, which significantly decreased the plume extent. Approximately 140 tons of PCE were extracted from the site subsurface and the plume extent was reduced to less than one hectare. The clean-up is still under operation at the site.

Several investigation methods, run between 2005 and 2008, allowed to gather information on the extent and pattern of the contamination plume (Larsen et al., 2008). The methods used included groundwater sampling from multi-level sampling points with filter not longer than 1.5 m, soil probing with membrane interface probing (MIP), tree core sampling, geophysical methods and soil core testing with hydrophobic dye. The survey revealed another contaminated plume which has not been influenced by remediation technique applied at the site. This part of the site was investigated in **Papers I** and **II**.

Maps and detailed descriptions of the geological structure and hydrogeochemical settings of the site are given in **Paper I** and related supporting information (SI).

2.1.2 Spolana, a HCHs contaminated site in Czech Republic (Papers III and IV)

Spolana Neratovice is one of the leading chemical companies active in the Czech industry. Its volume of revenues makes the company the fourth largest chemical plant in the Czech Republic. The primary scope of business involves the petrochemical and chemical production of suspension polyvinyl chloride and PVC granulates, linear alpha olefins (LAO) and LAO based products, caprolactam as an intermediate for polyamide fibers and engineering plastics, and inorganic compounds such as sodium hydroxide, liquid chlorine, hydrochloric acid, sulphuric acid, sodium hypochlorite, or ammonium sulphate (Klánová et al., 2006).

However, between 1952 and 1975, Spolana was one of the two largest producers of pesticides in former Czechoslovakia. Pesticides containing DDT were produced between 1958 and 1969, technical HCH since 1961 and lindane (pure γ-HCH) preparations until 1975. A total of 60,000 tons of technical HCH and more than 3,000 tons of pure lindane were produced.

Production buildings and their surroundings were heavily contaminated with pesticides, but, due to the applied technology, significant contamination of the buildings with polychlorinated dioxins (PCDDs) and furans (Fs) was also observed. PCDDs and Fs contaminated buildings have been subject to remediation in recent years using a base catalyzed decomposition (BCD) technology. Soil in the unsaturated zone was removed and decontaminated as well. Remediation was completed in 2008.

There are, however, areas of contaminated soils which were not subject to any remediation. Beside HCHs and DDTs, hexachlorobenzene (HCB) was also produced at Spolana and, and can be found at high concentrations in soils together with its starting material trichlorobenzene. As the soil contamination is several decades old, indications of active transport and microbial degradation processes are observed. While HCH contamination originated either from Lindane or technical HCH (majority of α-HCH) spills, β-HCH, which is most resistant to microbial degradation, is found at the highest concentration levels today.

Soil from this highly HCHs contaminated part of the site was sampled for incubation experiments in **Paper III** and CSIA-based investigation of HCHs contamination in **Paper IV**, where maps are also provided.

2.2 Groundwater and soil sampling

2.2.1 Groundwater and soil sampling at SAP (Papers I and II)

The groundwater (**Paper I**) and soil (**Paper II**) samplings at SAP site were carried out by AECOM, Czech Republic. Sampling locations, instrumentation and analyses are described in the papers.

For the site investigation (**Paper I**), 14 groundwater samples were collected from existing wells, according to US EPA guidelines (Hunkeler et al., 2008). The groundwater table level (GWT), pH, temperature, dissolved oxygen concentration and conductivity were measured in the field before sampling. CEs concentration analyses and inorganic parameters determination (chloride, nitrate, sulfate and soluble iron and manganese) were performed in the laboratory.

For the PCE and TCE degradation experiments (**Paper II**), three soil cores were taken in the contaminated zone of SAP, each exhibiting different levels of CEs contamination.

2.2.2 Soil sampling at Spolana (Papers III and IV)

Two sets of soil samples were collected at Spolana site by Masaryk University (MU), Czech Republic. Maps, instruments and physicochemical and biological parameters of the soil samples are reported in the papers and related SI.

Four stratified soil samples were collected from two HCHs contaminated locations in the grounds left after the destruction of a former pesticide production building. Samples were taken from the non-saturated (aerobic) surface (0-50 cm), as well as from the saturated (anaerobic) zone (180-220 cm) and were incubated for α-HCH degradation experiments (**Paper III**).

Ten other samples were taken from the top 10 cm soil layer and were selected to investigate the spatial distribution of HCHs contamination (**Paper IV**).

Soil samples were extracted by liquid-liquid extraction with dichloromethane (DCM) and HCHs concentration were analyzed by GCMS.

2.3 Degradation experiments (Papers II and III)

The degradation experiments were performed at the Hellenic Center for Marine Research (HCMR) in Heraklion, Crete, Greece.

2.3.1 Soil incubation and biodegradation experiments

For PCE, TCE (**Paper II**) and α-HCH (**Paper III**) degradation studies, all collected soil samples were incubated. However, in each case, one sample was selected for the implementation of the anaerobic degradation experiments. The soil was mixed with an anaerobic basal medium (Cole medium; Cole et al., 1994). The cultures were then spiked with a stock solution of the target compounds and a mixture of

butyric acid, propionic acid and ethanol as electron donor. The bottles were incubated at 30 °C, and the maintenance of anaerobic conditions during the preparation of cultures and throughout the biodegradation experiments was verified by a redox color indicator. The progress of biodegradation was monitored in all cultures by analyzing the concentration of PCE, TCE or α-HCH at regular time intervals.

2.3.2 DNA extraction, clone library construction and sequence analysis of the 16S rRNA genes

The microbial community composition changes during degradation of PCE (**Paper II**) and α-HCH (**Paper III**) were investigated via 16S rRNA gene clone library analysis, For each experiment, three samples were selected, corresponding to different remaining fraction (*f*) of PCE and α-HCH respectively, and representing the initial, middle and end stages of the degradation.

In both cases, 16S rRNA genes were PCR amplified from mixed genomic samples by using the universal eubacterial primers of 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-GGYTACCTTGTTACGACTT-3'). The operational taxonomic units (OTUs) were defined at a minimum sequence similarity of 98%. The 16S rRNA gene sequences were deposited in GenBank.

2.4 Carbon and chlorine CSIA (all papers)

2.4.1 Stable carbon isotope analysis – δ^{13}C-CSIA

All $\delta^{13}C$ analyses were performed at the University of Bristol (UB) using gas chromatograph connected via a combustion interface to an isotope ratio mass spectrometer (GC-C-IRMS) system.

The samples from SAP site (**Paper I**) and from the CEs degradation experiments (**Paper II**) were extracted with cyclopentane. The $\delta^{13}C$ values of PCE and TCE from the extracts were determined on a Thermo DeltaPlusXL IRMS coupled to a HP 6890 GC with split/splitless injector via a GC/C-III interface (HP, Palo Alto, California, United States; Thermo Finnigan, Bremen, Germany). The extracts were injected on to the GC column in splitless mode before separation on a 30-m BP-624 column (0.25 mm i.d.; 1.4 μm film thickness). Helium was used as the carrier gas at 1.2 $mL \cdot min^{-1}$ constant flow rate.

The $\delta^{13}C$ measurements of HCHs (**Papers III** and **IV**) were performed on a Thermo Finnigan Trace GC with a PTV inlet and a CTC Analytics GC Pal Autosampler, coupled to a Thermo DeltaPlusXP IRMS (HP, Palo Alto, California, United States; Thermo Finnigan, Bremen, Germany). The samples were injected on to a 50-m HP1 column (0.32 mm i.d.; 0.17 μm film thickness, J&W Scientific). Helium was used as the carrier gas at a constant flow rate of 1.1 $mL \cdot min^{-1}$.

Details about the sample extractions and GC temperature programs are provided in the respective papers. Replicate injections of target compounds (n = 3) led to an average standard deviation (SD) of the

$\delta^{13}C$ measurements ranging from ±0.1‰ to ±0.8‰ vs. VPDB depending on the study and analyzed compounds.

2.4.2 Stable chlorine isotope analysis – $\delta^{37}Cl$-CSIA

The $\delta^{37}Cl$ analyses were carried out using a gas chromatograph quadrupole mass spectrometer (GCqMS) system, following a procedure developed by Aeppli et al. (2010) within the frame of the isoSoil project. In this method, the analytes are bracketed five times with an isotopic standard of the same compound. For each sample, the concentrations of the isotopic standards solutions were adjusted to match the samples' concentrations within a 20% interval.

After liquid-liquid extraction with cyclopentane, the samples from SAP site (**Paper I**) and from the CEs degradation experiments (**Paper II**) were analyzed for $\delta^{37}Cl$ determination of PCE and TCE. The extracts (1 µL) were injected in the GCqMS system (GC 8000 gas chromatograph with MD-800 mass analyzer, Fisons, Manchester, UK) on to a 30-m SLB-5MS column (0.25 mm i.d., 0.25 µm film thickness; Supelco, Sigma-Aldrich, PA, USA). PCE and TCE were measured on masses of two molecular ions containing zero and one ^{37}Cl, respectively, i.e. *m/z* 130 and 132 for TCE, 164 and 166 for PCE. The $\delta^{37}Cl$ values are reported relative to the international Standard Mean Ocean Chlorine (SMOC). To this end, the $\delta^{37}Cl$ values of the PCE and TCE isotopic standards were determined vs SMOC using thermal ionization mass spectrometry (TIMS) according to published procedures (Aeppli et al., 2010; Holmstrand et al., 2004). The obtained average analytical precision of the $\delta^{37}Cl$ analysis was ±0.6‰ vs. SMOC. This includes the standard deviation from the GCqMS measurements (n = 5 sample/standard pairs) and the propagated standard deviation from the TIMS measurements of the authentic standards.

The $\delta^{37}Cl$ measurements of HCHs (**Papers III** and **IV**) were performed on a GCqMS instrument (HP 6890 Series GC/MS, Agilent Technologies, Germany), equipped with a HP 7673 Automatic Liquid Sampler and an on-column inlet. The extracts (1 µL) from the α-HCH degradation experiments (**Paper III**) were injected on to a 30-m SLB-5MS column (0.25 mm i.d., 0.25 µm film thickness; Supelco, Sigma-Aldrich, PA, USA), using Helium as the carrier gas at a constant column head pressure of 9.0 psi. For the field site investigation (**Paper IV**), the four present HCHs isomers were separated on to a 60-m SLB-5MS column (0.25 mm i.d., 0.25 µm film thickness; Supelco, Sigma-Aldrich, PA, USA), using Helium at a constant column head pressure of 19.0 psi. Two masses of the most abundant fragment, i.e. *m/z* = 181 and 183, were recorded in the single ion monitoring (SIM) mode using positive electron impact (EI+) ionization. The average analytical precision of the $\delta^{37}Cl$ measurements was ±0.5‰ vs. a reference standard of the analyzed isomer (n = 5), corresponding to the SD of the GCqMS measurements.

Detail about the sample extractions, GC temperature programs and calibration procedures are described in details in the respective papers and corresponding SI.

3. SUMMARY AND DISCUSSION OF THE MAIN RESULTS

3.1 CEs contamination at SAP (Papers I and II)

3.1.1 Overview of the extent of CEs contamination based on hydrogeochemical, concentrations and isotopic data

Investigations of contaminant propagation at field sites commonly look first at the hydrogeochemical parameters, which give qualitative information about the redox conditions present in different parts of a contaminated plume (Aeppli et al., 2010a; Christensen et al., 2000; Hunkeler et al., 2011a). In the investigated aquifer at SAP site, the concentrations of the hydrogeochemical parameters, i.e. nitrate, sulfate, oxygen, iron and manganese, suggest mixed redox conditions, as typical for numerous CEs' contaminated field sites (Amaral et al., 2011; Christensen et al., 2000). Therefore anoxic areas with e.g. sulfate reducing conditions are present in the aquifer. Most of the wells presented anaerobic conditions, where PCE and TCE reductive dechlorination is expected (Figure 1), although some oxic areas were identified. Since the site is located close to a river bank, some wells are influenced by seasonal river infiltration and switches from anaerobic to aerobic conditions.

In addition, analyses of the CEs' concentrations suggested ongoing PCE and TCE microbial hydrogenolysis with cDCE accumulation (**Paper I**). A decrease in PCE and TCE was indeed observed along the general groundwater flow path and cDCE and VC were detected at some wells. This was confirmed by the laboratory experiment performed for PCE degradation using an enriched mixed culture from the site, where dechlorination of PCE to cDCE via TCE was reported (**Paper II**).

The isotopic data also pointed towards ongoing PCE and TCE degradation (**Paper I**). In anoxic parts of the aquifer, enrichment in both $\delta^{13}C$ and $\delta^{37}Cl$ values was observed. In contrast, wells located in oxic parts of the site, showed no or little enrichment in $\delta^{13}C$ and depletion in $\delta^{37}Cl$, illustrating pure transport of the contaminant from the source zone. In some oxic wells, however, slight enrichment in isotopic values can be explained by transformation of the contaminant in anoxic areas during transport to the oxic pocket. In parts influenced by the river, the seasonal switch between aerobic and anaerobic conditions led to enrichments in both isotopes.

3.1.2 In depth interpretation of the extent of CEs contamination based on laboratory experiments

The laboratory experiments determined the microbial community changes during PCE degradation (**Paper II**). The microbial culture was first dominated by an OTU closely related to *Clostridium* sp. strain DR7, while *Desulfitobacterium aromaticivorans* UKTL took over at the end of the degradation process. Bacteria from the genus *Desulfitobacterium* are known for dechlorination processes (Villemur et al., 2006) and *Clostridium* spp. is known for fermentation processes (Chang et al., 2000; Smidt and de Vos,

2004). This suggested that both bacteria could be involved in PCE hydrogenolysis at SAP. In addition, fermentation by *Clostridium* spp. probably produced H_2 that could be further used as electron donor for *Desulfitobacterium* spp. (Flynn et al., 2000; Lee et al., 2011), explaining its growth.

The isotopic enrichments factors were determined for PCE and TCE and for both C and Cl isotopes. For PCE, the ε_C value was −5.6 ± 0.7‰ (95% CI; n = 11, R^2 = 0.96, SE 0.3‰) and is in the range of published values for enriched mixed cultures, i.e. −2‰ (Hunkeler et al., 1999) to −7‰ (Liang et al., 2007), but lower than values reported for abiotic processes. The ε_{Cl} value was −2.0 ± 0.5‰ (95% CI, n = 10, R^2 = 0.91, with 0.2‰ SE) and was comparable to the range −0.8 to −7.8‰ estimated from the field values in **Paper I**. The resulting process diagnostic $\varepsilon_{Cl}/\varepsilon_C$ ratio for PCE reductive dechlorination was 0.35 ± 0.11 (95% CI, n = 10, R^2 = 0.87, with 0.05‰ SE), and was lower than the field-derived values of 0.42 to 1.12. The related apparent kinetic isotope effect ratio $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ was 0.71.

For TCE, the ε_C value was −8.8 ± 2.0‰ (95% CI, n = 10, R^2 = 0.92, with 0.9‰ SE) and is in the range of literature values for enriched mixed cultures, i.e. −2.5‰ (Bloom et al., 2000) to −16.0‰ (Lee et al., 2007). The ε_{Cl} value was −3.5 ± 0.5‰ (95% CI, n = 10, r^2 = 0.97, with 0.2‰ SE). Both ε_C and ε_{Cl} values were also comparable to values reported for TCE abiotic degradation. The process diagnostic $\varepsilon_{Cl}/\varepsilon_C$ ratio for TCE biotic degradation was 0.37 ± 0.11 (95% CI, n = 10, r^2 = 0.88, with 0.04 SE), with a $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratio of 0.59. The difference from the value calculated for PCE is probably due to differences in the enrichment cultures or rate limiting but non-fractionating pre-equilibrium steps (Elsner et al., 2005).

Mechanistic study of PCE and TCE degradation suggested a dissociative electron transfer as initial reaction step (Glod et al., 1997). In contrast cDCE and VC form a carbon-cobalt bond and an $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratio of 0.08 was reported for the biotic transformation of cDCE to VC (Abe et al., 2009; Figure 4). The study in **Paper II** therefore supports that 2D-CSIA can be used to elucidate reaction mechanisms, through $\varepsilon_{Cl}/\varepsilon_C$ or $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratios.

3.1.3 Quantification of PCE extent of degradation and TCE source apportionment

The extent of PCE degradation B_{PCE} was calculated according to eq. 8. The average estimates from the $\delta^{13}C$ field values and based on two published ε_C values yielded $B_{C\text{-}PCE}$ of 13 ± 9% and 37 ± 20% depending on the applied ε_C values. The calculations with the laboratory determined ε_C values led to $B_{C\text{-}PCE}$ = 16 ± 10%, while the same calculations with ε_{Cl} values led to $B_{Cl\text{-}PCE}$ = 32 ± 21%, with an average residual B_{Cl}-B_C of 10%. Therefore both C and Cl based estimates are valid and determination of ε_C values using enriched culture from the investigated site help reducing the uncertainties.

At SAP, $\delta^{37}Cl$ values of TCE were above the $\delta^{37}Cl$ values for PCE. From this observation and since the TCE isotopic trends are dependent on its relative degradation rate to PCE degradation (Hunkeler and

Van Breukelen, 2009), TCE could either originate from pure PCE degradation, if TCE degradation rate is higher than PCE, or could be both a product of PCE degradation and an original source if PCE reacts faster, which is commonly assumed (Wiedemeier et al., 1999).

3.1.4 Implication for NA of CEs at SAP

The analysis of CEs concentrations, $\delta^{13}C$ and $\delta^{37}Cl$ data together with hydrogeochemical parameters and the microbial degradation study suggested ongoing reductive dechlorination of PCE and TCE and accumulation of cDCE in some parts of the site. This hypothesis was also supported by a reactive transport model. While NA would not be a suitable method for SAP remediation, the presence of anoxic areas would allow for enhanced or stimulated NA.

The process diagnostic $\varepsilon_{Cl}/\varepsilon_C$ ratios determined in the laboratory and in the field agreed to a large extent. Microbial variability can indeed translate into variability at the field site, whereas the laboratory experiments are more controlled. The laboratory derived $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratio is probably specific for the identified bacteria, and other bacteria might exhibit other values (see Figure 4 for comparison of some $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratios).

These two studies therefore showed that combining 2D CSIA and microbial data clearly allow for a thorough evaluation of groundwater contamination at a field site compared to concentration based methods.

3.2 HCHs contamination at Spolana (Papers III and IV)

3.2.1 Overview of the extent of HCHs contamination based on concentrations and isotopic data

The analysis of the HCHs concentrations at Spolana showed that HCHs originate from two buildings. Since Lindane production generates mostly α-HCH as byproduct (Vijgen et al., 2011), α-HCH was found at the highest concentration level, followed by γ-HCH, then by the more recalcitrant δ-HCH and β-HCH (Li et al., 2011; Willett et al., 1998).

Samples were taken from two different depths, i.e. in the aerobic unsaturated zone at 20 cm and in the anaerobic saturated zone at 200 cm depth, in order to investigate the degradation patterns under oxic vs. anoxic conditions. Since the HCHs wastes have been dumped on the soil surface, the isomers might have been dispersed laterally and downwards as solid HCH residues, by particle-mediated transport, and also as dissolved phase through precipitation. The $\delta^{13}C$ signatures were highly variable for all HCH isomers, both laterally in the top soil and vertically in the soil strata (**Paper IV**). However, the α, γ and δ isomers were slightly enriched in $\delta^{13}C$ at 200 cm compared to 20 cm depth, suggesting ongoing anaerobic degradation of these isomers. In contrast, the recalcitrant β-HCH was slightly depleted in $\delta^{13}C$ at depth.

During the microbial degradation experiment performed in the laboratory (**Paper III**), no evidence of degradation was observed after extensive incubation of surface soil. Although aerobic degradation has been shown for all isomers (Bhatt et al., 2009; Lal et al., 2010; Li et al., 2011), too high level of contamination can be toxic to the bacteria and inhibit their activity (Phillips et al., 2005).

For the surface soil samples, no significant correlation was found between the isomer specific $\delta^{13}C$ values and isomer concentrations when plotted as natural logarithms according to the linearized Rayleigh equation (eq. 7; Schmidt et al., 2004), with R^2 values from 0.11 to 0.23 for α-, β-, and γ-HCH, and slopes of the regression lines not significantly different from zero (95% CI), while the δ-HCH isomer showed a weak correlation ($R^2 = 0.48$). This is most probably due to random loading of HCHs over the site, leading to different degradation trends (**Paper IV**). Since aerobic degradation seems to be limited at this site, based on the laboratory results (**Paper III**), abiotic degradation or volatilization could have occurred, although these processes are generally considered to induce negligible isotope fractionation (Meckenstock et al., 2004).

3.2.2 In depth interpretation of the extent of HCHs contamination based on laboratory experiments

Both laboratory and field results suggested potential for anaerobic degradation at the site. During the α-HCH degradation (**Paper III**), the microbial community evolved towards a higher diversity. Among the bacteria, *Clostridium sphenoide* and *Dendrosporobacter quercicolous* strain DSM1736 were identified as anaerobic fermenting bacteria, that could have participated to the reaction (Heritage and MacRae, 1977; Walther et al., 1977). The increase in diversity can be explained by the decrease in toxicity as α-HCH is degraded (Phillips et al., 2005), which further allow the growth of other bacteria.

The ε_C value determined for anaerobic α-HCH degradation, using an enriched mixed culture from the site (**Paper III**), was −0.9 ± 0.3‰ (95% CI; $n = 11$, $R^2 = 0.83$, SE 0.4‰, P-value = 0.0001). This was less negative than previously determined ε_C values of −3.7 ± 0.8‰ and −3.9 ± 0.6‰ for anaerobic reductive dechlorination of α-HCH by pure cultures (Badea et al., 2009; Badea et al., 2011). For the first time, the ε_{Cl} value was also determined as −0.4 ± 0.3‰ (95% CI; $n = 11$, $R^2 = 0.50$, SE = 0.4‰, P-value = 0.015). Combining both values led to a $\varepsilon_{Cl}/\varepsilon_C$ ratio of 0.52 ± 0.23 (95% CI; $n = 11$, $R^2 = 0.75$, SE = 0.28‰, P-value = 0.0005), which is in the range of the values for PCE reductive dechlorination reported in **Paper I**. The corresponding $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratio was 0.44 if the mechanism is considered stepwise, as suggested in the literature, but 0.89 if concerted. The 0.44 value is smaller than determined for the abiotic reductive β-elimination of polychlorinated ethanes (Hofstetter et al., 2007), as well as the values reported for reductive dechlorination of CEs in laboratory experiments (**Paper II**; Figure 4). This suggests different reaction mechanisms. Furthermore both C and Cl isotopes showed a deviation from a typical

Rayleigh behavior. Thus, and since the microbial community diversity increases, rate-limiting steps could affect the degradation kinetics.

The extent of α-HCH degradation $B_{\alpha\text{-}HCH}$ at a sample in the deep soil was estimated based on both $\delta^{13}C$ and $\delta^{37}Cl$, and using the determined ε values (**Paper IV**). The values from a standard were used as hypothetical source value. Calculations led to $B_{\alpha\text{-}HCH}$ = 64 - 100% based on $\delta^{37}Cl$, and 85 - 100% based on $\delta^{13}C$. Both estimations agreed, such as for CEs (**Paper II**) and confirmed ongoing anaerobic degradation of α-HCH. The corresponding calculations for γ-HCH using a literature ε_C value (Badea et al., 2009) led to $B_{\gamma\text{-}HCH}$ = 16 - 42%. The differences between the α-HCH and γ-HCH estimates most probably reflect non representative ε values for γ-HCH, rather than a higher degradation rate for α-HCH.

3.2.3 Implication for NA of HCHs

The CSIA investigation at Spolana revealed potential for anaerobic degradation but inhibited aerobic degradation. 2D-CSIA bears the potential to quantify degradation and elucidate the underlying processes, which is not feasible by concentration-based assessment. However, initial isotopic composition should be known and appropriate ε values chosen for an accurate monitoring.

As for CEs in **Paper I** and **II**, the ε values determined for mixed consortium were lower than those published for pure cultures. Therefore transposition of pure cultures ε values to field investigation should be considered with care. In addition, the microbial community changes might be responsible for variations in the degradation pattern at a field site and microbial characterization might be necessary in order to select the most appropriate ε values.

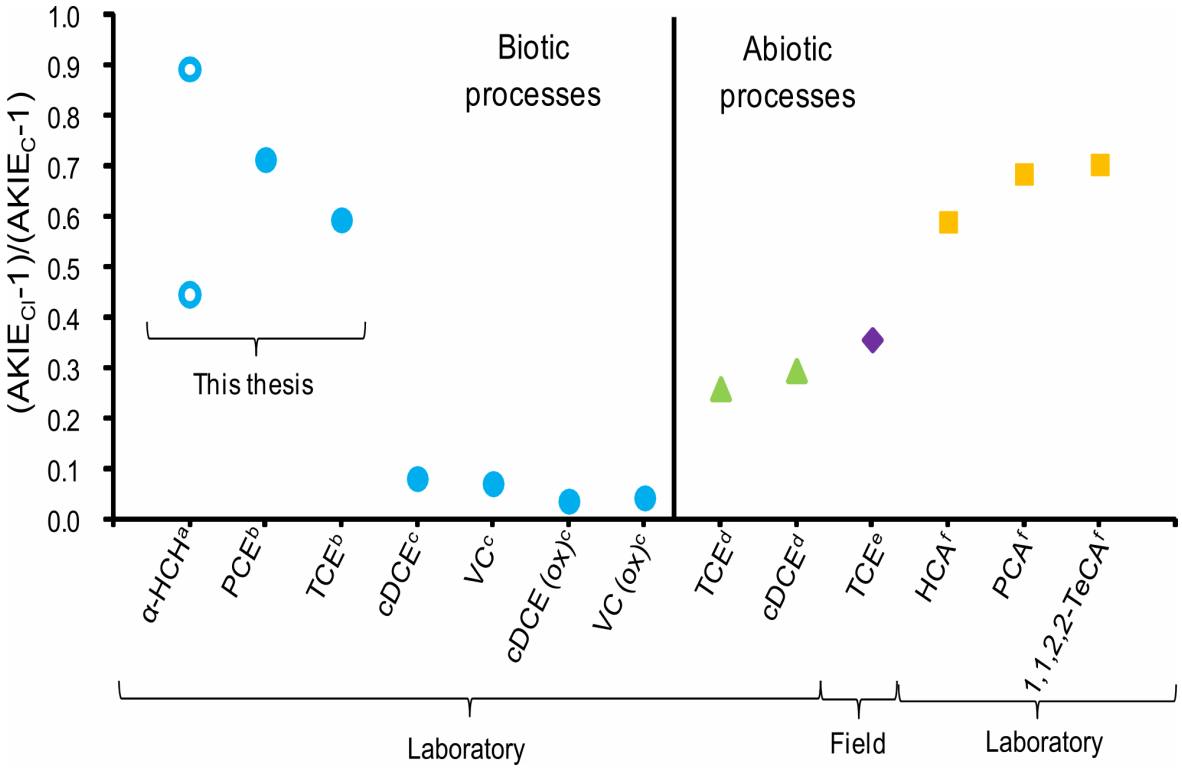


Figure 4. Contribution of this thesis to and comparison of the apparent kinetic isotope effect ratios $(AKIE_{Cl}-1)/(AKIE_C-1)$ from different field and laboratory studies (according to Abe et al., 2009), calculated from bulk ε values according to Elsner and Hunkeler (2008). Data were obtained from [a]**Paper III**, [b]**Paper II**, [c]Abe et al. (2009), [d]Audí-Miró et al. (2013), [e]Lojkasek-Lima et al. (2012). The mechanism was reductive dechlorination in all cases, except for the two aerobic oxidation data points cDCE(ox) and VC(ox) and for the date from [f]Hofstetter et al. (2007) for the reductive β-elimination by Chrome (II) of hexachloroethane (HCA), pentachloroethane (PCA) and 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA).

4. CONCLUSIONS

- The $\delta^{37}Cl$ values of CEs and HCHs can be determined by a GCqMS based analytical method. Since it only requires a benchtop GCqMS, many laboratories can use the method to determine the $\delta^{37}Cl$ of OCls. In addition, the extent of degradation of PCE and α-HCH at two contaminated field sites estimated with Cl isotopes led to results similar to C isotopes. Therefore it supports the use of $\delta^{37}Cl$-CSIA when $\delta^{13}C$ techniques are not available. This will broaden the applications of $\delta^{37}Cl$-CSIA to other OCls and for any organization dealing with contaminated sites monitoring.

- The combination of carbon and chlorine isotopes reduces the uncertainty in choosing the isotope enrichment factors needed to evaluate the sources and fate of OCls at contaminated sites. While single isotope ε values allow quantification of the degradation, the combination of $\delta^{13}C$ and $\delta^{37}Cl$ give information on potential secondary sources and ongoing reaction mechanisms.

- The ε_C and ε_{Cl} for the anaerobic degradation of PCE and TCE by a mixed culture enriched from a contaminated site were determined as $\varepsilon_C = -5.6 \pm 0.7‰$ (95% CI) and $\varepsilon_{Cl} = -2.0 \pm 0.5‰$ for PCE degradation, and $\varepsilon_C = -8.8 \pm 0.2‰$ and $\varepsilon_{Cl} = -3.5 \pm 0.5‰$ for TCE degradation. These values were in the lower range of previously determined ε_C and ε_{Cl} for reductive dechlorination of PCE and TCE using pure cultures, presumably because of the variability of the mixed consortium. The combination of both values led the mechanism-diagnostic $\varepsilon_{Cl}/\varepsilon_C$ ratios of 0.35 ± 0.11 and 0.37 ± 0.11 for PCE and TCE, respectively. The $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1)$ ratios were subsequently calculated as 0.71 and 0.59 for PCE and TCE respectively. The $\varepsilon_{Cl}/\varepsilon_C$ ratios were much higher than these determined for cDCE and VC reductive dechlorination, supporting the hypothesis that PCE and TCE degradation is initiated by another mechanism than degradation of cDCE and VC.

- The anaerobic degradation of α-HCH by a mixed culture enriched from a contaminated site yielded $\varepsilon_C = -0.9 \pm 0.3‰$ and $\varepsilon_{Cl} = -0.4 \pm 0.3‰$. The determined values are, as for PCE and TCE, lower than previously reported enrichment factors for the degradation of α-HCH, γ-HCH and polychlorinated ethanes by pure cultures. The subsequent $\varepsilon_{Cl}/\varepsilon_C$ ratio was 0.52 ± 0.23.and $(AKIE_{Cl}\text{-}1)/(AKIE_C\text{-}1) = 0.44$ if the mechanism is considered stepwise, as suggested in other laboratory studies and 0.89 if concerted.

- The microbial community composition changes during degradation of PCE and α-HCH were determined by clone library analysis of the 16S rRNA genes. This allowed the evaluation of the

microbial evolution occurring over the course of the degradation experiments and the identification of bacterial strains most probably responsible for the degradation process. Overall the microcosm changes observed at the laboratory scale reflect the variability in microbial community at the field level. It therefore explains the differences in ε values observed for degradation reactions performed with pure vs. mixed cultures, i.e. the ε values obtained with pure cultures are generally higher than these determined with mixed consortia.

- Application of CSIA to field sites studies requires a careful choice of ε values. In addition to consider the microbial diversity at a site vs. laboratory setup, the fact that the ε values might be bacteria specific should also be taken into account.

5. FUTURE PERSPECTIVES

This thesis further explored the applicability of 2D CSIA combining C and Cl isotopes for the assessment of natural attenuation potential at contaminated field sites. In order to make best use of this method, additional research areas were identified.

An extended use of δ^{37}Cl-CSIA still suffers from the lack of standardization. Although different instruments and methods can be used for δ^{37}Cl determination, the results should be comparable. This can be achieved by cross-calibration of standards between different laboratories as well as by the availability of authentic standards exhibiting a broad δ^{37}Cl range. These measures would improve the confidence in CSIA-based assessment studies.

Recent analytical advances allow for screening of a broader range of OCls, including the degradation products from prioritized pollutants such as CEs and HCHs. The analysis of these metabolites would allow building up an isotopic mass-balance, clearly adding valuable information to CSIA-based assessment.

This work provides a step further towards establishing a library of ε_C and ε_{Cl} and the resulting $\varepsilon_{Cl}/\varepsilon_C$ and $(AKIE_{Cl}-1)/(AKIE_C-1)$ ratios. However these laboratory-derived values must be carefully selected when applied to field situations. First the presence of mixed consortia in the field limits the use of pure cultures ε values. Second, $\varepsilon_{Cl}/\varepsilon_C$ ratios might be specific to bacteria. Therefore, more laboratory experiments are needed to constrain ε values for bacteria responsible for the degradation of OCls. Once at the field site, the microbial community can be characterized, in order to identify and quantify the bacteria that play a role in the degradation processes. Then, a field specific $\varepsilon_{Cl}/\varepsilon_C$ ratio could be estimated, taking into account the contribution of each bacterium and its related laboratory-derived $\varepsilon_{Cl}/\varepsilon_C$ and subsequent $(AKIE_{Cl}-1)/(AKIE_C-1)$ ratios.

Overall, more studies are needed to better transpose laboratory results to field evaluation, in order to choose the best appropriate remediation method. In this sense, CSIA-based assessments provide information on the NA potential at a contaminated site. With microbial characterization, the presence and activity of degrading bacteria can be inferred. Therefore combining CSIA and microbial characterization can help to determine which bacteria to use for e.g. enhanced NA.

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REFERENCES

Abe, Y., Aravena, R., Zopfi, J., Parker, B., Hunkeler, D., 2009. Evaluating the fate of chlorinated ethenes in streambed sediments by combining stable isotope, geochemical and microbial methods. Journal of Contaminant Hydrology 107, 10–21.

Abe, Y., Aravena, R., Zopfi, J., Shouakar-Stash, O., Cox, E., Roberts, J.D., Hunkeler, D., 2009. Carbon and Chlorine Isotope Fractionation during Aerobic Oxidation and Reductive Dechlorination of Vinyl Chloride and cis-1,2-Dichloroethene. Environmental Science & Technology 43, 101–107.

Aelion, C.M., 2010. Environmental isotopes in biodegradation and bioremediation. CRC Press, Boca Raton.

Aeppli, C., Hofstetter, T.B., Amaral, H.I.F., Kipfer, R., Schwarzenbach, R.P., Berg, M., 2010a. Quantifying In Situ Transformation Rates of Chlorinated Ethenes by Combining Compound-Specific Stable Isotope Analysis, Groundwater Dating, And Carbon Isotope Mass Balances. Environmental Science & Technology 44, 3705–3711.

Aeppli, C., Holmstrand, H., Andersson, P., Gustafsson, O., 2010b. Direct Compound-Specific Stable Chlorine Isotope Analysis of Organic Compounds with Quadrupole GC/MS Using Standard Isotope Bracketing. Analytical Chemistry 82, 420–426.

Alvarez, A., Benimeli, C., Saez, J., Fuentes, M., Cuozzo, S., Polti, M., Amoroso, M., 2012. Bacterial Bio-Resources for Remediation of Hexachlorocyclohexane. International Journal of Molecular Sciences 13, 15086–15106.

Amaral, H.I.F., Aeppli, C., Kipfer, R., Berg, M., 2011. Assessing the transformation of chlorinated ethenes in aquifers with limited potential for natural attenuation: Added values of compound-specific carbon isotope analysis and groundwater dating. Chemosphere 85, 774–781.

ATSDR, 1996. Toxicological Profile for 1,2-Dichloroethene, U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta,GA.

ATSDR, 1997a. Toxicological Profile for Trichloroethylene, U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta,GA.

ATSDR, 1997b. Toxicological Profile for Tetrachloroethylene, U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA.

ATSDR, 2005. Toxicological Profile for alpha-, beta-, gamma-, and delta-Hexachlorocyclohexane. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR): Atlanta,GA, 2005.

ATSDR, 2006. Toxicological Profile for Vinyl Chloride, U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta,GA.

Audí-Miró, C., Cretnik, S., Otero, N., Palau, J., Shouakar-Stash, O., Soler, A., Elsner, M., 2013. Cl and C isotope analysis to assess the effectiveness of chlorinated ethene degradation by zero-valent iron: Evidence from dual element and product isotope values. Applied Geochemistry 32, 175–183.

Badea, S.-L., Vogt, C., Gehre, M., Fischer, A., Danet, A.-F., Richnow, H.-H., 2011. Development of an enantiomer-specific stable carbon isotope analysis (ESIA) method for assessing the fate of α-hexachlorocyclo-hexane in the environment. Rapid Commun. Mass Spectrom. 25, 1363–1372.

Badea, S.-L., Vogt, C., Weber, S., Danet, A.-F., Richnow, H.-H., 2009. Stable Isotope Fractionation of γ-Hexachlorocyclohexane (Lindane) during Reductive Dechlorination by Two Strains of Sulfate-Reducing Bacteria. Environ. Sci. Technol. 43, 3155–3161.

Ballapragada, B.S., Stensel, H.D., Puhakka, J.A., Ferguson, J.F., 1997. Effect of Hydrogen on Reductive Dechlorination of Chlorinated Ethenes. Environmental Science & Technology 31, 1728–1734.

Bernstein, A., Shouakar-Stash, O., Ebert, K., Laskov, C., Hunkeler, D., Jeannottat, S., Sakaguchi-Söder, K., Laaks, J., Jochmann, M.A., Cretnik, S., Jager, J., Haderlein, S.B., Schmidt, T.C., Aravena, R., Elsner, M., 2011. Compound-Specific Chlorine Isotope Analysis: A Comparison of Gas Chromatography/Isotope Ratio Mass Spectrometry and Gas Chromatography/Quadrupole Mass Spectrometry Methods in an Interlaboratory Study. Anal. Chem. 83, 7624–7634.

Bhatt, P., Kumar, M.S., Chakrabarti, T., 2009. Fate and Degradation of POP-Hexachlorocyclohexane. Critical Reviews in Environmental Science and Technology 39, 655–695.

Bloom, Y., Aravena, R., Hunkeler, D., Edwards, E., Frape, S.K., 2000. Carbon Isotope Fractionation during Microbial Dechlorination of Trichloroethene, cis-1,2-Dichloroethene, and Vinyl Chloride: Implications for Assessment of Natural Attenuation. Environmental Science & Technology 34, 2768–2772.

Bombach, P., Richnow, H.H., Kästner, M., Fischer, A., 2010. Current approaches for the assessment of in situ biodegradation. Applied Microbiology and Biotechnology 86, 839–852.

Bouwer, E.J., Rittmann, B.E., McCarty, P.L., 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. Environmental Science & Technology 15, 596–599.

Bradley, P.M., 2000. Microbial degradation of chloroethenes in groundwater systems. Hydrogeology Journal, Volume 8, Number 1.

Bradley, P.M., 2003. History and Ecology of Chloroethene Biodegradation: A Review. Bioremediation Journal 7, 81–109.

Breivik, K., Pacyna, J.M., Münch, J., 1999. Use of α-, β- and γ-hexachlorocyclohexane in Europe, 1970–1996. Science of The Total Environment 239, 151–163.

Chang, Y.C., Hatsu, M., Jung, K., Yoo, Y.S., Takamizawa, K., 2000. Isolation and characterization of a tetrachloroethylene dechlorinating bacterium, Clostridium bifermentans DPH-1. Journal of Bioscience and Bioengineering 89, 489–491.

Christensen, T.H., Bjerg, P.L., Banwart, S.A., Jakobsen, R., Heron, G., Albrechtsen, H.-J., 2000. Characterization of redox conditions in groundwater contaminant plumes. Journal of Contaminant Hydrology 45, 165–241.

Cichocka, D., Nikolausz, M., Haest, P.J., Nijenhuis, I., 2010. Tetrachloroethene conversion to ethene by a Dehalococcoides-containing enrichment culture from Bitterfeld. FEMS Microbiology Ecology 72, 297–310.

Cole, J.R., Cascarelli, A.L., Mohn, W.W., Tiedje, J.M., 1994. Isolation and characterization of a novel bacterium growing via reductive dehalogenation of 2-chlorophenol. Applied and Environmental Microbiology 60, 3536–3542.

Cole, J.R., Fathepure, B.Z., Tiedje, J.M., 1995. Tetrachloroethene and 3-chlorobenzoate dechlorination activities are co-induced inDesulfomonile tiedjei DCB-1. Biodegradation 6, 167–172.

Criss, R.E., 1999. *Principles of Stable Isotope Distribution;* Oxford University Press, Inc.: New York, USA.

Cupples, A.M., 2008. Real-time PCR quantification of Dehalococcoides populations: Methods and applications. Journal of Microbiological Methods 72, 1–11.

DiStefano, T.D., Gossett, J.M., Zinder, S.H., 1991. Reductive dechlorination of high concentrations of tetrachloroethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. Appl. Environ. Microbiol. 57, 2287–2292.

Doherty, R.E., 2000. A History of the Production and Use of Carbon Tetrachloride, Tetrachloroethylene, Trichloroethylene and 1,1,1-Trichloroethane in the United States: Part 1--Historical Background; Carbon Tetrachloride and Tetrachloroethylene. Environmental Forensics 1, 69–81.

Duhamel, M., Wehr, S.D., Yu, L., Rizvi, H., Seepersad, D., Dworatzek, S., Cox, E.E., Edwards, E.A., 2002. Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-dichloroethene and vinyl chloride. Water Research 36, 4193–4202.

EEA, 2007. Progress in management of contaminated sites (CSI 015) - Assessment published Aug 2007 - Progress in management of contaminated sites.

EEA, 2010. Soil — SOER 2010 thematic assessment - Thematic assessments.

EU Council, 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Official Journal of the European Communities L 330/32

Elsner, M., 2010. Stable isotope fractionation to investigate natural transformation mechanisms of organic contaminants: principles, prospects and limitations. J. Environ. Monit. 12, 2005.

Elsner, M., Hunkeler, D., 2008. Evaluating Chlorine Isotope Effects from Isotope Ratios and Mass Spectra of Polychlorinated Molecules. Anal. Chem. 80, 4731–4740.

Elsner, M., Zwank, L., Hunkeler, D., Schwarzenbach, R.P., 2005. A New Concept Linking Observable Stable Isotope Fractionation to Transformation Pathways of Organic Pollutants. Environ. Sci. Technol. 39, 6896–6916.

Fathepure, B.Z., Nengu, J.P., Boyd, S.A., 1987. Anaerobic bacteria that dechlorinate perchloroethene. Appl. Environ. Microbiol. 53, 2671–2674.

Fetzner, S., 1998. Bacterial dehalogenation. Applied Microbiology and Biotechnology 50, 633–657.

Fischer, A., Theuerkorn, K., Stelzer, N., Gehre, M., Thullner, M., Richnow, H.H., 2007. Applicability of Stable Isotope Fractionation Analysis for the Characterization of Benzene Biodegradation in a BTEX-contaminated Aquifer. Environ. Sci. Technol. 41, 3689–3696.

Flynn, S.J., Löffler, F.E., Tiedje, J.M., 2000. Microbial Community Changes Associated with a Shift from Reductive Dechlorination of PCE to Reductive Dechlorination of cis-DCE and VC. Environmental Science & Technology 34, 1056–1061.

Freedman, D.L., Gossett, J.M., 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. [WWW Document]. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC203047/?page=1 (accessed 8.11.11).

Futagami, T., Goto, M., Furukawa, K., 2008. Biochemical and genetic bases of dehalorespiration. Chem Record 8, 1–12.

Gerritse, J., Renard, V., Gomes, T.M.P., Lawson, P.A., Collins, M.D., Gottschal, J.C., 1996. Desulfitobacterium sp. strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or ortho -chlorinated phenols. Archives of Microbiology 165, 132–140.

Glod, G., Angst, W., Holliger, C., Schwarzenbach, R.P., 1997. Corrinoid-Mediated Reduction of Tetrachloroethene, Trichloroethene, and Trichlorofluoroethene in Homogeneous Aqueous Solution: Reaction Kinetics and Reaction Mechanisms. Environmental Science & Technology 31, 253–260.

Gribble, G.W., 1998. Naturally Occurring Organohalogen Compounds†. Acc. Chem. Res. 31, 141–152.

Hauzenberger, I., 2004. Reports on substances scheduled for re-assessments under the UNECE POPs Protocol, Technical Review Report on Lindane, Austria.

He, J., Ritalahti, K.M., Aiello, M.R., Loffler, F.E., 2003. Complete Detoxification of Vinyl Chloride by an Anaerobic Enrichment Culture and Identification of the Reductively Dechlorinating Population as a Dehalococcoides Species. Appl. Environ. Microbiol. 69, 996–1003.

Heritage, A.D., MacRae, I.C., 1977. Degradation of lindane by cell-free preparations of Clostridium sphenoides. Applied and Environmental Microbiology 34, 222–224.

Hofstetter, T.B., Berg, M., 2010. Assessing transformation processes of organic contaminants by compound-specific stable isotope analysis. TrAC Trends in Analytical Chemistry.

Hofstetter, T.B., Reddy, C.M., Heraty, L.J., Berg, M., Sturchio, N.C., 2007. Carbon and Chlorine Isotope Effects During Abiotic Reductive Dechlorination of Polychlorinated Ethanes. Environ. Sci. Technol. 41, 4662–4668.

Holliger, C., Hahn, D., Harmsen, H., Ludwig, W., Schumacher, W., Tindall, B., Vazquez, F., Weiss, N., Zehnder, A.J.B., 1998. Dehalobacter restrictus gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra- and trichloroethene in an anaerobic respiration. Archives of Microbiology 169, 313–321.

Holliger, C., Schraa, G., Stams, A.J., Zehnder, A.J., 1993. A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Appl. Environ. Microbiol. 59, 2991–2997.

Holmstrand, H., Andersson, P., Gustafsson, Ö., 2004. Chlorine Isotope Analysis of Submicromole Organochlorine Samples by Sealed Tube Combustion and Thermal Ionization Mass Spectrometry. Anal. Chem. 76, 2336–2342.

Holt, B.D., Sturchio, N.C., Abrajano, T.A., Heraty, L.J., 1997. Conversion of Chlorinated Volatile Organic Compounds to Carbon Dioxide and Methyl Chloride for Isotopic Analysis of Carbon and Chlorine. Analytical Chemistry 69, 2727–2733.

Hunkeler, D., Abe, Y., Broholm, M.M., Jeannottat, S., Westergaard, C., Jacobsen, C.S., Aravena, R., Bjerg, P.L., 2011. Assessing chlorinated ethene degradation in a large scale contaminant plume by dual carbon–chlorine isotope analysis and quantitative PCR. Journal of Contaminant Hydrology 119, 69–79.

Hunkeler, D., Aravena, R., Butler, B.J., 1999. Monitoring Microbial Dechlorination of Tetrachloroethene (PCE) in Groundwater Using Compound-Specific Stable Carbon Isotope Ratios: Microcosm and Field Studies. Environmental Science & Technology 33, 2733–2738.

Hunkeler, D.; Meckenstock, R.U.; Sherwood-Lollar, B.; Schmidt, T.; Wilson, J.T., 2008. A Guide for Assessing Biodegradation and Source Identification of Organic Groundwater Contaminant using Compound Specific Isotope Analysis (CSIA). 600/R-08/148; U.S. Environmental Protection Agency: Washington, DC, 2008.

Hunkeler, D., Van Breukelen, B.M., 2009. Modeling Chlorine Isotope Trends during Sequential Transformation of Chlorinated Ethenes. Environmental Science & Technology 43, 6750–6756.

Häggblom, M.M., Bossert, 2003. Dehalogenation microbial processes and environmental applications. Kluwer Academic Pub., Boston.

Jeffrey, S., Gardi, C., Montanarella, L., Marmo, L., Miko, L., Ritz, K., Peres, G., Römbke, J., van der Putten, W., 2010. European Atlas of Soil Biodiversity.

Jin, B., Laskov, C., Rolle, M., Haderlein, S.B., 2011. Chlorine Isotope Analysis of Organic Contaminants Using GC–qMS: Method Optimization and Comparison of Different Evaluation Schemes. Environmental Science & Technology 45, 5279–5286.

Jollow, D.J., Bruckner, J.V., McMillan, D.C., Fisher, J.W., Hoel, D.G., Mohr, L.C., 2009. Trichloroethylene risk assessment: A review and commentary. Critical Reviews in Toxicology 39, 782–797.

Klánová, J., Kohoutek, J., Hamplová, L., Urbanová, P., Holoubek, I., 2006. Passive air sampler as a tool for long-term air pollution monitoring: Part 1. Performance assessment for seasonal and spatial variations. Environmental Pollution 144, 393–405.

Krumholz, L., Sharp, R., Fishbain, S., 1996. A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation. Appl. Environ. Microbiol. 62, 4108–4113.

Krumholz, L.R., 1997. Desulfuromonas chloroethenica sp. nov. Uses Tetrachloroethylene and Trichloroethylene as Electron Acceptors. International Journal of Systematic Bacteriology 47, 1262 –1263.

Lal, R., Pandey, G., Sharma, P., Kumari, K., Malhotra, S., Pandey, R., Raina, V., Kohler, H.-P.E., Holliger, C., Jackson, C., Oakeshott, J.G., 2010. Biochemistry of Microbial Degradation of Hexachlorocyclohexane and Prospects for Bioremediation. Microbiology and Molecular Biology Reviews 74, 58–80.

Larsen, M., Burken, J., Machackova, J., Karlson, U.G., Trapp, S., 2008. Using Tree Core Samples to Monitor Natural Attenuation and Plume Distribution After a PCE Spill. Environ. Sci. Technol. 42, 1711–1717.

Lee, P.K.H., Conrad, M.E., Alvarez-Cohen, L., 2007. Stable Carbon Isotope Fractionation of Chloroethenes by Dehalorespiring Isolates. Environmental Science & Technology 41, 4277–4285.

Lee, P.K.H., Warnecke, F., Brodie, E.L., Macbeth, T.W., Conrad, M.E., Andersen, G.L., Alvarez-Cohen, L., 2011. Phylogenetic Microarray Analysis of a Microbial Community Performing Reductive Dechlorination at a TCE-Contaminated Site. Environ. Sci. Technol. 46, 1044–1054.

Lee, W., Batchelor, B., 2002a. Abiotic Reductive Dechlorination of Chlorinated Ethylenes by Iron-Bearing Soil Minerals. 1. Pyrite and Magnetite. Environmental Science & Technology 36, 5147–5154.

Lee, W., Batchelor, B., 2002b. Abiotic Reductive Dechlorination of Chlorinated Ethylenes by Iron-Bearing Soil Minerals. 2. Green Rust. Environmental Science & Technology 36, 5348–5354.

Li, S., Elliott, D.W., Spear, S.T., Ma, L., Zhang, W.-X., 2011. Hexachlorocyclohexanes in the Environment: Mechanisms of Dechlorination. Critical Reviews in Environmental Science and Technology 41, 1747–1792.

Li, Y., 1999. Global technical hexachlorocyclohexane usage and its contamination consequences in the environment: from 1948 to 1997. Science of The Total Environment 232, 121–158.

Liang, X., Dong, Y., Kuder, T., Krumholz, L.R., Philp, R.P., Butler, E.C., 2007. Distinguishing Abiotic and Biotic Transformation of Tetrachloroethylene and Trichloroethylene by Stable Carbon Isotope Fractionation. Environmental Science & Technology 41, 7094–7100.

Lojkasek-Lima, P., Aravena, R., Shouakar-Stash, O., Frape, S.K., Marchesi, M., Fiorenza, S., Vogan, J., 2012. Evaluating TCE Abiotic and Biotic Degradation Pathways in a Permeable Reactive Barrier Using Compound Specific Isotope Analysis. Groundwater Monit R 32, 53–62.

López, Ó., Fernández-Bolaños, J.G., López, Ó., Fernández-Bolaños, J.G. (Eds.), n.d. Subject Index, in: RSC Green Chemistry. Royal Society of Chemistry, Cambridge, pp. 339–353.

Löffler, F.E., Sun, Q., Li, J., Tiedje, J.M., 2000. 16S rRNA Gene-Based Detection of Tetrachloroethene-Dechlorinating Desulfuromonas and Dehalococcoides Species. Appl. Environ. Microbiol. 66, 1369–1374.

Magenheim, A.., Spivack, A.., Volpe, C., Ransom, B., 1994. Precise determination of stable chlorine isotopic ratios in low-concentration natural samples. Geochimica et Cosmochimica Acta 58, 3117–3121.

Magnuson, J.K., Stern, R.V., Gossett, J.M., Zinder, S.H., Burris, D.R., 1998. Reductive Dechlorination of Tetrachloroethene to Ethene by a Two-Component Enzyme Pathway. Appl. Environ. Microbiol. 64, 1270–1275.

Maillard, J., Charnay, M.-P., Regeard, C., Rohrbach-Brandt, E., Rouzeau-Szynalski, K., Rossi, P., Holliger, C., 2011. Reductive dechlorination of tetrachloroethene by a stepwise catalysis of different organohalide respiring bacteria and reductive dehalogenases. Biodegradation 22, 949–960.

Mattes, T.E., Alexander, A.K., Coleman, N.V., 2010. Aerobic biodegradation of the chloroethenes: pathways, enzymes, ecology, and evolution. FEMS Microbiology Reviews 34, 445–475.

Maymó-Gatell, X., Anguish, T., Zinder, S.H., 1999. Reductive Dechlorination of Chlorinated Ethenes and 1,2-Dichloroethane by "Dehalococcoides ethenogenes" 195. Appl. Environ. Microbiol. 65, 3108–3113.

Maymó-Gatell, X., Chien, Y., Gossett, J.M., Zinder, S.H., 1997. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. Science 276, 1568–1571.

Maymó-Gatell, X., Tandoi, V., Gossett, J.M., Zinder, S.H., 1995. Characterization of an H2-utilizing enrichment culture that reductively dechlorinates tetrachloroethene to vinyl chloride and ethene in the absence of methanogenesis and acetogenesis. Appl. Environ. Microbiol. 61, 3928–3933.

Meckenstock, R.U., Morasch, B., Griebler, C., Richnow, H.H., 2004. Stable isotope fractionation analysis as a tool to monitor biodegradation in contaminated acquifers. Journal of Contaminant Hydrology 75, 215–255.

Neumann, A., Wohlfarth, G., Diekert, G., 1996. Purification and Characterization of Tetrachloroethene Reductive Dehalogenase from Dehalospirillum multivorans. Journal of Biological Chemistry 271, 16515 –16519.

NRC (National Research Council), 1980. Drinking Water and Health,Volume 3. The National Academies Press.

NRC - National Research Council, 2000. Committee on Intrinsic Remediation, Water Science and Technology Board, Board on Radioactive Waste Management,. *Natural Attenuation for Groundwater Remediation*; National Academy Press: Washington, D.C., 2000.

Phillips, T., Seech, A., Lee, H., Trevors, J., 2005. Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. Biodegradation 16, 363–392.

Phillips, T.M., Lee, H., Trevors, J.T., Seech, A.G., 2006. Full-scale in situ bioremediation of hexachlorocyclohexane-contaminated soil. J. Chem. Technol. Biotechnol. 81, 289–298.

Rosner, B., Mccarty, P., Spormann, A., 1997. In Vitro Studies on Reductive Vinyl Chloride Dehalogenation by an Anaerobic Mixed Culture. Appl. Environ. Microbiol. 63, 4139–4144.

Ryoo, D., Shim, H., Canada, K., Barbieri, P., Wood, T.K., 2000. Aerobic degradation of tetrachloroethylene by toluene-o-xylene monooxygenase of Pseudomonas stutzeri OX1. Nat Biotech 18, 775–778.

Sakaguchi-Söder, K., Jager, J., Grund, H., Matthäus, F., Schüth, C., 2007. Monitoring and evaluation of dechlorination processes using compound-specific chlorine isotope analysis. Rapid Commun. Mass Spectrom. 21, 3077–3084.

Schmidt, T.C., Zwank, L., Elsner, M., Berg, M., Meckenstock, R.U., Haderlein, S.B., 2004. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review

of the state of the art, prospects, and future challenges. Analytical and Bioanalytical Chemistry 378, 283–300.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 2003. Environmental organic chemistry. John Wiley & Sons, Hoboken, N.J.

Semprini, L., 1995. In situ bioremediation of chlorinated solvents. Environ Health Perspect, 103 (Suppl 5), 101–105..

Sharma, P., McCarty, P., 1996. Isolation and Characterization of a Facultatively Aerobic Bacterium That Reductively Dehalogenates Tetrachloroethene to cis-1,2-Dichloroethene. Appl. Environ. Microbiol. 62, 761–765.

Shouakar-Stash, O., Drimmie, R., Zhang, M., Frape, S., 2006. Compound-specific chlorine isotope ratios of TCE, PCE and DCE isomers by direct injection using CF-IRMS. Applied Geochemistry 21, 766–781.

Simonich, S., Hites, R., 1995. Global distribution of persistent organochlorine compounds. Science 269, 1851–1854.

Smidt, H., Akkermans, A.D.L., van der Oost, J., de Vos, W.M., 2000. Halorespiring bacteria-molecular characterization and detection. Enzyme and Microbial Technology 27, 812–820.

Smidt, H., de Vos, W.M., 2004. Anaerobic Microbial Dehalogenation. Annu. Rev. Microbiol. 58, 43–73.

Sturchio, N.C., Clausen, J.L., Heraty, L.J., Huang, L., Holt, B.D., Abrajano, T.A., 1998. Chlorine Isotope Investigation of Natural Attenuation of Trichloroethene in an Aerobic Aquifer. Environmental Science & Technology 32, 3037–3042.

Sung, Y., Ritalahti, K.M., Apkarian, R.P., Löffler, F.E., 2006. Quantitative PCR confirms purity of strain GT, a novel trichloroethene-to-ethene-respiring Dehalococcoides isolate. Appl. Environ. Microbiol. 72, 1980–1987.

Sung, Y., Ritalahti, K.M., Sanford, R.A., Urbance, J.W., Flynn, S.J., Tiedje, J.M., Loffler, F.E., 2003. Characterization of Two Tetrachloroethene-Reducing, Acetate-Oxidizing Anaerobic Bacteria and Their Description as Desulfuromonas michiganensis sp. nov. Appl. Environ. Microbiol. 69, 2964–2974.

Swartjes, F.A., 2011. Dealing with contaminated sites from theory towards practical application. Springer, Dordrecht.

Tandoi, V., DiStefano, T.D., Bowser, P.A., Gossett, J.M., Zinder, S.H., 1994. Reductive Dehalogenation of Chlorinated Ethenes and Halogenated Ethanes by a High-Rate Anaerobic Enrichment Culture. Environmental Science & Technology 28, 973–979.

Thullner, M., Centler, F., Richnow, H.-H., Fischer, A., 2012. Quantification of organic pollutant degradation in contaminated aquifers using compound specific stable isotope analysis – Review of recent developments. Organic Geochemistry 42, 1440–1460.

UNEP, 2009. Report of the Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants on the work of its fourth meeting. Convention on Persistent Organic Pollutants. Fourth meeting, Geneva, 4–8 May 2009.

U.S. EPA, 1996. Symposium Proceedings Natural Attenuation Of Chlorinated Organics In Groundwater.

US EPA, 2004. Cleaning up the nation's waste sites: markets and technology trends. Office of Solid Waste and Emergency Response, Washington DC.

US EPA, 2007. Treatment technologies for site cleanup, 12th edn. US EPA Report 542R07012, September 2007.

U.S. EPA, 2009. Monitored Natural Attenuation of Chlorinated Solvents. U.S. EPA remedial technology fact sheet – Office of Research and Development, Washington, DC.

Van Acker, M.R.M.D., Shahar, A., Young, E.D., Coleman, M.L., 2006. GC/Multiple Collector-ICPMS Method for Chlorine Stable Isotope Analysis of Chlorinated Aliphatic Hydrocarbons. Analytical Chemistry 78, 4663–4667.

Van Warmerdam, E.M., Frape, S.K., Aravena, R., Drimmie, R.J., Flatt, H., Cherry, J.A., 1995. Stable chlorine and carbon isotope measurements of selected chlorinated organic solvents. Applied Geochemistry 10, 547–552.

Vijgen, J., Abhilash, P.C., Li, Y., Lal, R., Forter, M., Torres, J., Singh, N., Yunus, M., Tian, C., Schäffer, A., Weber, R., 2011. Hexachlorocyclohexane (HCH) as new Stockholm Convention POPs—a global perspective on the management of Lindane and its waste isomers. Environ Sci Pollut Res 18, 152–162.

Villemur, R., Lanthier, M., Beaudet, R., Lépine, F., 2006. The Desulfitobacterium genus. FEMS Microbiology Reviews 30, 706–733.

Vogel, T.M., Criddle, C.S., McCarty, P.L., 1987. ES Critical Reviews: Transformations of halogenated aliphatic compounds. Environmental Science & Technology 21, 722–736.

Walker, K., Vallero, D.A., Lewis, R.G., 1999. Factors Influencing the Distribution of Lindane and Other Hexachlorocyclohexanes in the Environment. Environ. Sci. Technol. 33, 4373–4378.

Walther, R., Hippe, H., Gottschalk, G., 1977. Citrate, a specific substrate for the isolation of Clostridium sphenoides. Applied and Environmental Microbiology 33, 955–962.

Wania, F., MacKay, D., 1996. Peer Reviewed: Tracking the Distribution of Persistent Organic Pollutants. Environ. Sci. Technol. 30, 390A–396A.

Wiedemeier, T.H., Newell, C.J., Rifai, H.S., Wilson, J.T., 1999. *Natural attenuation of fuels and chlorinated solvents*; John Wiley & Sons Inc.: New York, USA.

Willett, K.L., Ulrich, E.M., Hites, R.A., 1998. Differential Toxicity and Environmental Fates of Hexachlorocyclohexane Isomers. Environmental Science & Technology 32, 2197–2207.

WHO - World Health Organization, 2011. Guidelines for drinking-water quality - 4th ed.; WHO Press, Geneva, Switzerland, 2011.

Xiao, Y.-K., Zhang, C.-G., 1992. High precision isotopic measurement of chlorine by thermal ionization mass spectrometry of the Cs2Cl+ ion. International Journal of Mass Spectrometry and Ion Processes 116, 183–192.

Zinder, S.H., Gossett, J.M., 1995. Reductive dechlorination of tetrachloroethene by a high rate anaerobic microbial consortium. Environ. Health Perspect. 103 Suppl 5, 5–7.