Contrasting dual (C, Cl) isotope fractionation offers potential to distinguish reductive chloroethene transformation from breakdown by permanganate

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Abstract

Cis-1,2-dichloroethene (*cis*-DCE) and trichloroethene (TCE) are persistent, toxic and mobile pollutants in groundwater systems. They are both conducive to reductive dehalogenation and to oxidation by permanganate. In this study, the potential of dual element (C,Cl) compound specific isotope analyses (CSIA) for distinguishing between chemical oxidation and anaerobic reductive dechlorination of *cis*-DCE and TCE was investigated. Well-controlled *cis*-DCE degradation batch tests gave similar carbon isotope enrichment factors ϵ_C (‰), but starkly contrasting dual element isotope slopes $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl}$ for permanganate oxidation ($\epsilon_C = -26\text{‰} \pm 6\text{‰}$, $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl} \approx -125 \pm 47$) compared to reductive dechlorination ($\epsilon_C = -18\text{‰} \pm 4\text{‰}$, $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl} \approx 4.5 \pm 3.4$). The difference can be tracked down to distinctly different chlorine isotope fractionation: an inverse isotope effect during chemical oxidation ($\epsilon_{\text{Cl}} = +0.2\text{‰} \pm 0.1\text{‰}$) compared to a large normal isotope effect in reductive dechlorination ($\epsilon_{\text{Cl}} = -3.3\text{‰} \pm 0.9\text{‰}$) ($p < 0.05$). A similar trend was observed for TCE. The dual isotope approach was evaluated in the field before and up to 443 days after a pilot scale permanganate injection in the subsurface. Our study indicates, for the first time, the potential of the dual element isotope approach for distinguishing *cis*-DCE (and TCE) concentration drops caused by dilution, oxidation by permanganate and reductive dechlorination both at laboratory and field scale.

Keywords: *cis*-DCE, TCE, dual element isotope, permanganate, reductive dechlorination

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1. Introduction

Chlorinated ethenes (CEs) like trichloroethene (TCE) and *cis*-1,2-dichloroethene (*cis*-DCE) are widespread groundwater contaminants often present as dense non-aqueous phase liquids (DNAPLs). During years, these DNAPLs slowly release the pollutant creating groundwater contamination plumes, leading to a common environmental problem (Stroo et al., 2012). As DNAPLs are hard to localize in the subsurface, remediation of CE-contaminated sites is challenging, *in situ* bioremediation and chemical oxidation (ISCO) are effective, well-established techniques for remediation of CEs contaminated sites. Anaerobic *in situ* bioremediation is based on microbial processes that replace chlorine with hydrogen, which requires the activity of a dehalogenase enzyme, an electron donor, a carbon source, while the CEs serve as electron acceptor. Several groups of bacteria such as *Desulfomonile*, *Dehalobacter*, *Desulfitobacterium* and *Desulfuromonas* are known for their dechlorination capacity, but only *Dehalococcoides mccartyi* is capable of performing the complete dechlorination from perchloroethene (PCE) to ethene (Tiehm and Schmidt, 2011) and has reductive dehalogenase (RDase) genes encoding the proteins responsible for the respiration of CEs. In respect to *in situ* studies, for instance, Atashgahi et al. (2016) performed an *in situ* glycerol injection to stimulate reductive dechlorination of *cis*-DCE and vinyl chloride (VC). The results indicated that in addition to monitoring dechlorinating guilds, supportive non-dechlorination community members and geochemical factors should be controlled to ensure robust dechlorination activity. ISCO, on the other hand, is based on complete oxidation of CEs by an oxidant (e.g. Fenton's reagent, persulfate) to generate chloride ion (Cl^-) and carbon dioxide. Amongst the different chemical oxidant agents, permanganate (MnO_4^- , PM) has been widely applied for *in situ* and *ex situ* remediation since it is less reactive, more persistent and relatively safe to handle in the field since large quantities of heat and gases are not produced (Tsail et al., 2008). Implementation of ISCO as a single treatment strategy may also not be a

sustainable remediation solution for soils with a mixed pollution and/or residual free phase (Lee et al., 2007) or a high soil heterogeneity (Hrapovic et al., 2005). Therefore, an approach of ISCO followed by bioremediation might be a cost-effective alternative in the subsurface and reduce CE concentrations to below regulatory limits by achieving desired removal efficiencies (Richardson et al., 2011). The study of Sutton et al. (2015) showed that mild permanganate treatments led to decreases in CE concentrations and had a stimulatory effect on dechlorination, while stronger oxidant loads can completely and irreversibly disrupt microorganisms.

When applying such a combined degradation strategy in the field, however, a decrease in the concentrations of CEs could potentially be due to three reasons: (i) chemical oxidation, (ii) biological processes or (iii) dilution through displacement of contaminated groundwater by the injected solution (Hunkeler et al., 2003). Compound specific isotope analysis (CSIA) of carbon isotopes has been used as a promising tool to detect the occurrence of chemical and biological degradation as opposed to dilution (Mancini et al., 2002; Hunkeler et al., 2003; Meckenstock et al., 2004). Gas chromatography-isotope ratio mass spectrometry is used to measure isotope ratios ($^{13}\text{C}/^{12}\text{C}$) at natural abundance inside organic molecules at low concentrations (Elsner et al., 2012). While biological or chemical degradation can profoundly change such carbon isotope ratios (Slater et al., 2001; Hunkeler et al., 2003; Elsner et al., 2005) physicochemical processes such as dispersion, sorption, dilution or volatilization have a minor impact on a pollutant's isotope signature (Schmidt et al., 2004; Hunkeler et al., 2008; Thullner et al., 2012). The reason is that stronger and less reactive molecular bonds are formed by heavy isotopes in comparison to light isotopes so that the heavy isotopes are degraded more slowly and become enriched among the remaining contaminant molecules. These characteristic changes in isotope values, in turn, can be measured by CSIA and provide

direct evidence for the occurrence of a degradation process (Elsner et al., 2012; Meckenstock et al., 2004; Thullner et al., 2012). The ability to distinguish between chemical oxidation (Poulson and Naraoka, 2002; Hunkeler et al., 2003) and reductive dechlorination (Hunkeler et al., 1999; Slater et al., 2001; Lee et al., 2007; Fletcher et al., 2011) during and after the combined approach is however much more difficult since both processes were found to result in similar changes in carbon isotope values. Dual-element isotope analysis based on the isotope analysis of the C as well as Cl elements offers a new opportunity to solve this problem (Abe et al., 2009; Wigert et al., 2013; Cretnik et al., 2013; Kuder et al., 2013). Several studies have indicated that a dual element isotope approach has the potential to distinguish different transformation pathways (Zwank et al., 2005; Elsner et al., 2005; Fischer et al., 2007; Elsner, 2010). However, analytical methods for dual element isotope analysis of $^{13}\text{C}/^{12}\text{C}$ and $^{37}\text{Cl}/^{35}\text{Cl}$ of chlorinated ethenes have only relatively recently become available and are still being improved (Shouakar-Stash et al., 2005; Sakaguchi-Söder et al., 2007; Bernstein et al., 2011; Aeppli et al., 2010; Jin et al., 2011). Abe et al. (2009) were the first to apply dual element (C,Cl) isotope fractionation to distinguish between microbial aerobic oxidation and microbial anaerobic reductive dechlorination of *cis*-DCE and VC. In a handful of other studies, dual element (C,Cl) isotope analysis was used to differentiate different CE removal mechanisms such as (1) abiotic transformation of *cis*-DCE by zero-valent iron (ZVI) and TCE by ZVI (Lojkasek-Lima et al., 2012; Audí-Miró et al., 2013) (2) reductive dechlorination of TCE by different microbial cultures (*Geobacter lovleyi* strain SZ and *Desulfitobacterium hafniense* Y51) (Cretnik et al., 2013), (3) transformation by the enzymatic cofactor cobalamin and by the simplified chemical model system cobaloxime (Cretnik et al., 2013), and (4) reductive dechlorination of PCE and TCE by reductive dehalogenases and enzymatic co-factors (Wiegert et al., 2013; Kuder et al., 2013; Cretnik et al., 2014; Badin et al., 2014; Renpenning et al., 2014). However, up to now, there are no studies on dual element (C, Cl) isotope

analysis to discriminate anaerobic reductive dechlorination and chemical oxidation by PM of *cis*-DCE or TCE.

In this study, we therefore evaluated the possibility of using stable isotope analyses of two elements ($^{13}\text{C}/^{12}\text{C}$ and $^{37}\text{Cl}/^{35}\text{Cl}$) to distinguish between different scenarios of *cis*-DCE and TCE concentration reduction i.e. (1) degradation by chemical oxidation, (2) reductive dechlorination, and (3) dilution. To lay the basis for field data, *cis*-DCE isotope enrichment factors (ϵ_{C} , ϵ_{Cl}) during chemical oxidation and reductive dechlorination were first determined in lab scale microcosm tests. In addition, a TCE oxidation batch test with PM was conducted to identify C,Cl isotope fractionation patterns. Next, groundwater samples from a field site were collected before and after PM injection and subjected to C and Cl isotope analyses of *cis*-DCE which was the main pollutant in groundwater. Specifically, the aim of the field test was to evaluate the potential of the dual element isotope approach (1) for revealing whether *cis*-DCE degradation processes occurred in the test area, (2) for differentiating between chemical oxidation by PM and reductive dechlorination, and (3) for evaluating the occurrence of (bio)degradation after a PM injection. Calculated dual element isotope slopes based on the lab scale tests were used as reference to identify the concurrent modes of *cis*-DCE degradation (chemical oxidation vs. reductive dehalogenation) and dilution in the field. In this sense, this study is the first to test the potential of dual element (C,Cl) isotope analysis for distinguishing between *cis*-DCE removal by chemical oxidation vs. anaerobic reductive dechlorination in a laboratory as well as field application.

2. Material and Methods

2.1. Laboratory Experiments

2.1.1. Oxidation experiments of *cis*-DCE by permanganate

Duplicate batch experiments were set up in 250 mL bottles equipped with Mininert valves (Supelco, Bellefonte, Pennsylvania, USA). Each bottle contained 150 mL of an aqueous *cis*-DCE solution, prepared with MilliQ water, at a concentration of 60 mg/L (99%, Acros Organics). Forty percent (w/v) of sodium permanganate solution (same solution as used for the field test) was added to each bottle to reach a final concentration of 240 mg/L PM, which is the theoretical demand to completely oxidize 60 mg/L of *cis*-DCE. Control bottles were filled with 150 mL of aqueous *cis*-DCE solutions (60 mg/L) and contained no PM. For the concentration analysis of CEs and for carbon and chlorine isotope analysis of *cis*-DCE, 10 mL subsamples were taken every two minutes over a period of 20 minutes. Samples for isotope analysis were subdivided into 2 mL and transferred to GC vials containing 0.1 mL of a quenching solution (0.5 M sodium thiosulfate and 0.06 M calcium nitrate) to stop PM reactivity. The same protocol was also followed for the TCE oxidation test. The quenching solution did not have any effect on *cis*-DCE or TCE degradation and C and Cl isotope values (data not shown).

2.1.2. Anaerobic biodegradation experiments of *cis*-DCE

Microcosms for anaerobic biodegradation were prepared in 160 mL glass serum bottles containing 40 g (wet) homogenized aquifer material (from 7 spots within the test area 2.4-8.0 m bgl; < 183 µg/kg CE) and 70 mL anaerobic groundwater (5-6 m bgl, pH = 6.5, ORP = -103 mV, EC= 2.17 mS/cm) from the field site described in section 2.2. The bottles were sealed with Teflon-lined butyl rubber stoppers. All preparations were performed under nitrogen in a glove box. The bottles were purged with a N₂/CO₂ (80/20%) mixture to remove any

preexisting volatile contaminants from the sediment/groundwater. Subsequently, *cis*-DCE solution was injected into each bottle to result in an initial concentration of 15 mg/L. The *cis*-DCE concentration in microcosms was monitored via direct sampling of the headspace. When the concentration of *cis*-DCE decreased to 10, 7, 5, 2 and 1 mg/L, 40 mL and 2 mL of aqueous samples were taken for carbon and chlorine isotope measurements, respectively, by sacrificing one bottle out of 14 at each concentration. These samples were adjusted with NaOH to pH 10-11 to stop microbial activity, and kept at -20 °C until isotope analysis. Control bottles were set up to monitor any change in *cis*-DCE and TCE concentration.

2.1.3. Compound specific isotope analysis

Carbon and chlorine isotope analysis of *cis*-DCE was conducted following the method described in Milosevic et al. (2013) and Audí-Miró et al. (2013), respectively. The carbon isotopic signature ($\delta^{13}\text{C}$) of the *cis*-DCE laboratory standard was $-25.5\text{‰} \pm 0.2\text{‰}$. The Cl isotopic signatures ($\delta^{37}\text{Cl}$) of the *cis*-DCE external standards, cisF and IS-63, were -1.52‰ and $+0.07\text{‰}$, respectively.

$$\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{compound}} - ({}^{13}\text{C}/{}^{12}\text{C})_{\text{VPDB}}] / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{VPDB}} \quad (1)$$

$$\delta^{37}\text{Cl} = [({}^{37}\text{Cl}/{}^{35}\text{Cl})_{\text{compound}} - ({}^{37}\text{Cl}/{}^{35}\text{Cl})_{\text{SMOC}}] / ({}^{37}\text{Cl}/{}^{35}\text{Cl})_{\text{SMOC}} \quad (2)$$

where VPDB and SMOC are the international reference standards of Vienna Pee Dee Belemnite and Standard Mean Ocean Chloride, respectively. Carbon and chlorine isotope analysis of TCE was conducted as described in SI-M1, M2 and M3, and illustrated in SI-Figures 1 & 2.

2.1.4. Calculations of isotope enrichment factors

Carbon and chlorine isotope enrichment factors (ϵ) were calculated according to the Rayleigh equation:

$$(\varepsilon) \cdot \ln(C_t/C_0) = \ln(R_t/R_0) \quad (3)$$

where C_t is the concentration of the compound at time t and C_0 is concentration of the compound at time zero.

The R_t/R_0 term was calculated according to the equation:

$$R_t/R_0 = ((1 + \delta^h E) / (1 + \delta^h E_0)) \quad (4)$$

where $\delta^h E_0$ is the carbon or chlorine isotopic signature of the compound at time zero and $\delta^h E$ is the carbon or chlorine isotopic signature at time t .

2.1.5. Chemical analyses

CEs were analyzed via direct headspace analyses of the test vials on a Varian GC-FID (CP-3800) (Middelburg, the Netherlands) to determine compound concentrations in the lab biodegradation test as described by Doğan-Subaşı et al. (2013). A Rt-U plot column was used for methane, ethane and ethene analyses, and a split-splitless injector (used in splitless mode, 250°C) followed by an Rt-X column (30 m length, 0.53 mm ID and 3 µm film thickness, Restek, Bellefonte, Pennsylvania, USA) and a DB-1 column (J&W Scientific, USA) for analysis of CAHs. The temperature program started at 50°C and ramped at 2°C/min to 60°C, ramped once again at 10°C/min to 155°C and finally ramped at 20°C/min to 180°C. The detector temperature was set at 250°C. The carrier gas was Helium at a flow of 6 mL/min (detection limit: 0.1-4.9 µg/L; coefficient of variance is between 1.5%-12%). For field samples, TCE, *cis*-DCE, VC, methane, ethene and ethane concentrations were measured using a Thermo Finnigan Trace GC-MS (Thermo Fisher, Bremen, Germany) equipped with a DB5-MS column (60 m length, 0.25 mm inner diameter and 0.25 µm film thickness, J&W Scientific, Folsom, California, USA) with He as carrier gas at a flow rate of 0.9 mL/min. The temperature program started at 35°C for 3 min and ramped at 5°C/min to 175°C. After equilibration for 30 min at 65°C, samples of 250 µL were taken from the headspace of the

sample vials using a headspace autosampler (CombiPal CTC Analytics, Zwingen, Switzerland). Depending on the specific compound, the detection limit was 0.18-3.46 µg/L, the variation coefficient was between 2.5% and 16%. The samples were treated with phosphoric acid to prevent further bioactivity and stored at 4 °C prior to measurements.

2.2. Field Test

The site is located in an industrial area near Antwerp, Belgium. At this site, a heterogeneous geology has been documented with dredged sand from 0 to 3 m below ground level (bgl), alluvial deposits of loamy sand and sandy loam with shells from 3 to 8 m bgl, sand with glauconite from 8 to 25 m bgl and a non-permeable clay layer at 25 m bgl (Uyttebroek et al., 2012). The groundwater flow direction is irregular due to different nearby groundwater-connected activities (like pumping), but mainly southwards (Figure 1). Negative redox potential values (Ag/AgCl) indicated that predominantly reduced conditions occurred in the subsurface of the site. A mixture of pollutants was detected in the groundwater with *cis*-DCE as the main contaminant (41 - 18,000 µg/L in the groundwater) and found its origin in multiple small spills that occurred over decades at different spots. Previously, membrane interface probe profiling and chemical analyses on core samples proved the heterogenic distribution of the pollutants in the subsurface, with pollutants varying highly in type and concentration in subsurface (x,y,z) without a clear pattern. An anaerobic biodegradation potential for reductive dechlorination was reported at the site as well as a natural high matrix demand for PM (Bastiaens et al., 2012). Via direct push technology, 1,896 L and 900 L of a 83 kg PM/m³ solution were injected at locations IN1 (2.1-5.1 m bgl) and IN2 (5.1-6.0 m bgl), respectively, located 2 m apart (Figure 1) (Uyttebroek et al., 2012). The injected PM came in contact with part of the pollution while migrating through the soil pores. As high injection pressures were used, preferential flow paths were created (Uyttebroek et al., 2012). Even

though heterogeneous conditions were observed at the site, for IN2 increased manganese concentrations in the soil indicated the presence of PM at 5.7 to 7.2 m bgl which correlates well with the injection of permanganate from 5.4 to 6.9 m bgl. This supports the conclusion that the oxidant reached the target area of the contaminants in the wells when the depths were considered (Uyttebroek et al., 2012). The injection spots were surrounded by monitoring wells (Figure 1) some of which were directly impacted by PM based on visual observations of the groundwater which turned purple (Figure 1). The pilot test performed was within the legal framework set by the Public Waste Agency of Flanders OVAM (Bodemdecreet (27/10/2016) betreffende de bodemsanering en de bodembescherming).

Groundwater samples were collected one day before PM injection and 15, 33, 55, 82, 305 and 443 days after PM injection using a peristaltic pump and polyethylene sample tubes (Eijkelkamp, Giesbeek, The Netherlands). Before each sampling event, groundwater was withdrawn from the wells until field parameters stabilized. The field parameters were measured using a flow-through cell (Eijkelkamp, Giesbeek, The Netherlands), and electrodes for temperature and electrical conductivity (TetraCon 325, WTW, Weilheim, Germany), pH (SenTix 41, WTW, Weilheim, Germany), dissolved oxygen (CellOx 325, WTW, Weilheim, Germany) and redox potential (ORP) (Oxitrode Platinum Hamilton, Bonaduz, Switzerland) controlled by a multimeter (MultiLine F/SET3, WTW, Weilheim, Germany). For CE quantification, duplicate samples of 5 mL were transferred into 10 mL glass vials containing 100 μ L concentrated H_3PO_4 (85%, Merck, Germany) to stop microbial activity. The vials were immediately closed by PTFE-lined caps. Samples for stable carbon and chlorine isotope analysis were collected in 250 mL amber bottles without leaving headspace, and immediately treated with NaOH pellets to reach pH above 10 in order to stop microbial activity. Chemical analyses and calculations were made as explained above.

3. Results and Discussion

3.1. Laboratory Reference Experiments

Batch experiments were performed to determine the carbon and chlorine isotope enrichment factors of *cis*-DCE and TCE during oxidation by PM (tested in *cis*-DCE and TCE solutions in MilliQ water) and those of *cis*-DCE during reductive dechlorination (tested in aquifer microcosms). The obtained carbon and chlorine isotope values of *cis*-DCE were plotted according to the Rayleigh equation (3) and the calculated isotope enrichment factors are presented in Figure 2A&D. Data reported by Cretnik et al. (2014) on reductive dehalogenation of TCE were plotted in Figure 2C&D along with the newly collected data related to oxidation of TCE by PM. Negative controls (without PM) did not show any change in *cis*-DCE and TCE concentration or their carbon and chlorine isotope signatures (data not shown).

Insight from Carbon Isotope Ratios Alone. The carbon isotope enrichment factor of *cis*-DCE during reductive dechlorination $\epsilon_C = -18\text{‰} \pm 4\text{‰}$ (Figure 2A) was in agreement with previous studies where ϵ_C ranged from -17‰ to -32‰ (Slater et al., 2001; Lee et al., 2007; Carreon-Diazconti et al., 2009; Abe et al., 2009; Fletcher et al., 2011; Schmidt et al., 2014). During oxidation of *cis*-DCE by PM, a value of $\epsilon_C = -26\text{‰} \pm 6\text{‰}$ was determined (Figure 2A), which also falls within the range of values ($\epsilon_C = -17\text{‰}$ to -27‰) previously reported for the oxidation of different CEs by PM (Poulson and Naraoka, 2002; Hunkeler et al., 2003). The carbon isotope fractionation for reductive dechlorination and oxidation by PM is large in both cases and can serve as a robust indicator for the occurrence of the *cis*-DCE degradation versus dilution. However, the similar trends and magnitude of the carbon isotopic enrichment make it difficult to distinguish the different pathways of chemical oxidation and/or reductive dechlorination.

Insight from Dual Element Isotope Plots. Figure 3 shows that such a pathway distinction becomes possible if in addition to $\delta^{13}\text{C}$ values also $\delta^{37}\text{Cl}$ values are measured during the experiments and if both values are plotted in a dual element isotope plot. Our study provides for the first time such dual element isotope fractionation data on chemical oxidation of *cis*-DCE by PM. The observed dual element isotope slope $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl}$ of -125 ± 47 was significantly different from the slopes observed for reductive dechlorination of *cis*-DCE in our study (4.5 ± 3.4) ($p < 0.05$) (Figure 3A). A similar difference is obtained in Figure 3B where the first TCE dual element isotope data from our PM experiment ($\delta^{13}\text{C}/\delta^{37}\text{Cl} = \infty$, $\delta^{37}\text{Cl}/\delta^{13}\text{C} = -0.001 \pm 0.006$) is compared to TCE data on reductive dechlorination of TCE from Cretnik et al. (2014) ($\delta^{13}\text{C}/\delta^{37}\text{Cl} = 3.4 \pm 0.1$; $\delta^{37}\text{Cl}/\delta^{13}\text{C} = 0.30 \pm 0.01$). As such, the negligible Cl fractionation for chemical oxidation results in distinctly different trends in dual element (C, Cl) isotope slopes, and delineates a promising new way to discriminate degradation of CEs by reductive dechlorination vs. oxidation by PM. Although the uncertainty of the $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl}$ slope in reductive dehalogenation of *cis*-DCE is relatively high, the difference indicates, moreover, that different underlying (bio)chemical reaction mechanisms lead to strongly different Cl isotope effects. Indeed, the chlorine isotopic enrichment factor observed during reductive dechlorination of *cis*-DCE was $\epsilon_{\text{Cl}} = -3.3\text{‰} \pm 0.9\text{‰}$ (Figure 2B). The value was larger than the one reported by Abe et al. (2009) ($\epsilon_{\text{Cl}} = -1.6\text{‰}$ to -1.4‰) indicating that the dual element isotope fractionation of *cis*-DCE in reductive dechlorination shows a larger variability than previously assumed. Irrespective of this, both chlorine isotopic enrichment factors were similar in direction and magnitude when compared to the data on chlorine isotope enrichment of *cis*-DCE and TCE during oxidation by PM. The chlorine enrichment factor for *cis*-DCE in reaction with PM ($\epsilon_{\text{Cl}} = 0.2\text{‰} \pm 0.1\text{‰}$) was (i) rather negligible and (ii) even inverse (i.e., ^{37}Cl reacted faster) (Figure 2B), and a similar trend was observed for

degradation of TCE by permanganate ($\epsilon_C = -25\text{‰} \pm 14\text{‰}$, $\epsilon_{Cl} = 0.1\text{‰} \pm 0.1\text{‰}$, Figure 2, C&D) ($p \gg 0.05$).

This negligible chlorine enrichment factor during oxidation with PM is consistent with secondary isotope effects, since a C-Cl bond is not directly broken in the initial step of oxidation of ethenes (Houk and Strassner, 1999; Morasch and Hunkeler, 2009) (Figure 3). During chemical oxidation, the large carbon isotope fractionation reflects a primary carbon isotope effect in the formation of C-O bonds with PM, while the chlorine does not participate in this first step of the reaction and causes only small, secondary isotope effects (Figure 3). Therefore, in contrast with reductive dechlorination, the degradation of *cis*-DCE and TCE through oxidation by permanganate is not associated with chlorine isotope fractionation. The results of our lab tests showed this specificity in the chlorine isotope fractionation mechanism for the first time, which enables us to distinguish between reductive dechlorination and chemical oxidation by permanganate of *cis*-DCE and TCE through dual isotope approach.

3.2. Field Experiments

The potential of dual C, Cl isotope fractionation to distinguish different degradation pathways was subsequently tested at a real contaminated site (main pollutant *cis*-DCE) by analyzing groundwater samples in the vicinity of PM injection points (Figure 1). The influence of PCE and TCE degradation on the *cis*-DCE isotope signatures was considered insignificant since their concentrations were close to zero in all the wells except for MW6b in which PCE and TCE were found reaching only 15% of the total CEs (Figure 1).

Insight from a Dual Element Isotope Plot of *cis*-DCE $\delta^{13}\text{C}$ vs. $\delta^{37}\text{Cl}$ Values.

Putative Source Values. Figure 4 summarizes all $\delta^{13}\text{C}$ and $\delta^{37}\text{Cl}$ values of *cis*-DCE measured in groundwater samples from the field in a dual element isotope graph. In contrast to the laboratory reference experiment, initial isotope values (i.e., values before *cis*-DCE underwent reductive dechlorination or oxidation) are not known. For original source contaminants, such initial isotope values are estimated from values measured upstream in a source zone (Meckenstock et al., 2004). For the current heterogeneous site with fluctuating groundwater flow directions, however, such information was not retrievable, and *cis*-DCE was, moreover, probably not the parent contaminant, but an accumulated daughter product of TCE or PCE. Considering that TCE had been degraded almost completely in all wells, however, and that *cis*-DCE represented the predominant daughter product, one can hypothesize that the average isotope values from the permanganate unimpacted well MW5b approximately represent initial *cis*-DCE isotope values. In Figure 4A&B, dual element isotope trends $\Delta\delta^{13}\text{C}/\Delta\delta^{37}\text{Cl}$ derived from our lab scale degradation tests were therefore sketched with these values ($\delta^{13}\text{C} = -29.6\text{‰} \pm 0.2\text{‰}$ and $\delta^{37}\text{Cl} = 5.0\text{‰} \pm 0.1\text{‰}$) as starting point.

Dual Element Isotope Values before PM Injection. Figure 4A displays the isotope values measured before PM injection and shows that besides MW5b, also the isotope values measured in MW5a ($\delta^{13}\text{C} = -28.9\text{‰} \pm 0.2\text{‰}$, $\delta^{37}\text{Cl} = 5.0\text{‰} \pm 0.0\text{‰}$) are close to the hypothesized origin. In contrast, the initial MW8b data point ($\delta^{13}\text{C} = -20.8\text{‰} \pm 1.1\text{‰}$ and $\delta^{37}\text{Cl} = 6.0\text{‰} \pm 0.0\text{‰}$) was shifted along the reductive dechlorination slope (area I) confirming that reductive dechlorination of *cis*-DCE was already ongoing at the first sampling date (Figure 4A). As expected, no points were measured in those areas that would be indicative of PM treatment: area II (oxidation by PM) and area III (removal by both reductive dechlorination and oxidation). Compared to MW5a and MW5b, the isotope values in MW6b

are situated in a different region (area IV): the $\delta^{13}\text{C}$ value was similar, but the $\delta^{37}\text{Cl}$ value was by 1‰ less positive (smaller $^{37}\text{Cl}/^{35}\text{Cl}$) ($\delta^{13}\text{C} = -29.5\text{‰} \pm 0.0\text{‰}$, $\delta^{37}\text{Cl} = 4.3\text{‰} \pm 0.0\text{‰}$). It is a fact that MW6b was the only well in which relevant concentrations of PCE (up to 15% of the CE mass balance) were observed, but for the reasons laid out in the Supporting Information this cannot explain the observed difference. Data points in area IV therefore suggest that *cis*-DCE in corresponding wells may be derived from a different original PCE/TCE source. Therefore, the initial *cis*-DCE isotope values could have covered a broader range as visualized in Figure 4B by the rectangular (Source zone range).

Dual Element Isotope Values after PM Injection. When looking also at the isotope values measured after the injection, the dual isotope plot (Figure 4B) gives evidence that data from numerous samples fell within a narrow range of carbon isotope values (between -30.0‰ and -26.0‰). Therefore, based on carbon isotope data alone, one could conclude that these samples stem from the same source. Additional information from chlorine isotope values, however, shows a high variability (between 4.0‰ and 6.0‰) and indicates that *cis*-DCE probably originated from TCE of different sources mirroring the complex history of solvent usage in the site (solvents from different origin were handled at a filling station over years). Indeed, the results of the lab experiments indicated that the oxidation by PM and reductive dechlorination were associated with pronounced increases in carbon isotope values giving the evidence of degradation. Therefore, based on the low variance in $\delta^{13}\text{C}$ a hypothetical source zone area was delineated in the dual isotope plot (Figure 4B), representing the high variability of the pollutant sources. Two data points - MW5b, 305 days after injection ($\delta^{13}\text{C} = -7.3\text{‰} \pm 0.3\text{‰}$) and MW8b, before injection ($\delta^{13}\text{C} = -20.8\text{‰} \pm 1.1\text{‰}$) – were clearly located outside the source zone area, giving unequivocal evidence of degradation. Degradation possibly also occurred in two more points: MW5a, 55 days after injection ($\delta^{13}\text{C} = -24.1\text{‰} \pm 0.7\text{‰}$) and

MW8b, 15 days after injection ($\delta^{13}\text{C} = -24.8\text{‰} \pm 0.0\text{‰}$) (Figure 4B). Further, MW6b, 15 days after PM injection ($\delta^{13}\text{C} = -26.4\text{‰} \pm 0.3\text{‰}$) is situated near the edge of the hypothesized source zone area. However, it would be impossible to distinguish reductive vs. oxidative nature of the degradation by carbon isotope data alone as both degradation pathways resulted in an increase in $\delta^{13}\text{C}$. In contrast, next to $\delta^{13}\text{C}$ also $\delta^{37}\text{Cl}$ increases for MW8b before injection, for MW5b 305 days after injection and for MW5a 55 days after injection (Figure 4B) indicated that reductive dechlorination was responsible for *cis*-DCE degradation in all these cases. Chemical oxidation instead of reductive dechlorination occurred possibly in two data points; MW6b, 15 days after PM injection ($\delta^{37}\text{Cl} = 3.7\text{‰} \pm 0.0\text{‰}$) and MW8b, 15 days after PM injection ($\delta^{37}\text{Cl} = 4.2\text{‰} \pm 0.1\text{‰}$) since the $\delta^{37}\text{Cl}$ did not increase. As such, evidence of *cis*-DCE degradation by PM was only observed after 15 days, whereas reductive dehalogenation took place before and even after injection. This indicates that the ability of microorganisms to perform biotic, or to create conditions for abiotic reductive dehalogenation was not affected by the oxidant in the long run. This observation was also evidenced by the changes in redox values for each well (Figure S3). For instance, while redox potentials were measured as -94 mV in MW6b and -112 mV in MW8b before injection, the values increased to 89 mV in MW6b and to 114 mV in MW8b on day 15 indicating the shift from anaerobic to less reduced conditions which is not favorable for reductive dehalogenation. Microbial data obtained via molecular analyses on gene transcripts (RNA) targeting 16S rRNA of *Dehalococcoides mccartyi* spp. and the *tceA* and *bvcA* genes (data not shown), were not conclusive, as in the samples collected 15 days after the injection, the analyses failed, possibly due to the presence of PM. At later time points (82 & 305 days after injection), gene transcripts were detected in all wells, with highest *bvcA* gene (most relevant for *cis*-DCE degradation) transcript numbers (1.0×10^7 /g aquifer) in MW5b and MW8b.

Comparison of isotope values and pollutant concentrations. In Figure 4C-F the carbon and chlorine signatures are plotted for each well in function of time, together with the measured concentrations of chlorinated ethenes and ethene. Time points where the 5 most predominant changes in the isotope signature were observed, are marked with rectangulars. For example, in well MW5a, between day 33 and 55, the carbon and chlorine isotope signatures changed from -26.7‰ to -24.1‰ and from 4.9‰ to 6.1‰, respectively (Figure 4C), with a small reduction of *cis*-DCE concentrations from 10 µM to 9 µM followed by ethene production providing an indicator of biodegradation. Similarly, in well MW5b, an increase in $\delta^{13}\text{C}$ values (from -29.4‰ $\pm$ 0.2‰ to -7.3‰ $\pm$ 0.3‰) and $\delta^{37}\text{Cl}$ values (from 5.1‰ $\pm$ 0.1‰ to 7.0‰ $\pm$ 0.1‰) measured on day 305 was indicative of an increase in reductive dechlorination, consistent with the reduction of *cis*-DCE concentrations from 250 µM to 53 µM, as well as the production of VC (51 µM) and ethene (15 µM) at that sampling date (Figure 4B and D). In well MW6b, a 3.1‰ increase in $\delta^{13}\text{C}$ signatures and *cis*-DCE reduction from 309 µM to 186 µM between injection and day 15 points towards degradation of *cis*-DCE (Figure 4E). Near MW8b, before and 15 days after the injection, the observed changes in isotope signatures are accompanied with a fast decrease in concentrations of *cis*-DCE and VC.

4. Conclusions

This study is the first to provide evidence that the dual element isotope approach (C, Cl) is a promising tool to differentiate two important mechanisms involved in abiotic and biotic degradation of *cis*-DCE and TCE, namely anaerobic reductive dechlorination and chemical oxidation via PM. Moreover, the dual element isotope approach was shown to be potentially helpful to identify the dominant CE removal mechanism in the field, which is of specific concern when a combined remediation approach is envisioned, such as chemical oxidation followed by reductive (bio)degradation.

In our study, the potential for such a distinction was obtained at a very heterogeneous site (complex geology, highly changing groundwater flow directions and flows, heterogeneous distribution of pollutants) where common monitoring approaches have failed to come to conclusions. The results of this pilot study further suggest that – even after permanganate treatment – biodegradation activity remained in some parts of the small pilot injection area indicating that – opposite to frequent concerns – reductive dechlorination capability was not completely suppressed. A next step would be the use of the dual element isotope approach for evaluating the impact after a larger scale remediation where the impact of PM can be more intensive to microorganisms.

5. Acknowledgment

The research was funded by the European Community's Seventh Framework Program *FP7/2007-2013* under grant agreement n° 212683 (GOODWATER) and European Union consortium UPSOIL, a Seventh Framework Programme within Theme 6. N° 226956. Contributions from ECOREM p.l.c. and of VITO colleagues, Maarten Uyttebroek, Guy Borgmans, Miranda Maesen and Queenie Simons are appreciated. Stefan Cretnik was funded by the German National Science Foundation (DFG, EL 266/3-1).

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CI = confidence interval

Figure 1. Location of the monitoring wells, depth of the filters and distances from the two injection points (-, not impacted by PM; ++, clearly impacted by PM; +++, heavily impacted by PM) (adapted from Uyttebroek et al. (2012)).

Figure 2. Isotope fractionation patterns in *cis*-DCE of $\delta^{13}\text{C}$ (A) and $\delta^{37}\text{Cl}$ (B) and in TCE of $\delta^{13}\text{C}$ (C) and $\delta^{37}\text{Cl}$ (D) measured during reductive dechlorination (aquifer microcosms) and chemical oxidation (MilliQ water). Enrichment factors were extracted from curve fittings according to the Rayleigh equation.

Uncertainties for given ϵ are 95% CI and number of replicates was 2.

Figure 3. Dual isotope plots: batch oxidation and biodegradation tests for *cis*-DCE (A) and for TCE (B). The slope of the current TCE study is given and compared to the result of Cretnik et al. (2014). Proposed mechanism for *cis*-DCE oxidation via permanganate (Morasch and Hunkeler, 2009) and anaerobic reductive dechlorination (Parsons, 2004) are given. Number of replicates was 2.

Figure 4. Dual isotope plot of *cis*-DCE for groundwater sampled over time near the PM injection points in the field, before injection (A) and before and after injection (B). The arrows indicate $\epsilon_{\text{C}}/\epsilon_{\text{Cl}}$ slopes (average, full lines; minimal and maximal, dashed lines with 95% Confidence interval (CI) of the batch scale

biodegradation and chemical oxidation tests. Values of $\delta^{13}\text{C} = -29.6\text{‰} \pm 0.2\text{‰}$ and $\delta^{37}\text{Cl} = 5.0\text{‰} \pm 0.1\text{‰}$ are assumed as the isotopic signatures of the field and origins of the arrows. Carbon and chlorine isotope signatures for *cis*-DCE as well as chlorinated ethene concentrations in the monitoring wells over time (C-F). Note that different scales are used for carbon isotope and chlorinated ethenes in Y axes (BI: Before injection). Data in rectangles indicate the significant changes in the isotope signatures. Error bars in C-F indicate the standard deviation of the duplicate samples.