

Estimating anaerobic reductive dechlorination of chlorinated compounds in groundwater by indigenous microorganisms

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Abstract. Tetrachloroethylene (PCE) and trichloroethylene (TCE), critical pollutants to human health and groundwater ecosystems, are managed by groundwater quality standards (GQS) in South Korea. However, there are no GQSs for their by-products, such as cis-dichloroethylene (DCE) and vinyl chloride (VC) produced through the dechlorination process of PCE and TCE. Therefore, in this study, we monitored PCE, TCE, cis-DCE, and VC in 111 national groundwater wells for three years (2016 to 2018) to evaluate their distributions, a biological dechlorination possibility, and human risk assessment. The detection frequency of them was 30.2% for PCE, 45.1% for TCE, 43.9% for cis-DCE and 13.4% for VC. The four chlorinated compounds were commonly detected in 21 out of 111 wells. In the results of statistical analysis with 21 wells data, DO and ORP also had a negative correlation with four organic chlorinated compounds, while EC and sulfate has a positive correlation with the compounds. This indicates that the 21 wells were relatively met with suitable environments for a biological dechlorination reaction compared to the other wells. Finally, cis-DCE had a non-carcinogenic risk of 10^{-1} and the carcinogenic risk of VC was 10^{-6} or higher. Through this study, the distribution status of the four chlorinated compounds in groundwater in South Korea and the necessity of preparing plans to manage cis-DCE and VC were confirmed.

Keywords: biological dichlorination; groundwater; risk assessment; PCE; TCE

1. Introduction

In recent years, the quality of the groundwater inside industrial complexes has become a critical issue as the advancement of industries results in using more tetrachloroethylene (PCE) and trichloroethylene (TCE) (Kim *et al.* 2019). The existence of chlorinated aliphatic hydrocarbons (CAHs) such as TCE, PCE, DCE (cis-dichloroethylene), and VC (vinyl chloride) causes groundwater contamination within industrial complexes (Kim *et al.* 2019). For non-drinking groundwater in South Korea, TCE and PCE are included in the criteria of groundwater quality guidelines under environmental quality regulations (Ministry of Environment 2019). The Ministry of Environment has started a program called 'Plans for the Installation of a Groundwater Quality Monitoring Network (GQMN) and Measurement of Water Pollution' to monitor domestic groundwater quality domestically. In the program, TCE and PCE levels are of major interest. Internationally, the World Health Organization is also interested in CAHs (e.g., TCE, PCE, DCE, VC) because they are major contaminants in water. In addition, PCE and TCE can be completely degraded into DCE and VC (carcinogenic compound) through a reductive dechlorination process (Xiao *et al.* 2020, Holliger *et al.* 1998), possibly by bacteria

(*Dehalococcoides ethenogens*) with complete dichlorination ability (Nijenhuis *et al.* 2016). There are two isomers of DCE: cis-DCE and trans-DCE. The isomer form of the DCE is usually cis-isomer in the water-related environment. VC could be created in the course of PVC production and it can exist as a metabolite from the dechlorination of TCE and PCE in groundwater (WHO 2017).

TCE and PCE are usually discharged from industrial complexes or ground storage facilities and flow into groundwater (Park *et al.* 2013). They move through the flow direction of groundwater at earlier stages. TCE is an example of the substance called DNAPL (dense non-aqueous phase liquid), of which specific gravity is higher than that of water. Therefore, TCE can move to lower positions in the groundwater due to its gravity. During its movement, it is transformed into other forms such as DCE and VC due to the generation of DNAPL pools (Kim 1998). If TCE goes into fractured rocks in particular, it is not easy to remove TCE from water (Kavanaugh *et al.* 2003, Kim *et al.* 2019, McCarty 2010). To remove chlorinated organic compounds (e.g., TCE) from groundwater, many water purifying methods have been developed (Kavanaugh *et al.* 2003) such as thermal process, oxidation/reduction reaction, and biological processes. Anaerobic organisms used for reductive dechlorination methods are reported as the most economical method compared to other purification methods (Yu *et al.* 2012), which eliminates highly concentrated TCE effectively (Park *et al.* 2012). This method with electron acceptor gradually transforms PCE into TCE, cis-DCE, and

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VC and the final product is ethylene. Reductive dichlorination preferentially takes place under anaerobic condition.

Anaerobic reductive dechlorination in groundwater contaminated with TCE, South Korea, was confirmed in earlier studies. Jeon *et al.* (2016) reported that once TCE increase, both increase of the cis-DCE and the detection of VC by following groundwater direction were monitored because of the anaerobic dechlorination. Lee *et al.* (2014) also suggested the need for the management of DCE and VC in groundwater, South Korea, because the by-products were detected in wells contaminated with TCE by the biological dechlorination process. Therefore, we evaluated an existing pattern of the four chlorinated compounds and the occurrence possibility of reductive dechlorination process to obtain management need of DCE and VC with PCE and TCE as groundwater standards, South Korea.

To confirm ideal groundwater condition for reductive dichlorination, we analyzed variation characteristics of four CAHs (TCE, PCE, cis-DCE, VC) compared with field components such as dissolved oxygen (DO), oxidation-reduction potential (ORP), and electric conductivity (EC), possibly related to anaerobic biological dechlorination in non-drinking groundwater wells. In addition, microbial dynamic analysis was performed to prove a biological reductive dechlorination process, and a risk assessment was conducted to determine whether the concentrations of the four chlorinated organic compounds were abnormally high.

2. Materials and methods

2.1 Groundwater collection

This study selects about 77 - 111 research points in areas with concerns about the contamination for non-drinking water in South Korea according to the GQMN while considering hydraulic and geological characteristics as well as contaminants. Through monitoring for three years between 2016 and 2018, the study focuses on the analysis of 580 samples. Items for analysis include field items for measurement (e.g., pH, ORP, DO, EC, Temp., HCO_3^-), cation & anion (e.g., Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Cl^- , NO_3^- , HCO_3^- , SO_4^{2-}) and chlorinated organic compounds (e.g., PCE, TCE, cis-DCE, VC). Based on such analysis, this study seeks the possibilities of natural attenuation of TCE in groundwater. Table 1, as shown below, indicates the information on the selected well:

In order to find more details of the various characteristics of chlorinated organic substances in each aquifer, there was monitoring for three years. By selecting monitoring wells with accumulated data for three halves or more (21 wells and 94 samples, except the wells with no detection of data), the survey tried to elicit variation characteristics.

Groundwater samples were taken by the guidelines of ISO 5667-11 and treated and stored by the guidelines of ISO 5667-3. Additional water was sampled in glass vials (40 mL) that were coated with polytetrafluoroethylene (PTFE) with septum attached without headspace for chlorinated organic compound analysis. For water samples, on-well chemical analysis was conducted at the study well using a mobile

Table 1 Environment and contamination sources around groundwater monitoring wells

Classification	Sources
Regional groundwater near various contaminant sources (114)	Farming (2) Agricultural water use area (2)
	Water (56) Pollution concern river area (5) Industrial area (51)
Waste (18)	General waste landfill area (9) Designated waste landfill area (2) The area near the manure treatment plant (7)
	Etc. (38) Resident health survey area (4) Amusement Park & Park area (3) Golf course area (3) Urban residential area (21) Storage tank area (7)

※ The number of monitoring wells are indicated parenthesis

water quality device (ProPlus Multiparameter, YSI, USA) with elements of temperature, pH, the electrodes of DO, ORP and EC installed. In addition, real-time field monitoring by using a flow cell was utilized in order to secure the background groundwater that flows into the actual aquifer during pumping. Water samples were stored in coolers after being wrapped in plastic bags to prevent breakage and the samples were stored in refrigeration at a temperature of 4°C.

2.2 Sample analysis

Cation's analysis was performed based on the guidelines suggested by Standard method 3120 (ASTM, USA). ICP-OES (Perkin Elmer, Optima 8300, USA) was used for cation analysis. Anions were analyzed based on the Standard for the Qualities of Drinking Water (ES05357.1c) of the South Korea with IC (Metrohm, 850 Professional, Switzerland). HCO_3^- was measured through titration by using 0.05 N-HCl, while samples were taken on time at the same time (Carranzo 2012). Chlorinated Organic compounds analysis was performed using Purge and Trap-gas chromatograph/mass (GC/MS), adopted by the Standard for the Qualities of Drinking Water (ES05601.1c) of the South Korea. A purge was carried out for 11 minutes after injecting 25 mL of samples. The absorbed sample was then desorbed for 2 minutes at 250°C before injection. The DB-VRX column was used on the GC/MS. The mass spectrum of each of the four volatile types of chlorinated organic compounds and internal standard substances was checked at the scan mode. After selecting characterized ions by substance, the selected ion monitoring was used for analysis. For sample doses, the internal standard method was applied. The initial rising-temperature conditions for the analysis device were set at 40°C so that the items for analysis could be erupted at a lower temperature and flow into the column.

2.3 Statistics analysis

With 580 sample data from the three years study, the Pearson's correlation analysis was performed by using SPSS21.0 to identify a linear correlation among four

Table 2 Risk assessment equation for groundwater exposure pathways (ingestion, dermal contact, and inhalation) (EPA 2004, Means 1989, Ministry of Environment 2007)

Exposure pathway	Equation	Cancer risk	Non-cancer risk
Ingestion	$ADD = \frac{C_w \times CR_w \times EF \times ED}{BW \times AT}$	Excess Cancer Risk = ADD (Average Daily Dose) × SF (Slope Factor)	HI (Hazard Index) $= \sum HQs \text{ (Hazard Quotients)}$ $HQ = \frac{ADD \text{ (AverageDailyDose)}}{RfD \text{ (Reference Dose)}}$
Dermal contact	$ADD = \frac{DA_{event} \times EV \times SA \times EF \times ED \times CF}{BW \times AT}$		
Inhalation	$ADD = \frac{C_w \times IR \times ET \times EF \times ED \times CF \times VF}{BW \times AT}$		

※ ADD (average daily dose), C_w (chemical concentration in groundwater), CR_w (intakes from ingestion of groundwater used as drinking water), EF (exposure frequency), ED (Exposure duration), BW (body weight), AT (averaging time), DA_{event} (absorbed dose per event), EV (event frequency), SA (skin surface area), CF (unit conversion factor), IR (ingestion rate), ET (exposure time), VF (volatilization factor)

Table 3 Toxicity and carcinogenicity of VC, cis-DCE, TCE and PCE

Substance for survey	Inhalation Exposure		Oral Exposure	
	Unit Risk	RfC	SF	RfD
	$(\mu\text{g}/\text{m}^3)^{-1}$	(mg/m^3)	$(\text{mg}/\text{kg}\cdot\text{day})^{-1}$	$(\text{mg}/\text{kg}\cdot\text{day})$
VC	4.4×10^{-6}	1×10^{-1}	7.2×10^{-1}	3×10^{-3}
cis-DCE	-	-	-	2.1×10^{-3}
TCE	4.1×10^{-6}	2×10^{-3}	4.6×10^{-2}	5×10^{-4}
PCE	2.6×10^{-7}	4×10^{-2}	2.1×10^{-3}	6×10^{-3}

※ (1): Proposed Guidelines for Carcinogen Risk Assessment (USEPA 1996)

(2): Guidelines for Carcinogen Risk Assessment (USEPA 2005)

chlorinated organic compounds (PCE, TCE, cis-DCE, and VC), well items (DO, pH, EC, ORP, temperature) and anions (NO_3^- , SO_4^- , Cl^-). In particular, the principal component analysis (PCA) was implemented to find factors that can affect concentration changes and the distribution of chlorinated organic compounds at every groundwater well.

For the analysis of the main components and factors of groundwater quality parameters, the rotation method called Varimax was applied: it was necessary to allow the correlation between factors so that water quality parameters can be precisely allocated to the relevant factors. Among the orthogonal rotation methods that enable researchers to find out meanings of factors and freely interpret the analysis results, the Varimax method can simplify and interpret results by maximizing the sum of distribution in order to reduce the number of parameters. To confirm the data validity before carrying out factor analysis, results of the Kaiser Meyer Okkin (KMO) test and Bartlett's test were used. The number of the extracted factors is limited to two while considering the total variance explained as well as the values of which the eigenvalue is larger than 1. The Varimax method was applied to analyze factor rotation. If the absolute value of loaded factors is 0.4 or higher, it is regarded as meaningful. Moreover, it is regarded as meaningful only if the P-value is 0.05 or lower.

2.4 Stable isotopes ($\delta^{13}\text{C}$, $\delta^{37}\text{Cl}$) analysis

This study requested the Environmental Isotope Lab, University of Waterloo, to carry out some tests: by applying the continuous flow isotope ratio mass spectrometry, the

analysis found out the isotope ratio of $\delta^{13}\text{C}$ and $\delta^{37}\text{Cl}$ by type of four chlorinated organic compounds that are included in the same sample. In order to implement the online analysis of the isotope of $\delta^{13}\text{C}$ and $\delta^{37}\text{Cl}$, the GC-IRMS system was used. Here, the device for mass spectrometry was made by Thermo, while the 7890 model (Agilent Technologies Inc, Santa Clara, CA, U.S.A.) was used for GC-IR.

2.5 Groundwater quality type assessment

The piper diagram was applied by using cations and anions: The piper diagram has the categories of Na^+ type (Na^+ and K^+ are dominant) and Ca^{2+} type (Ca^{2+} and Mg^{2+} are dominant) for cations, and HCO_3^- type (HCO_3^- is dominant) and Cl^- type (SO_4^{2-} , Cl^- and NO_3^- are dominant) for anions. The final types of water quality are divided into four types: $\text{Ca}^{2+}\text{-(Cl}^+\text{+NO}_3^-)$, $\text{Ca}^{2+}\text{-HCO}_3^-$, $\text{Na}^+\text{-(Cl}^+\text{+NO}_3^-)$, and $\text{Na}^+\text{-HCO}_3^-$. $\text{Ca}^{2+}\text{-HCO}_3^-$ indicates shallow groundwater conditions, $\text{Ca}^{2+}\text{-(Cl}^+\text{+NO}_3^-)$ for artificial contamination, $\text{Na}^+\text{-HCO}_3^-$ for the reaction when shallow groundwater meets an underground structure, and $\text{Na}^+\text{-(Cl}^+\text{+NO}_3^-)$ for the influence of seawater (Cho and Sung 2013, Jeon *et al.* 2002, Ministry of Environment 2013, Na and Son 2005).

2.6 Risk assessment by considering exposure passages of groundwater

The risk assessment was conducted to see whether the four CAHs are carcinogenic risk or not for human bodies. Table 2 suggests the relevant equations and factors. Among pollutants in groundwater, the concentration that is 95

percentiles following EPA risk assessment process (EPA 1992, EPA 2001) of the analysis results of each pollutant on this study by extracting through crystal ball; ver. 11.1.2.4 (Oracle Crystal ball, Oracle, Redwood City, CA, U.S.A.) was applied as C_w (chemical concentration in groundwater). The concentration of non-detect samples was selected by applying the half-value of each item's limit of quantification (LOQ). Toxicity information was collected for the risk assessment of chlorinated organic substances from US EPA IRIS (see Table 3) (EPA 2016). As per *cis* 1,2-DCE, this study could not conduct the risk assessment for exposure passage of intake and carcinogenic risk as there was no value for reference concentration (RfC) and cancer slope factor (SF). The risk assessment considers the following exposure passages of groundwater: intake by drinking, intake by non-drinking (unintentional intake), skin contact, and indoor inhalation of steam. For the passage of skin contact and intake by drinking/non-drinking, the study complies with the guidelines of the US EPA risk assessment. This study follows the guidelines of the US EPA for an indoor inhaling of steam through dishwashing, and it complies with the guidelines of MCP GW-1 Standards for the indoor inhaling of steam through showering (Massachusetts 2016).

2.7 Microorganism analysis

The program called Miseq made by Illumina was utilized for microorganism analysis. PCR amplification and Illumina sequencing were performed. First, PCR amplification was performed using primers targeting from the V3 to V4 regions of the 16S rRNA gene with extracted DNA. For bacterial amplification, primers of 341F (5'-TCGTCGGCAGCGTC-AGATGTGTATAAGAGACA-G-CCTACGGGNGGCWGCAG-3'; underlining sequence indicates the target region primer) and 805R (5'-GTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAG-GACTAC HVGGGTATCTAATCC-3') were used. The amplifications were carried out under the following conditions: initial denaturation at 95°C for 3 minutes, followed by 25 cycles of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, with a final elongation at 72°C for 5 minutes. Then, secondary amplification for attaching the Illumina NexTera barcode was performed with an i5 forward primer (5'-AATGATACGGCAGCACCAGGATCTACAC-XXX XXXXX-TCGTCGGCAGCGTC-3'; X indicates the barcode region) and i7 reverse primer (5'-CAAGCAGAAGACGGC ATACGAGAT-XXXXXXXX-AGTCTCGTGGGCTCGG-3'). The condition of secondary amplification was equal to the former one, except the amplification cycle was set to 8.

The PCR product was confirmed by using 2% agarose gel electrophoresis and visualized under a Gel Doc system (BioRad, Hercules, CA, U.S.A.). The amplified products were purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA, U.S.A.). Equal concentrations of purified products were pooled together and removed short fragments (non-target products) with the Ampure beads kit (Agencourt Bioscience, MA, U.S.A.). The quality and product size were assessed on a Bioanalyzer 2100 (Agilent, Palo Alto, CA, U.S.A.) using a DNA 7500 chip. Mixed

amplicons were pooled and the sequencing was carried out at Chunlab, Inc. (Seoul, South Korea), with an Illumina MiSeq Sequencing system (Illumina, U.S.A.) according to the manufacturer's instructions. For the Miseq pipeline method, raw processing reads start with a quality check and filtering of low quality (<Q25) reads by trimmomatic 0.321. After a QC pass, paired-end sequence data were merged using PandaSeq2. Primers were then trimmed with ChunLab's in-house program at a similarity cut-off of 0.8. Sequences are denoised using Mothur's 3 pre-clustering program, which merges sequences and extracts unique sequences allowing up to 2 differences between sequences. The EzTaxon database is used for Taxonomic Assignment using BLAST 2.2.224 and a pair-wise alignment of 5 is used to calculate similarity. Uchime 6 and the non-chimeric 16S rRNA database from EzTaxon are used to detect chimera on reads that contain a less than 97% best-hit similarity rate. Sequence data is then clustered using CD-Hit 7 and UCLUST 8, and an alpha diversity analysis is carried out.

3. Results and discussion

3.1 General information of on-well groundwater sample analysis across 3 years

As a result of field measurements for all wells (111 wells, 580 samples), the median values are 515.8 $\mu\text{S}/\text{cm}$ (minimum 14.5 ~ maximum 2,584 $\mu\text{S}/\text{cm}$) for EC, 190.5 mV (minimum -153 ~ maximum 2,166 mV) for ORP showing reductive conditions where ORP is less than 200 mV, and 4.1 mg/L (0.2 ~ 11.4 mg/L) for DO (Song *et al.* 2017).

Meanwhile, the median values for each item in wells (21 wells, 94 samples) with detection of four compounds show: 590 $\mu\text{S}/\text{cm}$ (109.8 ~ 1,747 $\mu\text{S}/\text{cm}$) for EC, 141.3 mV (-153 ~ 396.7 mV) for ORP, and 2.7 mg/L (0.2 ~ 11.4 mg/L) for DO. In comparison with the two groups, wells in 21 wells showed a relatively high tendency for EC, and relatively low values for DO and ORP. This shows that aquifers in 21 wells were relatively equipped with suitable environments, such as having a low oxidation state (reductive condition) for the biological reductive dechlorination of CAHs by microorganisms compared to the other wells (Löffler *et al.* 1999, WDNR 2014).

3.2 Piper diagram

Piper diagrams are used to evaluate groundwater type (Walter *et al.* 2017). As a result, a total of 580 groundwater samples are divided into four types: Ca^{2+} - $(\text{Cl}^- + \text{NO}_3^-)$ type (45%) indicates artificial contamination, Ca^{2+} - HCO_3^- type (50%) indicates shallow groundwater condition, Na^+ - $(\text{Cl}^- + \text{NO}_3^-)$ type (2%) indicates reaction when shallow groundwater meets an underground structure, and Na^+ - HCO_3^- type (3%) indicates the influence of seawater, respectively (Cho and Sung 2013, Jeon *et al.* 2002, Ministry of Environment 2013, Na and Son 2005).

Table 4 Summary of chlorinated organic compounds in groundwater during the study (n = 580, 2016~2018)

Parameters	PCE	TCE	cis-DCE	VC
Detection frequency (%)	30.2	45.1	43.9	13.4
Minimum Limit of Quantitation (LOQ) (µg/L)	0.17	0.156	0.115	0.17
Average	13.6	18.8	19.1	0.4
Maximum	2079.4	1088.6	727.9	30.7
Standard exceeding rate (%)*	5.4	10.8	8	5.3
Annual average concentration increase/decrease value (µg/L/year)	6.9	-5.3	-1.2	0.2
Annual average concentration increase/decrease rate (%/year)	+230.1	-21.9	-6.2	+168.5

* Standard exceeding the rate

- PCE, TCE : Korean groundwater quality standard concentration (PCE : 10 µg/L, TCE : 30 µg/L).

- cis-DCE : US EPA drinking water standards and health advisories tables (conc. : 70 µg/L).

- VC : Korean drinking water monitoring criteria (conc. : 2 µg/L).

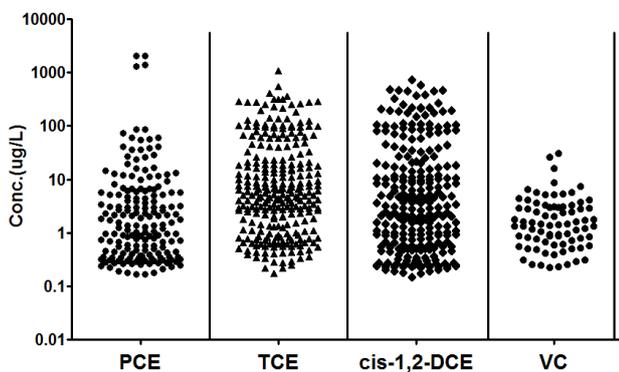


Fig. 1 The individual value of four chlorinated organic compounds in groundwater for 3 years

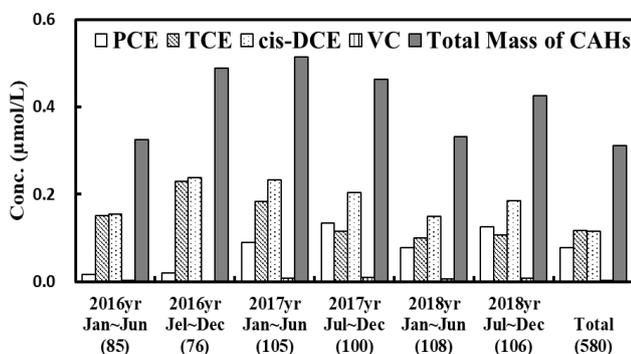
3.3 Distribution of chlorinated organic substances in groundwater

Detection frequency, average and maximum detection values, each of the four CAHs for three years (2016~2018) were listed in Table 4. Individual values for four CAHs monitored in 580 samples at 111 wells for three years are displayed in Fig. 1.

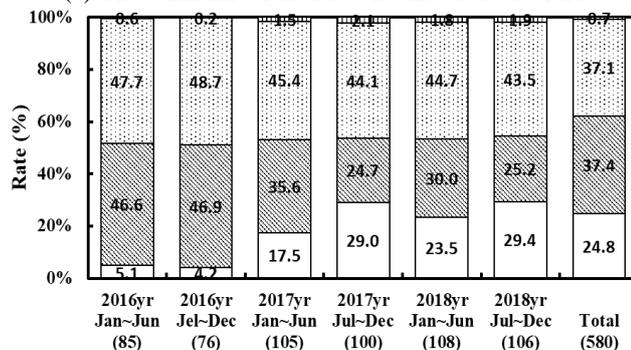
The concentration ranges of four CAHs are between ND (non-detection) and 2,079 µg/L for PCE, between ND and 1,088 µg/L for TCE, between ND and 727 µg/L for cis-DCE, and between ND and 30 µg/L for VC. The detection frequency is: 45.1% for TCE, 43.9% for cis-DCE, 30.2 % for PCE, and 13.4% for VC.

Trends in concentrations of four CAHs for about three years are shown in Fig. 2. Among the four CAHs, the fraction of PCE was 29.4% in 2018, above five fold higher than 5.1% in 2016. On the other hand, cis-DCE, a biological reduction by-product of PCE and TCE, were detected relatively constant (47.7% ~ 43.5%).

In particular, the detection level and frequency of cis-DCE confirm to be relatively higher than that of VC. Ahn *et al.* (2006) reported that intermediate products with low chlorine volumes such as cis-DCE or VC may have issues of degradation, in general, depending on field conditions, which may result in accumulation. The more chlorine water that is substituted from intermediate products in the course of dechlorination, the slower the dechlorination speed, which may result in the accumulation of intermediate products such as DCE and VC (Ministry of Environment 2009, Vogel *et al.* 1987). Based on the study results, it is regarded that the dechlorination turning PCE and TCE in groundwater into cis-DCE goes relatively smoothly as there are several substituted chlorines. In contrast, the complete dechlorination of PCE and TCE to ethylene gets delayed or suspended due to a lack of electron donors, nutrients, and microorganisms such as *Dehalococcoides spp.* The accumulation of cis-DCE, one of the intermediate product elements of dechlorination, was confirmed in monitored aquifers and the pattern was also monitored in previous studies (Aepli *et al.* 2010, Dolinová *et al.* 2017, Gafni *et al.* 2020, Hunkeler *et al.* 2011), showing that dechlorination



(a) The semi-molar concentration of CAH



(b) The mass ratio of each CAHs

Fig. 2 Concentration and rate of monitored chlorinated organic compounds (All wells)

Table 5 Simultaneous detection of two chlorinated organic compounds (n = 269)

Compounds	PCE (n = 179)	TCE (n = 269)	cis-DCE (n = 260)	VC (n = 80)
PCE	100%	62.1%	59.6%	68.8%
TCE	93.3%	100%	86.9%	97.5%
cis-DCE	86.6%	84%	100%	97.5%
VC	30.7%	29%	30%	100%

Table 6 The result of VOCs distribution percentage for six wells

Well numbers	n*	PCE (%)	TCE (%)	cis-DCE (%)	VC (%)
Well 1	14	67.76 ± 20.81	11.05 ± 6.27	21.09 ± 7.00	0.1 ± 0.04
Well 2	8	0.36 ± 0.34	32.85 ± 11.90	62.3 ± 22.55	4.49 ± 2.21
Well 3	8	1.56 ± 0.72	26.99 ± 9.96	59.85 ± 24.20	11.59 ± 5.78
Well 4	7	0.8 ± 0.33	63.23 ± 25.49	34.37 ± 13.68	1.61 ± 0.78
Well 5	12	4.83 ± 1.16	25.72 ± 7.79	67.52 ± 16.44	1.93 ± 0.69
Well 6	11	0.39 ± 0.15	98.83 ± 29.27	0.59 ± 0.43	0.19 ± 0.33

n*: The number of samples used

from cis-DCE to VC was slow.

The simultaneous detection pattern of four CAHs is shown in Table 5. TCE was detected in all wells (n = 269) as shown, but the detection rate of their by-products was sequentially decreased (TCE > cis-DCE > VC). Notably, the simultaneous detection rate of TCE and DCE in wells where VC was detected is 97.5%, respectively. That is, most of the TCE and cis-DCE were detected simultaneously in the VC detection wells. This seems to be the basis for the possibility of progression of dechlorination reactions in the field, considering the above-mentioned changes in the average concentration for each compound (VC increase, cis-DCE and TCE decrease trend) over time.

3.4 Monthly monitoring of chlorinated organic substances

To draw the variation characteristics by period from the aquifer in natural flow status, six wells with a relatively high concentration of PCE and TCE were selected for monthly monitoring (see Table 6).

As a result of the monthly monitoring, there are three degradation characteristics found that depend on biogeochemical characteristics in the aquifer: PCE, TCE → cis-DCE; well 1, PCE, TCE → VC; well 2, 3, 4, 5, and no-degradation; well 6. In most aquifers in this survey, it confirmed that there has been degradation up to the stage of cis-DCE. As per the seasonal variation characteristics of chlorinated organic substances, there was no obvious change of the substances by period. As time went on and the process went by TCE → cis-DCE → VC, dechlorination continued. However, the degradation rate varied. According to Fennel *et al.* (2001) and Hendrickson *et al.* (2002), there

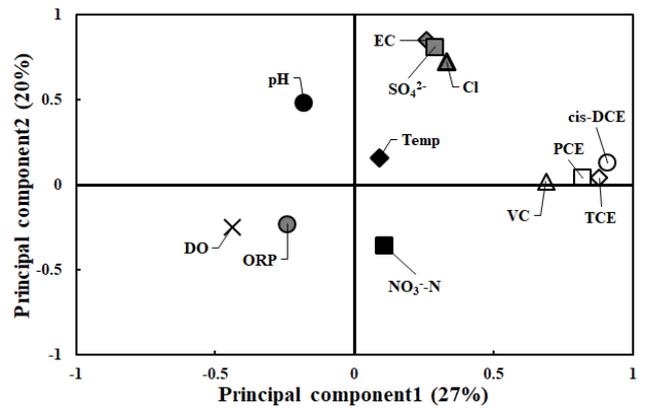


Fig. 3 Factor loading plot (PC1 vs PC2) by principal components analysis

is an accumulation in ethylene or intermediate products (e.g. cis-DCE, VC) by the complete dechlorination of TCE depending on the organisms within the underground aquifer. At well 1, chlorinated organic pollutant amounts decreased as there was intensive rainfall in July and August 2017 ($37.2_{\text{Total}} \mu\text{mol/L} \rightarrow 18.9_{\text{Total}} \mu\text{mol/L}$). The composition rate of chlorinated organic substances was almost the same after inputting PCE (3.5% → 42.0%) into the aquifer since 2017. That suggests that dilution due to rainfall or inflow differences may cause some changes, but it does not make huge differences for the composition rate. Here, it is confirmed that the decrease in the concentration of chlorinated organic substances is highly influenced by dichlorination.

3.5 Correlation characteristics analysis of groundwater quality

In over 50% of the entire number samples, the chlorinated organic compounds were not detected from the monitoring of this study. If the number is ignored or deleted, the total average value could have a great error. That is why this study replaced the concentration volume of ND samples with half of LOQ by Method 2 as mentioned by Hornung and Reed (Hornung and Reed 1990). After the replacement, all the concentration data logs became transformed. Other data logs of factors that did not go through normalization also became transformed.

The correlation characteristics analysis among parameters of groundwater quality was listed in Table 7. In particular, DO and ORP have a negative correlation with the four chlorinated organic compounds, while they react to complete anaerobic conditions (Aulenta *et al.* 2006). EC showed a positive correlation with the four compounds. In general, EC is an indicator of the inflow of pollutants in groundwater. Sulfate ions showed a positive correlation with chlorinated organic compounds, which is regarded to be related to sulfate reduction (Saiyari *et al.* 2018).

3.6 Principle components analysis for groundwater quality parameters

Table 8 and Fig. 3 indicate what the analysis results showed. PC1 can be used to accurately check the negative

Table 8 Rotated factor matrix extracted from principal components analysis

Variable	PC1	PC2
cis-DCE	0.91	0.13
TCE	0.88	0.04
PCE	0.82	0.04
VC	0.69	0.02
DO	-0.44	-0.25
ORP(Eh)	-0.24	-0.23
EC	0.26	0.85
SO ₄ ²⁻	0.29	0.81
Cl ⁻	0.33	0.72
pH	-0.18	0.48
NO ₃ ⁻	0.11	-0.36
Temp.	0.09	0.16
Eigenvalue	3.9	1.8
Variance explained (%)	27	20

Table 9 Risk assessment of organic chloride compounds for the 3-year study periods

Compounds	Cancer risk					
	Yes					
	Ingestion	Non-dietary ingestion	Dermal	Inhalation	Non-drinking	Drinking & Non-drinking
VC	●	○	●	○	●	●
cis-DCE	-	-	-	-	-	-
TCE	●	⊙	●	○	●	●
PCE	⊙	○	⊙	○	⊙	⊙
Compounds	No					
	Ingestion	Non-dietary ingestion	Dermal	Inhalation	Non-drinking	Drinking & Non-drinking
	VC	□	□	□	□	□
cis-DCE	■	□	■	-	■	■
TCE	■	▣	■	■	■	■
PCE	▣	□	▣	▣	■	■

※ Cancer Risk: ● (Cancer Risk > 10⁻⁶), ⊙ (10⁻⁷ ≤ Cancer Risk ≤ 10⁻⁶), ○ (Cancer Risk < 10⁻⁷)

Non-Cancer Risk(HI) : ■ (HI > 0.1), ▣ (0.01 ≤ HI ≤ 0.1), □ (HI < 0.01).

correlation between chlorinated organic compounds and items to indicate the redox degrees (DO, Eh). In particular, positive correlations among three of the chlorinated organic compounds except for VC (> 0.6) allowed this study to confirm the consecutive microbiological dechlorinating and cis-DCE accumulation (Ministry of Environment 2009, Vogel *et al.* 1987).

3.7 Risk assessment of chlorinated organic substances

Table 9 indicates the results of the risk assessment analysis for chlorinated organic substances. As a result of the risk assessment for chlorinated organic substances including TCE, the carcinogenic and the non-carcinogenic risk of compounds is calculated as in 10⁻⁵ (carcinogenic)

and 1 or more (non-carcinogenic). This suggests that there is a chance of overestimation for the 95th percentile concentration of some substances, as some wells are detected with high concentrations of such substances. In the end, however, all VC (by-product of dechlorination of TCE and PCE that are not included in the criteria of the current groundwater quality standards) and cis-DCE showed a certain level of risks to humans. Accordingly, there is a need for management measures. The carcinogenic risk of VC is at the level of 10⁻⁶, while the non-carcinogenic risk of cis-DCE is at the level of 10⁻¹.

3.8 Microorganisms in groundwater

This study selected five wells where the four chlorinated organic substances (PCE, TCE, cis-DCE and VC) were

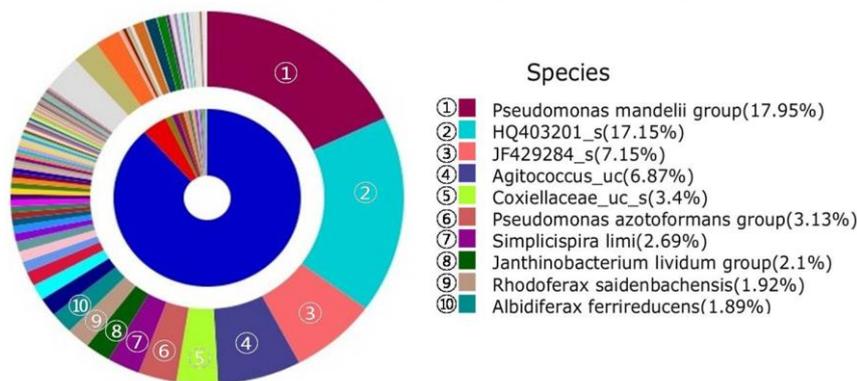
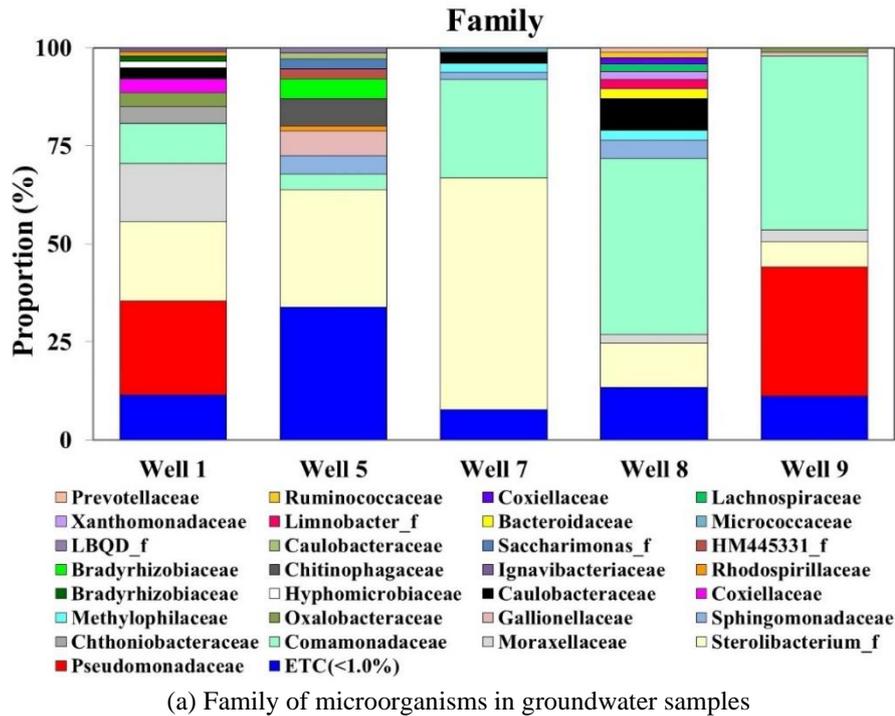


Fig. 4 Microorganism cluster in groundwater samples

detected to see microorganisms that are related to the dechlorination process in groundwater (see Fig. 4 (a)).

In-situ bioremediation (ISB) is a technique that uses reductive chlorination under the sulfate reducing conditions at a well where soil and groundwater are contaminated by TCE, which is known as an economical way of purification (Marco-Urrea *et al.* 2015, Saiyari *et al.* 2018). The reductive chlorination under the sulfate reducing conditions can happen along with denitrification, sulfuric acid, and steel reduction. In this case, nitrate, manganese, and CO₂ can replace hydrogen as electron acceptors (EPA 2006). In particular, nitrate is preferred for use over other electron acceptors for replacement compared to oxygen, manganese, and iron (Leeson *et al.* 2004). Taking all these into consideration, the ecosystem analysis of microorganisms checked whether those microorganisms have functions of denitrification and reduction of chlorinated organic compounds. As a result of the analysis, all five wells showed high distribution rates of 10 species including the

Pseudomonas veronii and *Pseudomonas mandelii* groups as well as 10 families such as *Sterolibacterium.f* and *Comamonadaceae* (see Fig. 4(b)). It turned out that microorganisms that belong to groups such as *Comamonadaceae*, *Pseudomonas veronii* and *Rhodoferax saidenbachensis* are related to denitrification, and *Comamonadaceae*, *Pseudomonadaceae*, and *Pseudomonas mandelii* are related to the degradation of organic compounds (Coleman *et al.* 2002, Elsner *et al.* 2005, Khan *et al.* 2002, KEMB 2020, Li *et al.* 2014). Also, *Geobacter*, *Desulfotobacterium*, *Desulfuromonas*, *Dehalobacter*, and *Dehalococcoides* were found from some wells that help dechlorination of TCE and PCE (Saiyari *et al.* 2018).

The isotopic analysis of $\delta^{13}\text{C}$ and $\delta^{37}\text{Cl}$ for TCE and DCE was conducted to see whether cis-DCE is created as a metabolite during the dechlorination process of TCE. As a result, the isotopic rate (%) of $\delta^{13}\text{C}$ for cis-DCE increased compared to that of $\delta^{13}\text{C}$ for TCE, while the isotopic rate (%) of $\delta^{37}\text{Cl}$ decreased (see Table 10).

Table 10 Comparison for stable isotopes of carbon and chloride between TCE and cis-DCE

WELL	TCE		cis-DCE	
	$\delta^{13}\text{C}$	$\delta^{37}\text{Cl}$	$\delta^{13}\text{C}$	$\delta^{37}\text{Cl}$
	-----‰-----			
GW1	-27.2	3.1	-22.8	1.6
GW4	-26.5	2.7	-24.6	0.9
GW10	-24	0.5	-22.1	-0.3
GW11	-27.7	1.1	-27.8	0

This was in line with previous findings that the TCE concentration decreased while the concentration of intermediate products (e.g., DCE) increased when TCE biodegradation occurred, and $\delta^{13}\text{C}$ rate (‰) of DCE increased while the rate (‰) of $\delta^{37}\text{Cl}$ (subject of bond cleavage, isotope of chlorine) decreased (Elsner *et al.* 2005, Park *et al.* 2013). Based on the results of the mentioned isotopic rate and precise survey, it is confirmed that cis-DCE and VC may be created as metabolites in the course of dechlorination of TCE.

4. Conclusions

This study was conducted to understand the distribution of chlorinated organic contaminants and to prepare groundwater management plans in South Korea. For three years (2016 to 2018), groundwater monitoring, risk assessment, and isotope analysis were conducted, and the followings are the conclusions:

- In the result of Pearson's correlation analysis and principal component analysis with data from wells commonly contaminated with PCE, TCE, DCE and VC, EC and sulfate have a positive correlation with four chlorinated organic compounds, while DO and ORP have a negative correlation with such four compounds. It is confirmed that 21 wells were relatively suitable with their environmental conditions for the dechlorination of chlorine compounds by microorganisms compared to all the wells.

- In evaluation of mass balance for chlorinated organic compounds at the 21 wells, the PCE distribution rate was relatively low (-19.3%) and the VC and cis-DCE rates were relatively high (+15.6%) at the wells where TCE degradation was confirmed. This suggests that PCE remains for a short period time at the wells where there was dechlorination progresses from the stage of the underground aquifer in a natural flow status to the VC stage. It also suggests that there is a chance of a complete dechlorination process. However, it was confirmed that cis-DCE was detected more than VC, which means the dechlorination of PCE and TCE towards the stage of cis-DCE relatively went smoothly although the dechlorination from cis-DCE to the VC and ethylene stage failed to reach the full dechlorination due to the shortage of electron donors, nutrients, and microorganisms at the wells. Therefore, DCE and VC concentrations can rapidly increase if the electron donors and nutrients are sufficiently provided for the dechlorination process. In fact, VC has continuously been

detected at six major points, and the VC concentration seems to drastically increase at some wells.

- Finally, as the result of human risk assessment of chlorine-based organic compounds in groundwater, TCE was found with the highest carcinogenic and non-carcinogenic risk. Furthermore, cis-DCE had a non-carcinogenic risk of 10^{-1} , and the carcinogenic risk of VC was 10^{-6} or higher. These results confirmed with a high possibility that cis-DCE and VC can no longer be neglected in South Korea. Therefore, it is necessary to establish Korean groundwater quality standards to manage DCE and VC in groundwater.

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