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Short communication

Associating potential 1,4-dioxane biodegradation activity with groundwater geochemical parameters at four different contaminated sites

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ABSTRACT

1,4-Dioxane (dioxane) is a groundwater contaminant of emerging concern for which bioremediation may become a practical remediation strategy. Therefore, it is important to advance our heuristic understanding of geochemical parameters that are most influential on the potential success of intrinsic bioremediation of dioxane-impacted sites. Here, Pearson's and Spearman's correlation and linear regression analyses were conducted to discern associations between 1,4-dioxane biodegradation activity measured in aerobic microcosms and groundwater geochemical parameters at four different contaminated sites. Dissolved oxygen, which is known to limit dioxane biodegradation, was excluded as a limiting factor in this analysis. Biodegradation activity was positively associated with dioxane concentrations ($p < 0.01$; $R < 0.70$) as well as the number of catabolic *thm*A gene copies ($p < 0.01$; $R = 0.80$) encoding dioxane monooxygenase. Thus, whereas environmental factors such as pH, temperature, and nutrients may influence dioxane biodegradation, these parameters did not exert as strong of an influence on potential biodegradation activity as the *in situ* concentration of substrate dioxane at the time of sampling. This analysis infers that aerobic sites with higher dioxane concentrations are more likely to select and sustain a thriving population of dioxane degraders, while sites with relatively low dioxane concentrations would be more difficult to attenuate naturally and may require alternative remediation strategies.

1. Introduction

1,4-Dioxane is a stabilizer added to chlorinated solvents such as 1,1,1-trichloroethane (1,1,1-TCA) and has been frequently reported as a co-occurring contaminant in groundwater impacted by chlorinated solvents (Anderson et al., 2012; Adamson et al., 2014). The Environmental Protection Agency (EPA) has classified dioxane as a probable human carcinogen (Class B2) and has proposed a provisional concentration limit of ≤0.35 μg/L based on a 10^{-6} cancer risk (EPA IRIS, 2013). Studies have shown that bacteria capable of degrading dioxane might be more widespread than previously assumed (Huang et al., 2014; Li et al., 2015; Parales et al., 1994; Sales et al., 2013; Sei et al., 2010, 2013), which has increased interest in bioremediation and monitored natural attenuation as potentially cost-effective alternatives to manage large dioxane plumes. Thus, assessing the potential biodegradation activity at contaminated sites is important for the selection (or rejection) of such remedial strategies.

Natural attenuation rates of dioxane at source zones have been previously estimated by evaluating temporal concentration changes within comprehensive field datasets, and linear discriminant analyses have been used to determine how site-specific conditions influence the estimated rates (Adamson et al., 2014). However, statistical analyses of field data cannot easily discern the contribution of biodegradation to the observed removal rate relative to all other natural attenuation processes (e.g., advection,

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dilution, dispersion). Moreover, considering that oxygen availability is a common limiting factor for dioxane degradation *in situ*, correlations based on field data may not reflect the potential biodegradation activity in a more favorable biostimulated environment where dissolved oxygen is plentiful. We postulate that potential biodegradation activity can be determined in microcosms under controlled favorable settings (e.g., sufficient oxygen availability) to assess how other geochemical parameters (e.g., dioxane, nutrient and co-contaminant concentrations, groundwater pH and temperature) affect the potential for bioremediation.

In this work, ordinary least squares regression as well as Pearson and Spearman correlation coefficients were used to assess possible associations between groundwater geochemical parameters (measured at 16 monitoring wells from four different contaminated sites) and the corresponding potential dioxane biodegradation activity, which was determined in aerobic microcosms. These heuristic relationships may provide valuable insight on dioxane contamination scenarios that are amenable to remediation by monitored natural attenuation.

2. Materials and methods

2.1. Groundwater monitoring data

Groundwater geochemical data were compiled from various monitoring wells (MWs) installed at four separate contaminated sites in the US. Two MWs were located in Alaska and 14 MWs were located in three independent sites in Southern California. Aquifer material used for determination of potential dioxane biodegradation activity in microcosms was representative of the time groundwater was collected for geochemical analysis. Possible correlations between dioxane biodegradation rates and geochemical variables [e.g., nutrients (NO_3^-/NO_2^-, NH_3 and PO_4^{3-}), temperature, pH and conductivity] were explored. It is important to recognize that other co-contaminants were also reported [e.g., tetrachloroethylene (PCE), trichloroethylene (TCE), 1,1-dichloroethylene (1,1-DCE), trichloroethane (TCA), and 1,1-dichloroethane (DCA), methyl-*tert*-butyl ether (MTBE), benzene, toluene, ethyl benzene and isomers of xylene (BTEX), 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene, sec-butylbenzene, tert-butylbenzene, n-butylbenzene, isopropylbenzene, n-propylbenzene, p-isopropyl toluene, naphthalene, 2-methylnaphthalene, chloroform, and trichloropropane]. Other non-volatile co-contaminants were also found at trace levels and in only a few of the MW's considered. Analysis of these parameters were not statistically dependable due to insufficient number of data and therefore not explored here. Groundwater concentrations of volatile organic compounds (EPA method 5030B or 8260B), PO_4^{-3}, NO_3^-, NO_2^- (EPA method 300.0), NH_3 (EPA 350.3), DO (APHA 4500-O or membrane electrode probe corrected for temperature), Total organic carbon (TOC, APHA 5310D) as well as data on conductivity (APHA 2510B) and pH (APHA 4500 H+B) were obtained by independent laboratories.

2.2. Determination of potential dioxane biodegradation activity

Dioxane biodegradation rates were determined using microcosms prepared with aquifer materials (10–50 g) and groundwater (50–150 mL) samples collected from four contaminated sites (Li et al. 2010, 2013). Microcosms were maintained at controlled temperatures that matched site-specific groundwater temperatures. Autoclaved and poisoned (200 mg/L of $HgCl_2$) microcosms served as controls to discern dioxane biodegradation from abiotic losses. The concentrations of dioxane were monitored over time using a frozen microextraction method followed by gas chromatography/mass spectrometry (Li et al., 2011) (detection limit of $\geq$1 ppb). In each microcosm, the dioxane biodegradation rate was calculated as the average of the removal rate (concentration versus time slope) corrected for the losses in autoclaved controls.

2.3. Determination of thmA/dxmA genes concentrations

At the end of microcosm experiments, aquifer material was collected from microcosms and the bacteria DNA was extracted using commercially available kits (PowerSoil DNA Isolation Kit-MoBio). Real-time quantitative PCR was performed using a previously developed set of primers and probes to target naturally occurring thmA/dxmA genes coding for soluble di-iron monooxygenase (SDIMO) known to be involved in the initiation of dioxane and tetrahydrofuran metabolism (Li et al., 2013).

2.4. Statistical analyses

Associations between dioxane biodegradation rates (k′; μg/L/d) and groundwater physical-chemical parameters were assessed using linear regression analyses and Pearson and Spearman correlation coefficients. The Kruskal-Wallis test for dioxane concentrations from the 4 sites yielded a *p*-value of 0.12 while the test for biodegradation rate yielded a *p*-value of 0.35. This implies that the dioxane concentrations and biodegradation rate distributions are statistically undiscernible at the 95% confidence level and can be aggregated for further analyses. Linear regression analyses were performed using normally distributed biodegradation rates. Because the observed biodegradation rates violated the normality assumption by being non-symmetric and right-skewed, a logarithmic transformation was used for correction. To ensure that any positive correlations were not driven by outliers, 16 $\log_{10}$ dioxane concentrations were binned by 25th, 50th, and 75 th percentiles into 4 clusters to compare $\log_{10}$ biodegradation rates. For each cluster of $\log_{10}$ dioxane concentrations, a boxplot of $\log_{10}$ biodegradation rates were generated to verify a positive trend. The detection limit for observed dioxane biodegradation rates introduces zero values, which are undefined under a logarithmic transformation. Thus, dioxane biodegradation rates were translated by +0.01, corresponding to the detection limit. Any small, positive value less than the detection limit would be suitable, but +0.01 was chosen for simplicity and numerical convenience. Chemical parameters and contaminants were also translated and $\log_{10}$ transformed in situations where the quantity of interest was right skewed with values below the detection limit (e.g., PCE, TCE, DCE, TCA, and DCA).

Additional regression analyses were performed to test for linear relationships between initial concentrations of dioxane and chlorinated solvents. Due to missing values for various chemical parameters, three groups (n = 16, n = 14, n = 12) were considered for correlation analyses: *thmA* copy numbers (n = 16); dissolved oxygen, temperature, pH, and conductivity (n = 14); and the concentrations of TOC, NO_3^-, NO_2^-, and PO_4^{3-} (n = 12).

Pearson and Spearman correlation coefficients were also calculated for the three groups to determine strength of linear relationships and non-linear, monotonic relationships, respectively. Significance of Pearson and Spearman correlation coefficients was assessed using test statistics based on the t and F distributions, respectively. More specifically, the test statistics check if the correlation value (R) is not equal to zero at the 95% confidence level. Thus, *p*-values $\leq$ 0.05 denote significant correlations.

The Kruskal-Wallis test, regression analysis, and correlations were performed in R, a statistical software package (https://cran.r-project.org/) using built in functions for Kruskal-Wallis tests and linear regressions; Harrell Miscellaneous (HMisc) package version 4.0–1 for correlations (https://cran.r-project.org/web/packages/Hmisc/index.html); and ggplot2 (http://ggplot2.org) for graphics.

Table 1
Associations between dioxane and chlorinated solvent concentrations per Pearson correlation analysis. Significant associations were assumed for *p*-values ≤ 0.05, as depicted by gray shaded areas. Sample size n = 16 unless otherwise noted.

	Log_{10} PCE		Log_{10} TCE		Log_{10} DCE		Log_{10} TCA		Log_{10} DCA	
	p[a]	R[b]	*p*	R	*p*	R	*p*	R	*p*	R
Log_{10} Dioxane	0.507	−0.18	0.02	0.58	0.04	0.53	<0.01	0.87	0.236	−0.31
Log_{10} TCE	0.230	0.32	–	–	–	–	–	–	–	–
Log_{10} DCE	0.315	0.27	<0.01	0.79	–	–	–	–	–	–
Log_{10} TCA	0.534	−0.17	0.09	0.43	0.357	0.25	–	–	–	–
Log_{10} DCA	0.01	0.624	0.71	−0.10	0.728	−0.09	0.654	−0.12	–	–

[a] *p*-values for Pearson correlation.
[b] Pearson correlation.

R markdown and Knitr (http://rmarkdown.rstudio.com) were used to prepare the Supporting information.

3. Results and discussion

Dioxane concentrations showed positive associations with the concentrations of commonly found co-contaminants, i.e., TCA ($p < 0.01$; R = 0.87), TCE ($p = 0.02$; R = 0.58), and their dechlorinated byproduct DCE ($p = 0.04$; R = 0.53) (Table 1). The strongest association was observed with TCA, which might reflect the fact that dioxane was primarily used as a stabilizer for this specific chlorinated solvent (Mohr et al., 2010).

The Kruskal-Wallis test for dioxane concentrations from the 4 sites yielded a *p*-value of 0.12 while the test for biodegradation rate yielded a *p*-value of 0.35. This implies that the dioxane concentrations and biodegradation rate distributions are statistically undiscernible at the 95% confidence level and could be aggregated for further analyses. 1,4-Dioxane biodegradation rate was significantly associated with the concentrations of dioxane present in the monitoring wells at the time of sampling ($p < 0.01$; R = 0.70). This is paradoxical because groundwater from high-concentration monitoring wells should experience faster degradation rates that rapidly decrease dioxane concentrations, which is not always observed. However, quantitative interpretation of natural attenuation can be confounded by poorly-understood source dynamics, such as release of dioxane that had been entrapped in clay pockets that serve as a sustained source for groundwater contamination (Adamson et al., 2016). Replenishment of dioxane from such overlooked sources would offset removal by biodegradation. Our microcosm experiments avoided such confounding factors and quantitatively discerned potential biodegradation activity. Furthermore, this statistical inference is consistent with the law of mass action; i.e., degradation rates are faster with higher (non-toxic) reactant concentrations (Alvarez and Illman, 2005), and trace contaminant concentrations may pose a challenge to meet enzyme induction thresholds (Schmidt et al., 1987; Lechner and Straube, 1984) or minimum cell maintenance energy requirements (Bosma et al., 1996; Schmidt et al., 1985).

Biodegradation rates were also significantly associated with the catabolic *thm*A gene copy numbers ($p < 0.01$; R = 0.80) (Table 2). The correlation between *thm*A gene copy numbers and dioxane biodegradation rate was discussed elsewhere (Li et al., 2013). The potential involvement of other important monooxygenases, dehydrogenases, and or hydroxylases that are also known to participate in co-metabolic biodegradation of dioxane should not be ruled out (Gedalanga et al., 2014; Lan et al., 2013; Mahendra and Alvarez-Cohen, 2006). Nonetheless, the strong association between *thm*A abundance and dioxane biodegradation rates suggested that *thm*A was a relevant biomarker to infer on biodegradation at these sites.

Dioxane biodegradation rates ranged from 0.03 to 493 μg/L/d (average of 68.2 ± 129.1 μg/L/d) and followed zero-order kinetics. Adamson et al. (2015) have previously evaluated data from a large number of contaminated sites and reported the importance of dissolved oxygen on the natural attenuation of dioxane (Adamson et al., 2014). Therefore, the influence of oxygen concentrations on dioxane biodegradation is already well established. In this work, dioxane biodegradation rates were measured in microcosms under unlimited oxygen conditions. Accordingly, a non-significant association was observed between degradation rates and the dissolved oxygen concentration (Table 2) present at these sites at average concentrations of 2.5 ± 1.8 mg/L. The presence of nitrogen (NO_3^- + NO_2^-) and phosphorus (PO_4^{3-}) as nutrients in groundwater at average concentrations of 15.3 ± 21.1 mg/L and 10.4 ± 22.9 mg/L, respectively, did not significantly affect potential dioxane biodegradation activity. Other physical-chemical parameters such as pH (ranging from 6.1 to 7.1), conductivity (ranging from 0.2 to 2.5 mS/cm) and temperature (varying from 5.1 to 25 °C) were also not significantly associated with the measured degradation activity.

Most volatile organic compounds present in the groundwater may have been lost through volatilization during sample collection

Table 2
Associations between dioxane biodegradation rates (k′) and geochemical data per Pearson's or Spearman's correlation analyses. Significant associations were assumed for *p*-values ≤ 0.05, as depicted by gray shaded areas. Sample size n = 14 unless otherwise noted.

	Pearson												Spearman[d]			
	Log Dioxane (μg/L)		Log_{10} *thm*A[c] (gene copy numbers/g-soil)		DO (mg/L)		pH		Groundwater (°C)		Log_{10} Conductivity (mS/cm)		NO_3^- + NO_2^- as N (mg/L)		Log_{10} PO_4^{3-} as P (mg/L)	
	p[a]	R[b]	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R	*p*	R
Log_{10} (k′) (μg/L/d)	<0.01	0.70	<0.01	0.80	0.483	0.21	0.248	−0.33	0.259	0.32	0.239	−0.34	0.372	−0.28	0.712	−0.12

[a] *p*-values for Pearson or Spearman correlations.
[b] Pearson correlation.
[c] n = 16.
[d] n = 12.

and/or transportation, as well as during microcosm preparation and samplings. Therefore, determination of dependable associations between dioxane biodegradation rates and the concentrations of volatile compounds present at the time of sampling could be misleading and were not explored here. Note that while some chlorinated solvents hinder dioxane biodegradation by some bacteria (e.g., *Pseudonocardia dioxanivorans* CB1190) (Zhang et al., 2016), an inhibitory effect was not observed for other dioxane degraders using different enzymes [e.g., *Pseudonomas mendocina* KR1 and *Escherichia coli* TG1 (T4MO)] (Mahendra et al., 2013). It is unclear whether potential inhibitory effects mainly take place immediately after microorganisms are first exposed to chlorinated solvents, or if inhibition occurs to a lesser extent (or not at all) for acclimated bacteria with exposure history.

Determining the variation in lithology among the 16 different contaminated sites was beyond the scope of this study. In general, these sites were characterized by unconsolidated sediments (e.g., sands, silts, and clays) and were thus comparable. An evaluation of how the site specific sediment composition (Xia et al., 2011) and background organic matter type and concentration (Yang et al., 2011) as well as associated hydrogeologic characteristics may impact dioxane biodegradation rates and correlations would be valuable. However, such an evaluation was not possible in this study due to the relatively limited number of sites available, all with similar lithology and monitoring well construction (i.e., fully submerged well screens). Epidemiologists often account for such confounding factors by considering very large databases that randomize them, which was impossible in this study.

Overall, these results suggest that while many geochemical factors (pH, temperature, nutrients) may be important for dioxane biodegradation, these factors may not exert as strong of an influence on the potential biodegradation activity as the concentration of dioxane. Unequivocally, when oxygen is not limiting *in situ* dioxane concentrations significantly influences the observed dioxane biodegradation rates (Fig. 1) with higher dioxane concentrations possibly selecting for a thriving microbial population, enhancing the sustenance of bacteria metabolic capabilities (Grostern et al., 2012) and/or inducing specific enzymes (Sales et al., 2013). This suggests that enhanced aerobic bioremediation strategies would be most effectively employed in source areas with higher dioxane concentrations and acclimated microbial communities.

This work provides valuable insight into site-specific scenarios where significant dioxane biodegradation might be expected. Specifically, while sites with higher dioxane concentrations are more likely to select for and sustain dioxane degraders, sites with relatively low dioxane concentrations would be more challenging to attenuate naturally and may require implementation of alternative remediation strategies. Whether there is a site-specific threshold dioxane concentration of regulatory concern below which sustained biodegradation is unlikely to proceed remains to be determined.

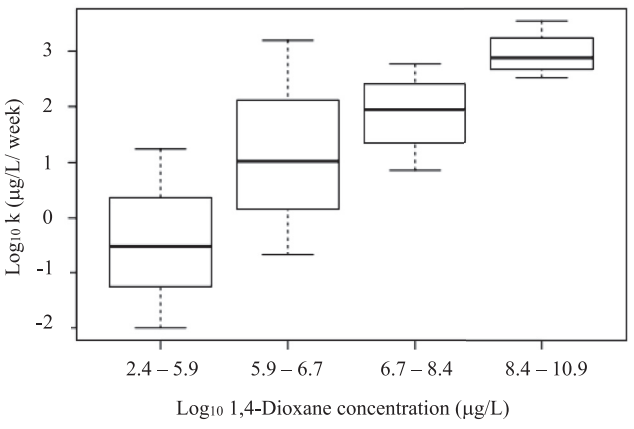

Fig. 1. Boxplots of logarithmically transformed biodegradation rates and 1,4-dioxane concentrations from 16 monitoring wells. Dioxane concentrations were binned into quartiles resulting in data from 4 wells per bin. For each bin, a boxplot was constructed using log biodegradation rates for the respective site.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2017.10.031.

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