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Degradation of chloroethenes in the transition zone between aquifers and aquitards

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Abstract

In the transition zone between aquifers and basal aquitards, the perchloroethene-pools are more recalcitrant than those elsewhere in the aquifer. The aim of this study is to demonstrate that the transition zone is an ecotone where the biodegradation of chloroethenes from aged-pools of perchloroethene is favored. A field site was selected where an aged pool exists at the bottom of a transition zone. Two boreholes were drilled to obtain sediment and groundwater samples to perform chemical, isotopic and molecular analyses. The main results were: i) the transition zone is an ecotone with a high microbial richness; ii) partial reductive dechlorination coexists with denitrification, Fe and Mn reduction, and sulfate-reduction; iii) the high concentrations of perchloroethene in this zone resulted in a decrease in microbial richness; iv) however, the reductive dechlorination in this area is not inhibited. These findings suggest that biostimulation and/or bioaugmentation could be applied to promote complete reductive dechlorination and to enhance the dissolution of more DNAPL.
Keywords: aged PCE-pool, transition zone to a basal aquitard, biotic reductive dechlorination, biodegradation halo, richness and degree of development of microbial communities.

1 Introduction

Chloroethenes are chlorinated solvents that belong to the group of non-aqueous phase liquids (DNAPL) and have been involved in numerous contamination episodes [1]. Chloroethenes have an elevated toxicity, and in the case of perchloroethene (PCE), trichloroethene (TCE) and vinyl chloride (VC), the risk of cancer is increased when exposed to them.

The transition zones between granular aquifers and basal aquitards were described by Parker *et al.* [2] as a reasonable paradigm for the DNAPL source area architecture in granular aquifers. Such zones are located at the bottom of many aquifers and are characterized by the presence of numerous thin silty-clay layers interstratified with coarser-grained layers. The low contaminant mobility in transition zones should be pointed out since it implies that DNAPL sources in these zones are recalcitrant (much more than those in the rest of the aquifer), which has far reaching implications for the environment.

The core sampling is necessary to study the biogeochemical conditions under which the biodegradation processes occur in the subsurface [2] and to analyze the depth variation of contaminants that result from degradation processes expressed as biodegradation haloes of parent and metabolite compounds in the depth profile of concentrations. According to the observations of Puigserver *et al.* [3], a biodegradation halo is a depth interval in the profile of concentrations where a gradual increase in a metabolite (e.g., TCE) reaches a maximum and then progressively decreases which results in a steep concentration gradient of this compound. An opposite variation of the parental compound (e.g., PCE), is produced parallel to the evolution of this metabolite.

Groundwater chemistry in transition zones where DNAPL is present also provides the chemical characterization of the dissolved phase contaminants at the depths of the source of contamination. Changes in the concentrations of contaminants, oxidants, and metabolites can be used to confirm the activity of microorganisms and identify the processes involved. These processes include aerobic respiration; the reduction of nitrate, Mn and Fe, which result in the dissolution of these metals in groundwater, sulfate and CO_2 (methanogenesis]; and the fermentation and reductive dechlorination (RD) of chlorinated solvents.

Chloroethenes may be recalcitrant under some conditions over long periods. However, they can also be biodegraded, for example, under anoxic conditions by biotic RD [4], which is carried out by organohalide respiring bacteria (OHRB). Moreover, some studies show that the presence of dechlorinating activity can significantly enhance the dissolution rate of the source of PCE [5]. RD of chloroethenes takes place by sequential dechlorination from PCE to ethene or ethane [1]. RD of PCE and TCE may take place under nitrate-, Mn- and Fe-reducing conditions, under sulfate-reducing and methanogenic conditions, especially if an excess of electron donors is supplied to achieve substantial dechlorination [6].

The reductive dechlorinating sequence may be wholly or partially inhibited by competition for electron donors depending on the environmental conditions. This competition is between communities of OHRB and communities of anaerobic

hydrogenotrophic (including reducers of NO_3^-, Mn^{4+}, Fe^{3+} and SO_4^{2-}), autotrophic methanogenic, and homoacetogenic microorganisms [7].

The present study aims to analyze the RD occurring in the transition zone in a source area and to study how it is affected by other biogeochemical processes. To this end, two working hypotheses were formulated: 1) the RD of chloroethenes from aged-pools of PCE residual-DNAPL in transition zones can take place when these transition zones are ecotones, and 2) the RD of chloroethenes in these transition zones occurs despite the potential inhibition that arises as a result of microbial competition and the partial inhibition of microbial communities caused by the high concentrations of PCE.

2 Site description

The site consists of a confined aquifer made up of Pliocene materials. Chloroethene contamination was detected at the site in 1980, but it is not known when this originated. The main contaminant is PCE, which was used as a degreaser of vehicle parts at a nearby industrial plant serving the automotive industry. PCE as DNAPL was most likely dumped into an old abandoned agricultural well located in the plant. Groundwater contamination resulted in concentrations of PCE that remained high in 2012, the maximum value being 17,300 μg/L, whereas TCE and cDCE were 39 and 25 μg/L, respectively.

The monitoring network consists of 12 conventional wells. In addition, two boreholes (B-F1UB and B-F2UB) were drilled by our research group in January 2011 and were subsequently equipped as multilevel wells.

Five hydrostratigraphic units of Pliocene age were differentiated from surface to bottom: 1) the Unsaturated Zone (USZ), which is made up of gravels and coarse, medium and fine sands; 2) the Upper Aquitard (UDTA) (upper discontinuous thin aquitard), which mostly consisted of clays and was crossed by subvertical microfractures; 3) the Upper Part of the Aquifer (UPA) was mainly constituted by gravels (hydraulic conductivities ranged from 10 to 20 m/day); 4) the lower part of the aquifer, which comprised the Transition Zone to the Basal Aquitard (TZBA), was made up of paleochannels of alternating gravels and coarse sands with numerous interbedded layers of medium to fine sands and silts at the centimeter to decimeter scale (hydraulic conductivities ranged between 1 and 10 m/day). These paleochannels act as drainage lines, coinciding with the general flow toward the northeast. 5) Lastly, the Basal Aquitard (BA) consisted of fine laminar sands and silts crossed by a dense network of subvertical microfractures.

3 Materials and methods

3.1 Borehole drilling and installation of multilevel wells

Boreholes B-F1UB and B-F2UB (17.00- and 20.20-m depth, respectively) were drilled by rotary drilling. A core-sampler was used to recover the core. The drilling operations and the core-sampler are described by Puigserver *et al.* [3]. The

boreholes were equipped as multilevel wells (multi-level CMT-type 7 channels, Solinst) following the protocol established by Einarson and Cherry [8].

3.2 Core sampling, conservation protocols and analytical determinations in porewater

Cores were exhaustively sampled to characterize the vertical distribution of PCE, TCE, cDCE, tDCE, 1,1-DCE, VC in porewater, the percentage of organic carbon (f_{oc}), total Fe and Mn sorbed in the fine fraction and the richness and degree of development of microbial communities in the sediments. The sampling procedure and conservation protocol for chloroethene analysis, as well as the calculations of porewater concentrations were an adaptation of the protocol followed by Parker *et al.* [2] and Chapman and Parker [9]. To minimize volatilizations, a methanol trap (methanol, Merck, ISO Pro analysis) was used in accordance with EPA SW-846, Method 5035. The samples collected for the analysis of Fe, Mn, and f_{oc} were stored and frozen on site at -20ºC.

3.3 Groundwater sampling and conservation protocols

In all cases, the sampling and conservation protocols reported by Puls and Barcelona [10], Trevors [11] and Johnston [12] were followed. Groundwater from ports 3 to 7 was sampled to analyze chloroethenes, the $\delta^{13}C$ of chloroethenes, nitrate, nitrite, sulfate, Fe, Mn and dissolved oxygen (DO) in the aquifer. Sodium azide (N_3Na Fluka) was added to the groundwater samples for chloroethene concentration and $\delta^{13}C$ analyses immediately after being collected to inhibit bacterial activity.

3.4 Sample pretreatments and analytical techniques

3.4.1 Chemical and carbon isotopic analyses

All chemical analyses were conducted in the laboratories of Scientific-Technical Services at the University of Barcelona. The chloroethenes were extracted from the core samples (sorbed and in porewater) in the laboratory by adapting the protocol described in Dincutoiu *et al.* [13]. Gas Chromatography-Mass Spectrometry was used to determine the chloroethenes in core and groundwater samples. The pretreatment to measure the Fe and Mn consisted of conducting an extraction with *aqua regia* of a selected fine fraction of the sample (according to the ISO/DIS 11466:1995 protocol). The elemental analyses were performed by gas chromatography with a thermal conductivity detector. Samples for TOC in groundwater were also taken, and analyzed using the TOC analyzer TOC-5000 (Shimadzu). Compound Specific Isotope Analysis was performed by Gas Chromatography Combustion Isotope Ratio Mass Spectrometry to determine the $\delta^{13}C$ values in chloroethenes of groundwater samples and they determined by Elemental Analyzer Flash EA 1112 coupled to an IRMS delta C Thermo Finnigan. The nitrate, nitrite and sulfate in groundwater were analyzed by Ion Chromatography, and the Fe and Mn in groundwater were analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy.

3.4.2 Molecular analyses

Molecular analyses were performed to verify the presence of microbial communities in samples and to study their role in the degradation of chloroethenes. The richness and degree of development of microbial communities were also assessed. The former refers to the number of different species in a community and the latter constitutes a semiquantitative indirect estimate of the microbial biomass. The number of sediment samples was 21 for molecular analysis using the Terminal-Restriction Fragment Length Polymorphism (T-RFLP) technique. The analyses were performed at the Helmholtz Centre for Environmental Research–UFZ (Leipzig-Germany). Genomic DNA was extracted from 1.10 g of sediment with NucleoSpin® soil of Macherey-Nagel following the manufacturer's protocol to perform the T-RFLP. The microbial richness and degree of development were assessed by T-RFLP analysis of the 16S rRNA gene.

Microbial richness in each sample was assessed with the number of restriction fragments (RF) greater than 50 bp and larger than 1% of the total area. From the three different results obtained (one for each restriction enzyme), the larger one was taken as valid. The actual microbial richness is 3 or 4 times higher than the number of RF, according to Liu *et al.* [14] and Marsh *et al.* [15]. The microbial community degree of development was estimated by averaging the total area of the RF greater than 50 bp and larger than 1% and standardizing on a scale from 0 to 10. The results of this parameter were treated as semiquantitative data agreeing with Liu *et al.* [14] and Bruce [16].

4 Results and discussion

4.1 Biogeochemical processes in porewater and groundwater

4.1.1 Reductive dechorination in the UPA

Pronounced peaks of PCE were located immediately below the contact UDTA-UPA at depths of 4.80 m and 4.51 m for B-F1UB and B-F2UB, respectively (Fig. 1(a), labels $1A_3$ and $2A_4$). A fraction of the PCE-DNAPL that previously penetrated into the UDTA through the vertical microfractures would have reached these depths in the UPA and remained as residual-DNAPL trapped interstitially in the gravels and sands of the top of this unit, where groundwater conditions were oxidizing (DO concentration of 8.00 mg/L). Nitrification took place in F1UB (Fig. 2(a), port 3) owing to these oxidizing conditions, which are consistent with the low groundwater concentrations of TCE, suggesting a lower PCE degradation than in the ports located in the TZBA.

Redox conditions continued to be oxidizing in this part of the unit, which accounts for the absence of PCE RD as corroborated by the low concentrations of metabolites in porewater (Fig. 1(a)) and in groundwater (Fig. 2(f), (g); port 4). In addition to these low concentrations, the isotopic composition of PCE in groundwater at port 4 in F1UB (-25.04 ± 0.18‰) was the lightest in the whole profile, which is evidence that PCE degradation does not occur in this unit. Since PCE degradation was very little in this unit at these depths, it is reasonable to suppose that, although residual-DNAPL was not currently observed, the registered

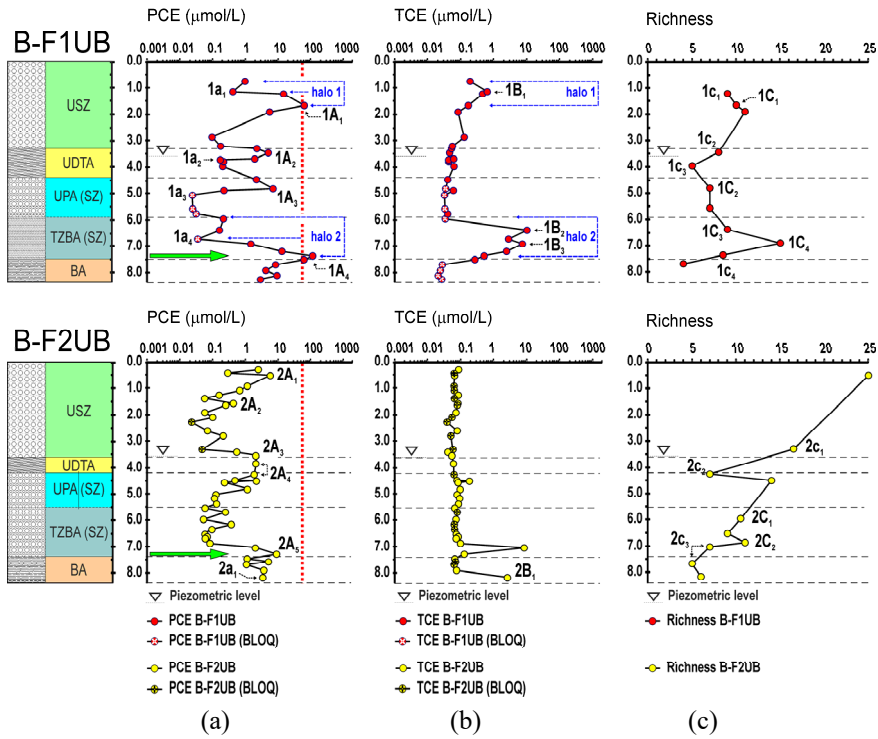

Figure 1: Porewater profiles in B-F1UB and B-F2UB of (a) PCE; (b) TCE. BLOQ: samples below the Limit of Quantification; and (c) Richness of microbial communities (i.e., the number of restriction fragments in each sample). Vertical scales in meters below ground surface. Dashed red line indicates the concentration of PCE above which residual PCE-DNAPL has been observed. Green arrows show the depth at which a residual-DNAPL pool of PCE was detected.

molar concentrations of PCE and TCE in porewater represent an estimation of the initial molar composition of the DNAPL that penetrated.

4.1.2 Reductive dechorination in the TZBA

Reducing conditions prevailed throughout the year in the groundwater in this unit (DO varied between 1.25 and 0.89 mg/L), giving rise to denitrification with increasing depth as evidenced by the decrease in nitrate and the formation of nitrite from port 5 to 7 in F1UB (Fig. 2(a), (b)) and from 4 to 7 in F2UB (not shown in Fig. 2). Furthermore, reduction processes of Mn were identified in sediments at a depth of 5.69 m (Fig. 3(b), label $2b_1$) accompanied by an increase in Mn in the groundwater from these ports (see Fig. 2(d) for the case of F1UB). In addition, evidence of the current presence of residual-DNAPL on the geological contact with the BA exists, which accounts for the two large peaks of PCE detected near this geological contact (Fig. 1(a), label $1A_4$ and label $2A_5$) in porewater and the

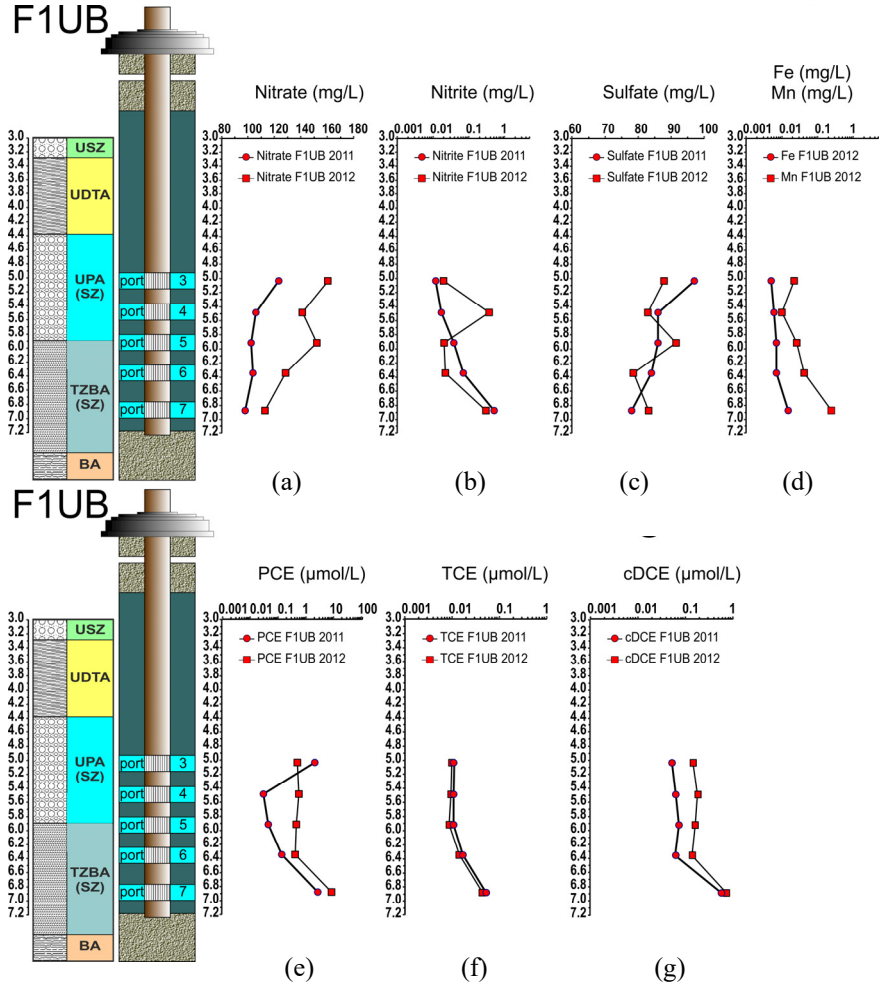

Figure 2: Variation with depth of nitrate (a), nitrite (b), sulfate (c), Fe and Mn (d), PCE (e), TCE (f), and cDCE (g) in groundwater at the multilevel piezometer F1UB (March 2011 and 2012).

high groundwater concentrations of PCE observed at port 7 (see Fig. 2(e) for the case of F1UB).

The reduction of sulfate was observed in ports 6 and 7 (see Fig. 2(c) for the case of F1UB), which suggests that the conditions (especially in port 7) were sulfate-reducing, created as a result of the high concentration of PCE because of the residual-DNAPL. In this zone near the contact with the BA, the presence of a steep concentration gradient of Fe (Fig. 3(a), label $1A_1$) and another of Mn (Fig. 3(b), label $1B_1$) in the sediments of B-F1UB at a 7.35 m depth is consistent with the aforementioned sulfate-reducing conditions. Thus, water-sediment

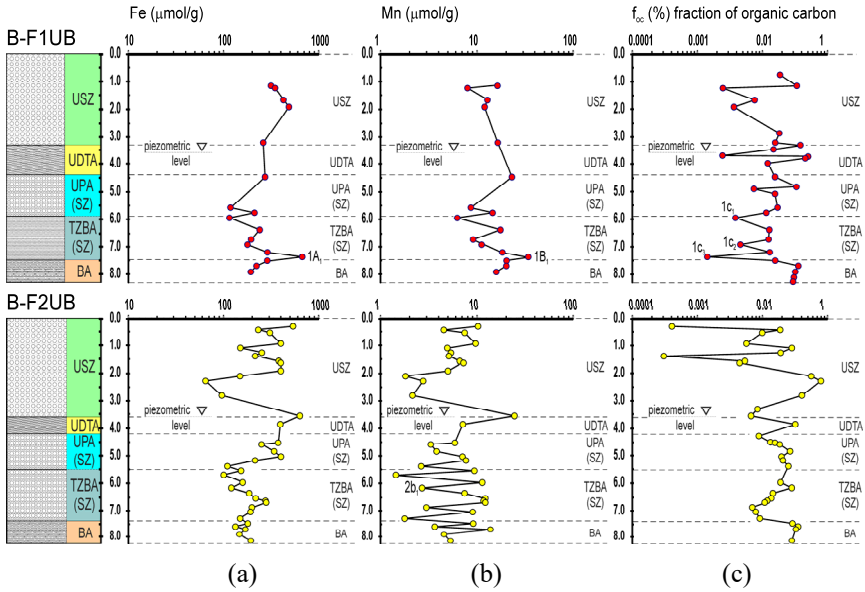

Figure 3: Profiles of Fe (a), Mn (b), and fraction of organic carbon (c) in subsurface sediments in BF1UB and B-F2UB.

equilibrium is achieved at this depth in which a fraction of the dissolved Fe and Mn precipitates as sulfide and/or carbonate minerals under reducing conditions.

The TZBA is heterogeneous, which led to the accumulation of a pool of PCE on the geological contact TZBA in the past. Concentrations of the two large peaks of PCE detected near the contact with the BA in the two boreholes and the lower concentrations than the effective solubility of PCE are consistent with the presence of residual PCE-DNAPL at DNAPL saturation lower than the residual saturation. Therefore, the aforementioned pool is currently an aged-pool (i.e., composed of remnants of PCE free-phase found in an immobile residual form).

The storage of PCE by molecular diffusion in the fine-grained sediments of the TZBA contributed to the persistence of PCE in this unit, as evidenced by the PCE increase in the porewater of the boreholes (Fig. 1(a), $1A_4$ and $2A_5$).

As for the depth evolution of chloroethene concentrations, the existence of two depths with steep concentration gradients in TCE in the porewater at depths of 6.36 and 6.90 m in B-F1UB (Fig. 2(b), labels $1B_2$, $1B_3$) related to a maximum in cDCE (1.05 μmol/L) and VC (0.26 μmol/L). These four maxima of concentration were centered on a PCE minimum at a depth of 6.71 m (Fig. 1(a), label $1a_4$) and constituted a biodegradation halo (halo 2), which is similar to another halo in the USZ (halo 1; Fig. 1(a), (b)), indicating the biotic RD of PCE and metabolites, as described by Puigserver *et al.* [3]. In addition, the PCE groundwater at port 7 of the multilevel well F1UB (located at the zone of halo 2) showed isotopic fractionation, with a $\delta^{13}C$ value of -23.53 ± 0.07‰. This value corresponded to a Δδ of +1.5‰ with respect to the UPA. This isotopic shift is compatible with the

Rayleigh fractionation curves of PCE by RD with enrichment factors ε ranging from -0.4 to -16.4‰, which is more evidence of PCE biodegradation forming TCE. Moreover, the $\delta^{13}C$ value of the TCE in groundwater from port 7 of F1UB (-22.10 ± 0.30‰) was heavier than that of the parent PCE, suggesting that a fraction of the TCE was also biodegraded by RD to form cDCE.

As for F2UB, isotopic fractionation of groundwater PCE was apparently not observed given a $\delta^{13}C$ value in port 7 of -25.02 +/-0.16, which is a similar value to that obtained in the UPA. However, the fact that the RD of PCE to cDCE occurred in some depth intervals provides evidence that lack of isotopic fractionation PCE must likely due to the continuous dissolution of DNAPL at this depth masking any fractionation effect.

Although the aged-pool has led to an increase in PCE concentration in the groundwater in port 7 of the two multilevel wells, and despite the biogeochemical processes that take place compete with one another, it is noteworthy that these circumstances does not completely inhibit the RD of PCE to cDCE because steep increases from port 6 to port 7 in TCE and cDCE were recorded at both boreholes (see Fig. 2(f), (g) for the case of F1UB). The predominance of metabolites such as TCE and cDCE in the groundwater and porewater suggests that the RD sequence was not completed, according to Bradley [4], although low VC concentrations in port 7 of F1UB were also detected.

4.2 Effects of high concentrations of chloroethenes on microbial communities

The peaks of PCE immediately below the contact USZ-UDTA (Fig. 1(a), labels $1A_2$ and $2A_3$) coincided with a decrease in richness, (Fig. 1(c), labels $1c_2$ and $2c_1$) when compared with the upper unit. This decrease is attributable to an increase in the specialization of microbial populations because many of these cannot adapt to high concentrations of PCE [17]. In contrast, the degree of development increased slightly at B-F1UB (from 7.5 to 8 units), which suggests that despite the decline in microbial populations, those that have adapted to high levels of PCE show an increase in their degree of development owing to less competition with other populations [17]. Moreover, the minimum value of PCE at a depth of 3.96 m (Fig. 1(a), label $1a_2$) coincided with a minimum of richness (Fig. 1(c), label $1c_3$) and degree of development (2.5 units, not shown in Fig. 1). These minimums are related to the absence of groundwater flow in these sands, which prevents microbial communities from gaining access to the nutrients, electron donors, carbon sources, and growth factors (natural substances that stimulate the growth, proliferation, differentiation and cellular healing) that they need. Therefore, the most suitable conditions for the development of microbial communities were not met in these sands.

The concentrations of PCE recorded in the aforementioned pronounced peaks immediately below the contact UDTA-UPA (Fig. 1(a), labels $1A_3$ and $2A_4$), corresponded with lower richness and degrees of development (Fig. 1(c), labels $1C_2$ and $2c_2$, for richness, and 4.5 and 5.9 for development, data not shown in Fig. 1) than those on the contact USZ-UDTA (Fig. 1(c), labels $1c_2$ and $2c_1$, for

richness, and 8 and 6 units, for development in B-F1UB and B-F2UB, respectively, data not shown in Fig. 1).

In the biodegradation halo 2 (Fig. 1(a)), a significant increase in the microbial richness and degree of development of microbial communities was recorded in B-F1UB at a depth of 6.90 m (Fig. 1(c), with maximum relative values of 9 for the richness, label $1C_4$, and 7.1 for degree of development, data not shown in Fig. 1) compared with the top of the TZBA and the UPA. This increase is consistent with the coexistence of the aforementioned biogeochemical processes (denitrification, reduction of Fe and Mn, sulfate-reduction, and RD). The aforementioned high peaks of PCE in the TZBA near the contact with the BA (Fig. 1(a), label $1A_4$ and $2A_5$), diminished the richness (Fig. 1(c), labels $1c_4$ and $2c_3$; with relative average values of 8 and 6 in B-F1UB and B-F2UB, respectively) and the degree of development of microbial communities (with relative values of 4.4 and 5.4 units in B-F1UB and B-F2UB, respectively, data not shown in Fig. 1) compared with the upper part of the TZBA in contact with the UPA (Fig. 1(c), labels 1C3 and $2C_1$, for richness and 7.2 and 5.7 for degree of development, data not shown in Fig. 1). These findings would suggest that high concentrations of PCE negatively affect these two parameters of the structure of microbial communities. Also supporting this conclusion, the higher concentration of the PCE peak in B-F1UB than in B-F2UB (Fig. 1(a), label $1A_4$ and $2A_5$; respectively) was responsible for the lower degree of development in B-F1UB in the contact TZBA.

4.3 Characterization of the TZBA as an ecotone and relevance for the reductive dechlorination of chloroethenes

Several pieces of evidence support the observation that the TZBA is an important ecotone sustaining microbial degradation: 1) the numerous geological heterogeneities and textural changes in the TZBA enable the different microorganisms to gain access to nutrients, electron acceptors, electron donors, carbon sources and growth factors reaching this unit along the layers of larger grain size materials. Microorganisms also have access to the solid organic matter and chloroethenes along the contact surface with fine materials similar to that described by Puigserver *et al.* [18] in an alluvial aquifer. 2) The TZBA is located at the interface between the oxic medium of the UPA and the anoxic medium generated by the presence of the aged PCE-pool, which resulted in a high microbial richness in this unit. 3) Numerous biogeochemical processes, such as denitrification, the reduction of Fe and Mn, sulfate-reduction, and the RD of PCE and TCE, related to a large degree of development of microbial communities (with relative values of 9.9 and 7.0 in B-F1UB and B-F2UB, respectively) coexist in this zone.

In contrast to the UPA, the TZBA groundwater showed presence of the metabolites of PCE and TCE and the isotopic fractionation of PCE, hence, reveal the presence of RD. This biodegradation is favored because the TZBA is an ecotone characterized by a richness and a large degree of development. However, RD is not significant, and major amounts of TCE and cDCE accumulate in the TZBA. Also the fact that no significant variations in the PCE, TCE, and cDCE concentrations were recorded in the UPA natural attenuation suggest that one of

the major limiting factors for completing RD may be a small supply of bioavailable electron donors. This suggests that an adequate supply of fermentable substrates is needed for the production of dissolved hydrogen, which is used directly by OHRB as the electron donor. For this reason, biostimulation (and/or bioaugmentation) in the TZBA by adding electron donors could be more efficient than in the UPA, where the richness and degree of development of microbial communities tend to be lower.

5 Conclusions

The transition zone is an ecotone characterized by a high microbial richness and highly developed microbial communities. The isotopic fractionation of groundwater PCE and TCE showed RD and this process of the partial RD of PCE to cDCE coexisted with denitrification, Fe and Mn reduction, and sulfate-reduction despite competition between microbial communities. The high concentrations of PCE in the zone of this pool resulted in a decrease in richness and degree of development.

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