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A Multidisciplinary Evaluation of Hyporheic VOC Plume Natural Attenuation Including Stable Isotope and Microbial Analyses-22438

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ABSTRACT

Pen Branch hyporheic sediments are the upwelling zone for a PCE plume flowing from the closed CMP Pits (RCRA-CERCLA site) at Savannah River Site. Although these hyporheic zone sediments consistently display concentrations of PCE, TCE, cis-DCE, and VC, VOC is not detected in Pen Branch surface waters. Our efforts have documented apparent natural attenuation; helping to account for VOC absence in surface waters. However, plume natural attenuation variability must be quantified temporally and spatially over long-term hydrological conditions to assess efficacy as an acceptable EPA Record of Decision (ROD). Additionally, biotic natural attenuation must be identified and contrasted with abiotic pathways. As final plume downslope destinations are aquifers and emergent surface streams and rivers, hyporheic zone sampling is essential. Comprehensive hyporheic sampling documents plume groundwater remediation prior to upwelling into stream benthic strata and attenuation actions within this dynamic zone itself.

Additional assessment concerns must address causal mechanisms behind apparent attenuation. Verification of whether declines in VOC daughter products at our hyporheic stations were due to physical actions of dilution and dispersion rather than true biochemical dechlorination was an expanded objective for current analyses. To gain a more complete quantification of natural attenuation, the use of compound-specific isotope ratio analysis (CSIA) and microbial 16S rRNA with qPCR were included for Pen Branch. To account for potential microbial degradation of source VOC along the groundwater pathway upslope from Pen Branch, CSIA was also planned for samples from deep monitoring wells. Changes in isotope ratios of parent to daughter compounds can help verify microbial actions in strictly groundwater flows. Our intensive sampling of the Savannah River Site (SRS) Chemical, Metals, and Pesticides (CMP) Pits VOC plume-fringe from 2005 to 2021 previously documented PCE natural attenuation and effectiveness of In Situ Thermal Treatment (ISTT).

At Pen Branch most plume hyporheic upwelling was delimited to a 20m inflow reach. Our study utilized corroborative sampling methodologies: ambient hole-water grab samples: passive diffusion bags (PDB); and hole-core sediment samples to confirm station to station spatial and temporal trends. VOC hot spot detections were confirmed from both groundwater and soil core samples and became the focus of our CSIA and microbial sampling. VOC pathways through the Pen Branch hyporheic zone were assessed at hole-installation using sequential soil samples from augered cores and grab-samples of hole-bottom water. Longer term, composite water samples were collected from PDBs deployed for a minimum two-week equilibration period. Soil samples were collected from the 15 and 65 cm core levels in December 2017 and June 2018 at six stations. Additional core depths were sampled in June 2018, 2020, and 2021. All samples were analyzed in an EPA certified lab by purge and trap GC-MS. Effectiveness of previous ISTT and soil vapor extraction (SVE) for this CMP Pits

PCE plume, was clearly documented by significant downward trends in VOC at hyporheic zone stations prior to December 2015. Achieving final stage conversions of PCE daughter products to ethene and CO2 may be limited depending upon suitable interactions between hyporheic zone sediment morphology, hydrology, and redox conditions. Our preliminary CSIA results for stable carbon isotope ratios show apparent bacterial fractionation in cis-DCE becoming vinyl chloride at hot-spot stations. Microbial samples from these same hyporheic sediments also detected higher abundance of dehalogenating bacteria, *Dehalogenimonas* sp. With these promising results for biotic attenuation, our continuing research is focused on more clearly delineating functional linkages between CSIA and 16S rRNA/qPCR to document biotic plume natural attenuation. Continued, long-term biotic natural attenuation represents the strongest case for non-engineered plume fringe remediation.

INTRODUCTION

Along with successful production of Cold War nuclear materials at the US Department of Energy (DOE) Savannah River Site (SRS) came the ancillary issues of nuclear, toxic, and mixed waste disposal. Cleanup and recovery operations at SRS have progressed since reactor shut-downs with many environmental restoration and remediation projects at various stages of completion. As a designated EPA CERCLA site (Superfund), SRS has addressed numerous cleanup priorities, especially those related to potential contaminant migration off-site to vital water-supply resources such as the Savannah River (Fig. 1). As identified for numerous other Superfund sites (EPA 2018a), common SRS contaminants impacting groundwater are chlorinated volatile organic compounds (cVOC); producing plumes of tetrachloroethylene (PCE) and trichloroethylene (TCE).

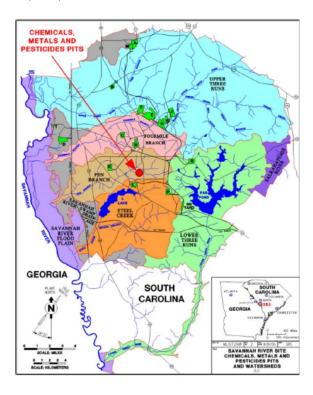


Fig. 1. US Department of Energy (DOE) Savannah River Site (SRS) showing location of CMP Pits and Pen Branch watershed drainage and flow to Savannah River (From SRNS (2018)).

Among the many SRS Operable Units (OU) under CERCLA, one OU heavily impacted through the 1970's by cVOC disposal into pit trenches was the Chemical, Metals, and Pesticides (CMP) Pits (SRNS 2018). All of the remediation categories detailed by EPA (2018b) ranging from: excavation of waste source soils; pump and treat of Dense Non-Aqueous Phase Liquids (DNAPL); *In Situ* Thermal Treatment (ISTT); and Soil Vapor Extraction (SVE) to more localized *in-situ* biogeochemical remedies collectively labeled: natural attenuation (Weidemeier *et al.* 1998; EPA 1999; Wilson 2010) were sequentially-implemented at CMP Pits following closure of these unlined pits in 1979 (SRNS 2018). Even with these extensive efforts, beginning with soil-excavation in 1984 (SRNS 2018), contaminated soil-remnants and deep-seepage of cVOC to underlying aquifers produced a primarily PCE plume moving downslope to the nearby Pen Branch stream valley (Fig. 2) (SRNS 2018). Contaminants of concern (COC) for this plume were PCE and TCE, however, EPA determined that previous treatments of plume source materials were successful enough to designate natural attenuation as the remedial Record of Decision (ROD) (SRNS 2012, 2018).

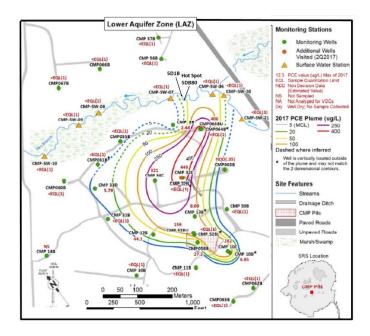


Fig. 2. Modeled PCE plume flow downslope from CMP Pits towards Pen at SRS (From SRNS (2018)).

The dynamics of adsorbed soil/sediment cVOC exchange with groundwater represent a key component in modelling natural attenuation effectiveness. Predictions of mass transfer budgets for cVOC entering the dynamic hyporheic zone where natural attenuation is maximized (Atashgahi *et al.* 2013) must include the rates of cVOC desorption from soil particles and transport within conductive groundwater. Soil percentage organic matter enhances adsorption and inhibits desorption (Zytner 1992). Different cVOC compounds, e.g. PCE, desorb and migrate at different rates compared to other compounds. Desorbed PCE was found to be less mobile and migrated more slowly in groundwater (Conant *et al.* 2004). Sediment geological analyses and cVOC isotopic tracking were beyond the scope of our study. However, our investigation sought to relate cVOC concentration differences within hyporheic zone strata to fluctuations in groundwater elevation and movements.

By determining plume fringe longitudinal and vertical extent intersecting the Pen Branch hyporheic zone, two important remediation objectives could be achieved: 1). Plume movement and attenuation ground-truthing would yield more accurate model forecasting. 2) Delimiting actual plume hyporheic entry level will better assist any future efforts utilizing augmented or bioenhanced remediation approaches.

Applying comprehensive plume assessment technologies similar to Ottosen, *et al.* (2020) to Pen Branch, MSI faculty and students aquired advanced research experiences with PNNL collaboration including: training with PNNL for microbial biotechnology ((16S rRNA gene sequencing with qPCR) and gas chromatography-isotope ratio mass spectrometry (GC-IRMS) with compoundspecific isotope analysis (CSIA). (Technologies described by Weatherill *et al.* (2018) as decisive evidence for biotic NA pathways.) Results of this PNNL collaboration will enable documentation of expected plume final stage (Bradley and Chapelle, 2011) natural attenuation (NA) at SRS; potentially generating significant cost-savings via EPA accepting SRNS NA plans with technology-transfer to other SRS plumes.

An additional benefit of this DOE-Minority Serving Institutions Partnership Program (MSIPP) research was the training and professional development of minority students, who conducted the bulk of field sampling and laboratory sample preparations.

DESCRIPTION

To determine natural attenuation efficacy for the plume-fringe, intensive sampling of shallow groundwater and wetland ecosystem components was begun in 2009. Fifty-six hyporheic zone piezometer stations were established between the upstream surface water (SW) station CMP-SW-21 and downstream station CMP-SW-09, ca. 0.45 km apart, (Fig. 2). These preliminary samples helped delimit the extent of the plume front and focus future monitoring. Placement of screening stations was designed to encompass the cVOC plume-fringe and determine optimal long-term station locations to detect any plume influx to Pen Branch hyporheic zone and SW. These station arrays determined both small-scale plume variability (piezometer triplicate-clusters 1.0 m apart) and stream reach variability with spacing of 10 to 20 m intervals along the Pen Branch channel. Screening stations were sampled through 2015 and relative cVOC station concentrations were exemplified by 2011 results (Fig. 3). Plume-fringe efforts benefited from collaborative larger-scale efforts of Savannah River Nuclear Solutions, LLC (SRNS) addressing longer-term groundwater and surface water monitoring for this OU.

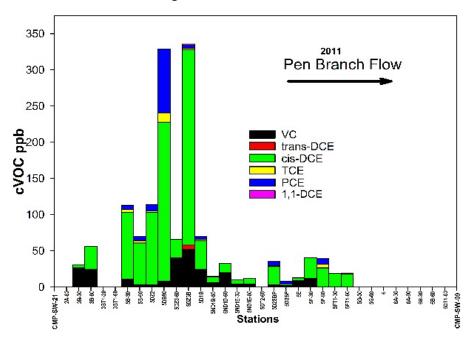


Fig. 3. Hyporheic cVOC at Pen Branch screening stations during summer 2011.

Pen Branch sampling was refined in 2016 to a priority set of ten stations positioned along the ca. 400 m reach between SRNS SW stations CMP-SW-21 and CMP-SW-09. These ten piezometers focused on six stations within a 40 m reach containing the hot spot station 5DB80 (Fig. 3), but with continued sampling of four sentinel stations above and below this impacted reach. Seasonal variability was addressed in 2017-2018 by repeating piezometer insertions in spring and summer 2017 and winter 2018. Piezometers were installed by manually-driving a 10.2 cm diameter polyvinyl chloride (PVC) pipe through compacted substrata with no annular space to a depth of ca. 0.7 to 0.75 m below stream bottom (Fig. 4). After



Fig. 4. PVC hole-liners were manually driven through sediment layers and augered into the local Pen Branch confining layer

residual stream water in the pipe was bailed out, a stainless steel 8.25 cm diameter auger was used to core through sediment layers to a hole-depth of 70 to 80 cm below the surrounding stream bottom (Fig. 4). The PVC hole-liner was solid with no screened intervals. Sample water resulted from hyporheic waters upwelling into the pipe from surrounding substrates. For piezometers installed during June 2017, December 2017, and June 2018, sediment core cVOC samples were collected at different depths as the hole progressed. South Carolina Department of Health & Environmental Control (DHEC) permit approvals were obtained prior to installing any piezometers. Nine surface water stations were also established along the Pen Branch study reach and sampled on the same schedule as the piezometers.

Hole-water and Pen Branch surface water were sampled using passive diffusion bags (PDB) (Vroblesky and Hyde 1997). PDB deployment at each station always exceeded the recommended 14-day minimum equilibration time (ITRC 2002) before transferal to an EPA-certified laboratory (Shealy Environmental Services, 106 Vantage Point Drive, West Columbia, SC 29172 (SES)) for purge and trap gas chromatograph mass spectrometer (GC/MS) analysis. Sediment vial samples were collected according to EPA Method 5035A protocol and both water and sediment samples were analyzed using EPA Method 8260B for purge and trap GC/MS.

DISCUSSION

The main reach of the Pen Branch hyporheic zone impacted by the CMP Pits cVOC plume was clearly identified by the 2009 to 2015 screening samples (typified by cVOC results for 2011 in Fig. 3). As previously stated, this impacted reach extended along *ca.* 40 m between upstream station 5B and downstream station 5D2BP (Fig. 3). Results in Fig. 3 revealed the upstream and downstream plume limits; plume hot spot near station 5D880; and verified natural attenuation products of cis-DCE and VC. The main plume hot spot was consistently displayed at station 5D880 throughout the 2009 to 2015 screening period (Fig. 5) with the exception of December 2016. Highest cVOC and PCE shifted to station 5D1B; resulting from extreme hydrological events (Williams and Shull 2018).

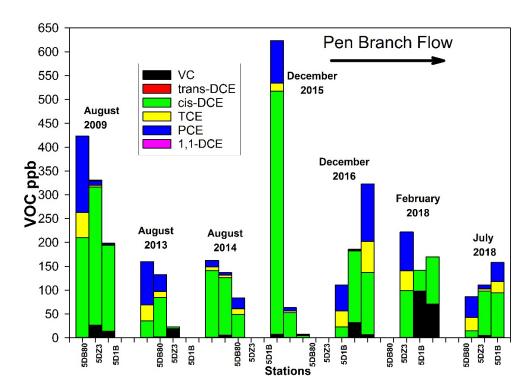


Fig. 5. Changes in cVOC from 2009 to 2018 for Pen Branch hot spot stations.

The impacts of plume source-reduction by ERH and SVE in the upslope vadose zone during 2008 to 2009 (SRNS 2018) were dramatically seen as an over sixty-percent reduction in total VOC levels from 2009 to 2014 at Station 5DB80 along the plume-fringe (Fig. 5). However, this trend was reversed with the extreme cVOC rise at station 5DB80 in December 2015 (Fig. 5). This peak was likely connected to elevated aquifers beneath the CMP Pits plume pathway resulting from much higher rainfall during October to December in 2015 (Williams and Shull 2018). Rainfall at SRS for fall 2015 and 2016 was greatly elevated over the 20-year average (SRNS 2018). For October and November 2015, it was nearly 250% higher than the 20-year average (Williams and Shull 2018). Elevated aquifer levels resulting from these hydrological events continued at SRNS monitoring wells into 2017 (SRNS 2018); potentially raising plume VOC concentrations with new mobilization of adsorbed PCE. Increased soil moisture content (Chiou and Shoup 1985) and soil pore-space humidity (Guigard et al. 1996) were shown to displace adsorbed PCE and chlorinated benzene compounds respectively.

Plume fringe hydrology can be inferred from the repeated patterns of relative cVOC concentrations at the hot spot stations 5DB80, 5DZ3, and 5D1B (Fig. 5). Main plume hyporheic inflow appears to be channeled into a narrow entry reach at station 5DB80. Longitudinal plume movement within the hyporheic zone could then move successively to downgradient stations 5DZ3 and 5D1B. This hypothesis is supported by apparent movement of the pulse of increased cVOC with elevated PCE at station 5DB80 to downstream station 5D1B in 2016 (Fig.5). The one-year sampling time interval between these two detections may not be the actual cVOC travel-time duration between these two stations (ca. 8.5 m distance). However, cVOC movement could be quite slow in saturated layers adjacent to Pen Branch and within the hyporheic zone silty sand deposits and sandy clay layers. Conant *et al.* (2004) present PCE migration rates of 4.7 years to travel through 2 m of silty sand deposits. This slow PCE migration rate supports the evidence of a moving pulse of cVOC from station 5DB80 to station 5D1B. Dynamic natural attenuation actions on plume cVOC along this pathway are also demonstrated by the higher occurrence of VC at stations 5DZ3 and 5D1B which are further from the plume source; allowing more time for hyporheic microbial attenuation (Fig. 5).

Comparisons of cVOC levels in sediment cores and hole-water for the same piezometer (Fig. 6) provide additional support for groundwater plume movements and sediment natural attenuation interactions described for Fig. 5. Sediment cores collected beneath Pen Branch during piezometer installation from the upper 15 cm and from the hole bottom core were compared for stations 5DB80 and 5D1B (Fig. 6). Holewater grab samples were collected at least 24 h after piezometer installation from the bottom water layer using a peristaltic pump and clear pvc tubing. CMP Pits plume trends observed for longer-term PDB hole water samples (Fig. 5) were reflected in sediment core samples (Fig. 6) and included: much higher cVOC at station 5DB80 compared to 5D1B and a continued decline in total cVOC over time; indicating the continued success of ERH and SVE at CMP Pits in 2008 (SRNS 2018). Since sediment cores were not collected for the 2015 to 2016 hydrological pulse of apparent desorbed cVOC (Fig. 5), no detection of a plume pulse moving down-gradient from station 5DB80 could be determined for sediment cores. However, the presence of PCE in the bottom core layer and hole-water for station 5D1B in June 2018 may be an indication of fresh plume groundwater inflow (Fig. 6). Previous hole-water at station 5D1B in December 2017 displayed evidence of natural attenuation products of cis-DCE and VC, but no PCE (Fig. 6). For both stations, sediment cores from the hole bottom were a greater reflection of groundwater inflow; showing no degradation products at station 5DB80 and only small amounts of cis-DCE at station 5D1B for both dates (Fig. 6). In contrast, sediments from the top cores and piezometer hole-water at both stations showed more evidence of PCE degradation products, cis-DCE and VC (Fig. 6). Natural attenuation progress in these shallower sediment layers and hole-water may be related to exposure to more aerobic conditions (Conant, et al. 2004; Atashgahi et al. 2013; Mattes et al. 2010)

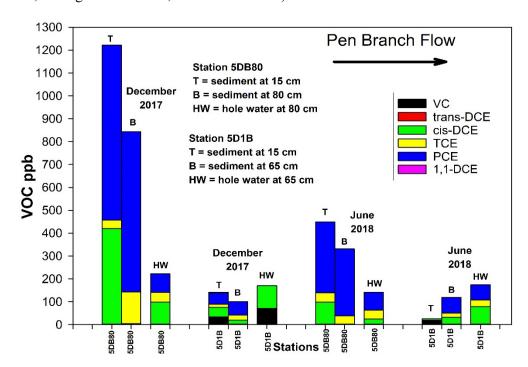


Figure 6. Pen Branch hyporheic sediment core cVOC versus hole-water cVOC at stations 5DB80 and 5D1B for December 2017 and June 2018.

Microbial samples were sent to the laboratory overnight in dry ice and were stored at -80C until analysis. The DNA was extracted using a ZymoBIOMICS DNA Microprep Kit (Zymo Research, Irvine, CA, US) according to the manufacturer's instructions after the following modifications. Approximately 10 g of each homogenized sample was weighed into a 50 ml Falcon tube and centrifuged at 7000 xg for 5 min to pellet the sediment. Excess water supernatant was removed

and discarded. The remaining sediment was used for DNA extraction by weighing 100 mg samples into the ZR BashingBead Lysis Tubes and following the protocol from the manufacturer. DNA was eluted in 20 ul DNA elution buffer.

The qPCR assays were optimized with SsoAdvanced Universal Inhibitor-Tolerant SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), 250 nM forward and reverse primer, and 2 µl of template. Cycling conditions were 2 min at 98°C followed by 35 cycles of 98°C for 15 sec, 58°C for 60 sec. Melt curves were generated per the instrument recommendations. Samples were assayed on an Applied Biosystems StepOnePlus (Applied Biosystems/Fisher Scientific, Waltham, MA, US). Standard curves were generated for qPCR by using the E.coli genome 16s sequence. The standard DNA was used to generate triplicate standard curves by diluting the standard pool in tenfold dilutions. No template controls were also added for quality control.

Sequencing was performed on an Illumina MiSeq instrument. Triplicate, separate 16S rRNA gene amplification reactions were performed on gDNA template from each extraction. The 16S primers targeted the V4 hypervariable region of the 16S SSU rRNA gene using the V4 forward primer (515F) and V4 reverse primer (806R) (Caporaso, 2012).

Amplicon data was processed via hundo. Briefly, paired-end sequence reads are quality trimmed, trimmed of adapters, and filtered of contaminant sequences using BBDuk2 of the BBTools package (v37.17). Passing reads are then merged using VSEARCH (v2.6.0) and aggregated into a single FASTA file with headers describing the origin and count of the sequence. Prior to creating clusters, reads are filtered again based on expected error rates of 1. Cluster were then created, the aggregated, merged reads are dereplicated to remove singletons by default using VSEARCH. Sequences are preclustered into centroids using VSEARCH to accelerate chimera filtering, which is completed in two steps: de novo and then reference based using the entire annotation database. Following chimera filtering, sequences are placed into clusters using distance-based, greedy cluster with VSEARCH based on 97% similarity of the configuration. After OTU sequences have been determined, BLAST (v2.9.0) is used to align sequences to the reference database. Reference databases for 16S were curated by the CREST team and hundo incorporates the CREST LCA method. Counts are assigned to OTUs using the global alignment method of VSEARCH, which outputs the final OTU table as a tab-delimited text file. Relative abundance values were calculated for for each OTU by dividing the sequence numbers of each OTU by the sum of sequences per sample. To determine whether bacteria involved in dehalogenation were more abundant at one location than another we used the Mann-Whitney U test due to the non-parametric nature of the data.

Preliminary CSIA results for stable carbon isotope ratios show apparent bacterial fractionation in cis-DCE becoming vinyl chloride. Microbial analyses also detected higher abundance of Dehalogenimonas in some hyporheic sediments along the plume fringe. Dehalogenimonas has been shown to reductively dechlorinate PCE to TCE and TCE to cis-DCE anaerobically (Schieflera, et al., 2018). However, taxa associated with dehalogenation activity did not show clear trends in abundance in the two locations samples. We found that the Dehalococcoidaceae, a family with known dehalogenation activity, had higher abundance at 5DZ3 than 5DB80 (P<0.001; Figure 1A). In contrast, Dehalobacter was more abundant in 5DB80 than in 5DZ3 (P=0.049; Figure 1B), while Pseudomonadaceae showed no difference in abundance (P=0.28; Figure 1C).

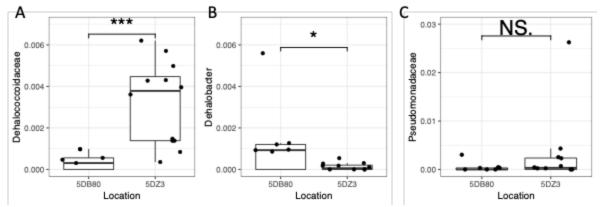


Figure 1. Microbial taxa associated with dehalogenation showed a mixed response to perchloroethylene exposure. (A) Dehalococcoidaceae. (B) Dehalobacter. (C) Psuedomonadaceae.

CMP plume impacts to different hyporheic sediment layers (Fig. 6) showed that the cVOC plume entering this reach of Pen Branch had a potential vertical flow of at least 65 to 80 cm in depth. In order to analyze these groundwater inputs more precisely, additional sediment cores (15, 40, 65, and 85 cm deep) were sampled for station 5DB80 in June 2018 (Fig. 7). Similar to patterns in Figure 6, samples from the shallow 15 cm core depth resembled cVOC in the hole-water (Fig. 7); reflecting more access to probable aerobic microbial dechlorination (Conant *et al.* 2004; Atashgahi *et al.* 2013; Mattes *et al.* 2010). Deeper core cVOC (65 and 85 cm cores) displayed plume cVOC without degradation products. Sediment cVOC for 65 cm and deeper clearly reflects groundwater

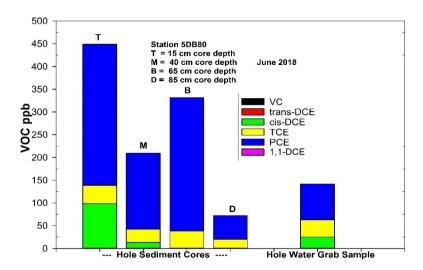


Figure 7. Pen Branch hyporheic sediment core cVOC versus hole-water cVOC for station 5DB80 in June 2018.

plume flow with no natural attenuation. The lower concentrations of PCE and TCE for the 85 cm core (Fig. 7) likely indicate this layer is near the bottom of the plume inflow. Sediments in this 85 cm core were more compacted with a higher clay composition probably reflecting the Pen Branch semi-confining layer. Plume inflow would not penetrate easily into such a compacted layer with greatly reduced PCE flow rates (Conant, et al. 2004). Additionally, Student's t-test showed PCE levels at station 5DB80 for the 15, 40, and 65 cm cores were significantly higher than PCE in the 85 cm core; further indicating this bottom core may be

within the confining layer; restricting plume inflow due to more compacted clay inhibiting plume inflow from above.

Additional groundwater and hyporheic zone sediment interactions can be inferred from sample statistical variability for each core stratum. Greater hydrological mixing and bioturbation actions could affect the relative availability of aerobic or anaerobic conditions and produce small scale mixtures of both conditions within localized areas similar to descriptions by Briggs *et al.* (2015). Samples of sediment cVOC collected from more heterogeneous natural attenuation conditions would be expected to show greater statistical variance and a broader standard deviation from the mean. Conversely, sediments displaying less mixing and more stable redox conditions would be expected to display lower standard deviations. To explore this hypothesis, standard deviations for PCE and cis-DCE concentrations from the four sediment core depths at station 5DB80 were compared. Three replicate samples were collected from each core depth and compared in Fig. 8.

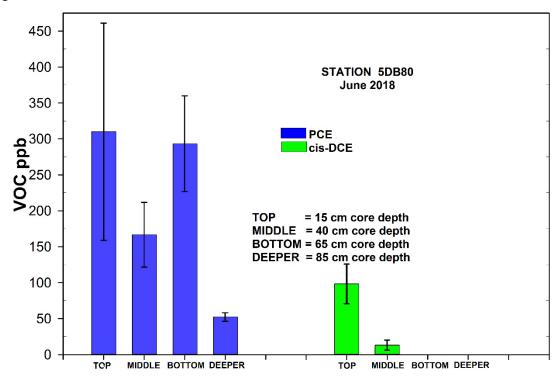


Fig. 8. Means and standard deviations for PCE and cis-DCE samples (n=3) within four sediment core depths at station 5DB80, June 2018.

Sediment standard deviations for both PCE and cis-DCE were larger in the shallow, more hydrologically-impacted 15 cm core depth. Lowest standard deviation was for PCE in the deepest 85 cm core sample. Similarly, cis-DCE showed a higher standard deviation in the 15 cm sediment core than the very tight standard deviation in the 40 cm core. No cis-DCE was detected below the 40 cm core depth. Future sampling should address microscale sediment geochemical differences in redox and porosity for this layer similar to Briggs *et al.* (2015) to help verify these patterns.

CONCLUSIONS

Our results conclusively show natural attenuation is effectively reducing VOC contaminant loads along the CMP Pits plume-fringe and the dynamic hyporheic biogeochemical pathways are playing a major role.

Groundwater elevation fluctuations along the Pen Branch valley vadose zone lower boundary may contribute to desorbing and transporting new source VOCs into groundwater flows. Our previous 'hot spots' (stations 5DB80, 5DZ3, and 5D1B) displayed a progression of cVOC plume pulses; indicating introduction of new plume PCE followed by natural attenuation at downgradient stations.

Plume fringe depth and entry into the Pen Branch hyporheic zone are very limited vertically and longitudinally. Sediment core cVOC indicated the sandy-clay semi-confining layer is restricting plume groundwater penetration beneath Pen Branch and natural attenuation in the upper hyporheic sediments revealed a mixture of aerobic and anaerobic probable microbial degradation pathways.

Preliminary CSIA results for stable carbon isotope ratios show apparent bacterial fractionation in cis-DCE becoming vinyl chloride. Microbial analyses also detected higher abundance of *Dehalogenimonas* in some hyporheic sediments along the plume fringe. *Dehalogenimonas* has been shown to reductively dechlorinate PCE to TCE and TCE to cis-DCE anaerobically (Schieflera, *et al.*, 2018).

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