

Research Article

Spatial Distribution of PCB Dechlorinating Bacteria and Activities in Contaminated Soil

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Soil samples contaminated with Aroclor 1260 were analyzed for microbial PCB dechlorination potential, which is the rate-limiting step for complete PCB degradation. The average chlorines per biphenyl varied throughout the site suggesting that different rates of *in situ* dechlorination had occurred over time. Analysis of PCB transforming (aerobic and anaerobic) microbial communities and dechlorinating potential revealed spatial heterogeneity of both putative PCB transforming phylotypes and dechlorination activity. Some soil samples inhibited PCB dechlorination in active sediment from Baltimore Harbor indicating that metal or organic cocontaminants might cause the observed heterogeneity of *in situ* dechlorination. Bioaugmentation of soil samples contaminated with PCBs ranging from 4.6 to 265 ppm with a pure culture of the PCB dechlorinating bacterium *Dehalobium chlorocoercia* DF-1 also yielded heterologous results with significant dechlorination of weathered PCBs observed in one location. The detection of indigenous PCB dehalorespiring activity combined with the detection of putative dechlorinating bacteria and biphenyl dioxygenase genes in the soil aggregates suggests that the potential exists for complete mineralization of PCBs in soils. However, in contrast to sediments, the heterologous distribution of microorganisms, PCBs, and inhibitory cocontaminants is a significant challenge for the development of *in situ* microbial treatment of PCB impacted soils.

1. Introduction

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that are still present in the environment despite a U.S. production ban in 1976 [1]. Prior to this, commercial mixtures of PCBs (trade name Aroclor in the U.S.) were used for a range of industrial applications such as high-voltage transformers, insulating materials, and hydraulic liquids [2, 3]. PCBs are hydrophobic with a high affinity for adsorption to soil particles and for bioaccumulation in lipids causing hepato- and immunotoxicity, carcinogenesis, and affecting endocrine organs and reproduction in humans [4, 5] and animals [6]. Removal of PCBs from impacted sites has, therefore, been a regulatory priority for several decades [7].

Soils contaminated with PCBs can be found worldwide as a result of industrial activity [8]. However, large heterogeneities in contaminant concentration and microbial populations were observed, when the total concentration of PCBs and other contaminants were evaluated on both macro- and microscales [9, 10]. In some cases, the reason for the heterogeneity was caused by the source of contamination such as organic contaminants from an aluminum plant, where there was a decreasing contaminant gradient away from the plant [11]. Even in cases without a specific source, the physical and environmental conditions in soil involved in formation of soil aggregates impacted the spatial distribution of PCBs leading to heterogeneity of microbial activity [12, 13]. Despite the physical heterogeneities in soil, prior studies

have shown that this variability is not a crucial factor for the composition of the microbial soil communities [14]. Previous reports suggested that sites contaminated with weathered PCBs are recalcitrant to microbial dechlorination [15] due to sequestering of PCB molecules in the pores of the soil particles [16]. Desorption of PCBs depends on the partitioning of PCB molecule between the pore water and the associated organic matter as well as the diffusion of the molecules. It has been suggested that formation of strong bonds between the PCB molecule and the soil particles could cause desorption resistance [16].

Complete microbial degradation of PCBs requires anaerobic reductive dechlorination of extensively chlorinated congeners followed by subsequent aerobic cleavage of the biphenyl ring and mineralization of the less extensively chlorinated congeners [17, 18]. Several anaerobic bacteria within the *Chloroflexi* have been confirmed to have PCB dechlorinating activity including *Dehalococcoides ethanogenes*, [19], *Dehalococcoides* sp. CBDB1 [20], *Dehalobium chloroocercia* DF-1, bacterium o-17 [21], and phylotypes SF-1 and DH-10 [22]. In contrast, aerobic PCB degradation can be performed by many bacterial species such as *Burkholderia xenovorans* strain LB400 [23], *Rhodococcus* sp. strain RHA1 [24], and bacteria utilizing biphenyls are ubiquitously distributed in the environment [25]. Biphenyl 2,3-dioxygenases are considered the key enzymes in the oxidative pathway for PCB degradation, where bphA1 can degrade specific PCB congeners [26] and bphC is involved in extradiol *meta* cleavage [27]. In the soil environment, bioaugmentation with aerobic PCB degrading bacteria has successfully been applied [28, 29], whereas anaerobic bioaugmentation has previously only shown success in sequential anaerobic-aerobic treatment in granular sludge and a mixture of sediment and soil [30].

In this study, the spatial distribution of putative aerobic and anaerobic bacteria involved in PCB transformation was investigated for the first time in PCB-contaminated soil. The indigenous anaerobic dechlorination potential was mapped spatially throughout the site using a fixed grid established for previous sampling events. In addition, the effect of anaerobic bioaugmentation with a PCB dechlorinating microorganism was evaluated.

2. Materials and Methods

2.1. Bacterial Cultures for Bioaugmentation. *Dehalobium chloroocercia* DF-1 isolated from sediments in Charleston Harbor was maintained in the laboratory as described previously [31]. *D. chloroocercia* dechlorinates doubly flanked in the *para* or *meta* positions.

2.2. Sample Collection. Samples were collected from storm water drainage ditch in Mechanicsburg, PA ranging from its source at 40°13'46.16" N, 76°59'38.51" W to approximately 730 meters downstream to 40°14'06.60" N, 76°59'32.50" W. The open drainage ditch, which collects storm water runoff from the Naval Support Activities base and surrounding off-base properties, extends approximately 2.4 kilometers from

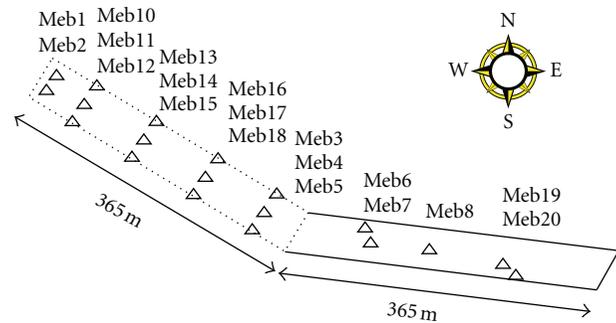


FIGURE 1: Sampling locations in the drainage ditch.

its origin to its confluence with Trindle Spring Run. Samples contaminated with different levels of PCBs were collected with a 60 cm by 5 cm (OD) core sampler from the top 30 cm of soil. In total, 19 soil samples were collected (Figure 1) and stored anaerobically in sealed glass jars at 4°C in the dark and processed 1–3 weeks later.

2.3. Extraction and Analysis of PCBs from Soil Samples and Microcosms. The total concentration of PCBs in the samples was measured in sacrificed samples as described previously [32]. Briefly, the samples were weighed prior to extraction, the overlying water was separated from the soil by decanting, and the remaining sample was mixed with anhydrous sodium sulfate. Following the addition of the surrogates PCB-14, PCB-65, and PCB-166, the overlying water samples were shaken in hexane, while the solid samples were extracted by sonication in 25 mL of 1:1 acetone:hexane for six minutes following EPA method 3550B. The extracts were then pooled and solvent exchange was performed to replace the solvents with hexane. PCB cleanup was based on EPA SW846 Methods 3660B (activated copper treatment), and 3630C (silica gel treatment). The volume obtained after cleanup was diluted according to QA protocols, and the PCB congeners were analyzed using an Agilent 6890 Gas Chromatograph equipped with microelectron capture detector.

2.4. Microbial Activity Assays. A microbial dechlorination activity assay was used to determine the potential dechlorination activity of indigenous microbial communities in the soil samples as described previously [33]. Briefly, 10 mL of low-sulfate mineral F-medium [34] was prepared anaerobically and the congener 2,3,4,5,6-CB (AccuStandard, CT) was added to a final concentration of 50 ppm in acetone (10 µL added). Triplicate cultures were inoculated with 4.0 g of soil (wet weight). Negative controls were prepared by autoclaving twice for 20 min at 121°C one and three days after inoculation prior to adding PCB. The cultures were incubated at 30°C in the dark and 1 mL subsamples were collected for PCB analysis in an anaerobic glove box after 0, 52, 75, 105, 130, 163, and 200 days.

Inhibition of the dechlorination activity was evaluated with a modification of the activity assay described above. Sediment from Baltimore Harbor (2 g) with confirmed dechlorination activity was mixed with the test soil sample

(2 g) in E-Cl medium and analyzed for PCB dechlorination. Sampling for PCB analysis was performed after 0, 50, 75, 100, 150, and 214 days.

2.5. DNA Extraction. DNA was extracted (in triplicate) by transferring 0.75 g of soil to a sterile microcentrifuge tube containing approximately 1 g of 0.1 mm Zirconia/Silica Beads (BioSpec Products, Inc. OK) followed by addition of 200 μ L of 1x TE buffer, 150 μ L of phosphate buffer pH 8.0 (0.12 M) and 150 μ L 1x TS-SDS buffer. The sample and buffers were mixed by hand shaking prior to 30 s of bead beating at speed "4.5" using a FastPrep120 (Q-Biogene, CA). Nucleic acid extraction and purification were performed using a phenol/chloroform-based protocol described previously [35].

2.6. Detection and Enumeration of Putative PCB Dechlorinating Bacteria. Enumeration of putative dechlorinating bacteria in the soil samples was performed in triplicate by a competitive PCR assay as described previously [33] using the 348F-884R 16S rRNA gene primer set. To confirm that potential PCR inhibitors were not coextracted, all samples were tested in parallel with the universal 16S rRNA gene primer set 341F/907R [36, 37]. All samples from the site showed positive results with the universal primer set indicating that the assay would detect putative dechlorinating phylotypes in abundances greater than the detection limit of 2×10^2 gene copies μ L⁻¹. The enumerated 16S rRNA genes copies from the cPCR assay were normalized to the dry weight content of the soil sample. One 16S rRNA gene copy per cell was assumed based on the genome sequences of *Dehalococcoides ethenogenes* [38] and CBDB1 [39]. DNA extraction efficiency was tested by extracting different amounts of homogenized soil and measuring the DNA concentration on a spectrophotometer at 280 nm. The results showed linear extraction efficiency in the range from 0–4 g dry wt of soil (data not shown).

2.7. Detection of Potential Aerobic PCB Degrading Bacteria. Putative PCB degrading aerobic bacteria were detected by PCR amplification of the functional genes *bphA* and *bphC*, which encode the PCB transforming enzymes biphenyl dioxygenase and 2,3-dihydroxybiphenyl 1,2-dioxygenase, respectively. Detection of *bphA* was performed with primer set *bphA40-F/bphA50-R* [26], whereas detection of *bphC* was performed with primer set *P42D-F/P43U-R* [27]. PCR was conducted in 50 μ L reaction volumes using the following GeneAmp reagents (Applied Biosystems, Foster City, CA): 10 mM Tris-HCl, 75 mM KCl, 0.2 mM of each dNTP in a mix, 1.5 mM MgCl₂, 2.5 units of AmpliTaq DNA Polymerase, 50 pM of each primer, 1 μ L of DNA template, and 34.5 μ L of nuclease free water. Amplification of the *bphA* fragment was performed as follows (40 cycles): denaturation at 45°C for 60 s, primer annealing at 31–49°C for 3 min, elongation at 72°C for 4 min, and a final holding step at 4°C. For amplification of the *bphC* fragment (35 cycles), the following conditions were applied: denaturation at 95°C for 30 s, primer annealing at 35°C for 60 s, elongation at 72°C

for 3 min, a final extension step at 72°C for 10 min, and a final holding step at 4°C. PCR products of the correct length were confirmed by electrophoresis using a 1.5% agarose gel. *Burkholderia xenovorans* LB400 [23] was used for verification of the PCR protocols for *bphA* and *bphC* and as the positive control during PCR amplification.

2.8. Bioaugmentation in Microcosms. In total, 4 g of soil (wet weight) was inoculated into triplicate 10 mL volumes of anaerobically prepared mineral medium [41] in 25 mL anaerobe tubes sealed under N₂-CO₂ (80:20) with Teflon septa. No electron donors were added except for the components in the medium (0.0125% w/v cysteine) and residual hydrogen ($\leq 5\%$ v/v) present in the atmosphere of the anaerobic glove box used for inoculating and sampling of the microcosms. *D. chlorocoercia* DF-1 was grown to approximately 10⁷ cells per mL with PCE, which was purged with N₂/CO₂ to remove residual PCE and chloroethene products that could result in "priming" of dechlorination activity. 2 mL of culture were inoculated into the soil microcosms. Nonbioaugmented controls included medium and soil containing indigenous microorganisms without DF-1. Controls for abiotic activity containing medium, soil containing indigenous microorganisms, and DF-1 were sterilized by sequential autoclaving for 1 hour on days 0, 2, and 4.

2.9. Microbial Community Analysis. Community analyses were performed by denaturing HPLC (DHPLC) using a WAVE 3500 HT system (Transgenomic, Omaha, NE) and the 16S rRNA gene primers 348F/884R as described previously [33]. The 16S rRNA gene fragments were analyzed in 20 μ L injection volumes and the fractions were collected in 96 well plates (Biorad, Hercules, CA). Fractions were dried using a Savant SpeedVac system (Thermo Electron Corporation, Waltham, MA) followed by dissolution in 15 μ L nuclease-free water. Each DHPLC fraction was sequenced in both the 5' and 3' direction with 250 pM of primer 348F or 884R, respectively, in 5% DMSO to reduce effects from potential secondary structure using the BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA) kit per the manufacturer's instructions and sequenced on an ABI 3130 XL automated capillary DNA sequencer (Applied Biosystems, CA) as previously described [33]. The 16S rRNA gene sequences and submitted gene sequences obtained from NCBI (<http://ncbi.nlm.nih.gov/BLAST>) were compiled and aligned using the automatic nucleic acid aligner in the BioEdit sequence alignment editor. A total of 13 sequences containing approximately 530 nucleotides were unambiguously aligned and used for calculation of trees by the neighbor joining and FITCH approaches using default settings in the PHYLIP software (<http://evolution.genetics.washington.edu/phylip.html>). Bootstrap analyses (1000 replicates) were performed using the PHYLIP package.

2.10. Nucleotide Accession Numbers. The Genbank accession numbers for the 16S rRNA sequences reported in this paper are JF412634-JF412646.

TABLE 1: Analyses of the dry matter content, the total PCB concentration, the average number and percentage of chlorines less than 4 and 6, respectively, and in soil samples and commercial Aroclor mixtures [40]. ^{a,b,c}refer to the homolog distribution profiles, where ^ais the most typical distribution, ^bis the most dechlorinated, and ^cis the least dechlorinated among the soil analyzed soil samples. Sample Meb9 was not collected.

Location	Dry matter (mg/g)	PCB conc. (ppm)	Average no. of chlorines	≤6 chlorines (%)	≤4 chlorines (%)
Meb1	18.92	34.8	6.52	52.1	0.4
Meb2 ^a	17.54	264.6	6.47	53.6	0.5
Meb3	13.71	12.5	6.51	52.5	0.5
Meb4	28.25	36.6	6.54	50.7	0.4
Meb5	23.17	15.1	6.54	50.5	0.4
Meb6	26.61	3.1	6.43	54.7	1.7
Meb7	16.71	5.6	6.50	52.6	0.2
Meb8	23.71	45.3	6.54	50.5	0.5
Meb10 ^b	15.91	4.6	6.36	55.9	4.3
Meb11 ^c	26.68	2.3	6.61	45.8	1.0
Meb12	33.61	8.2	6.48	52.3	2.4
Meb13	13.55	12.1	6.47	51.8	3.2
Meb14	27.34	12.3	6.47	52.3	2.6
Meb15	29.22	35.6	6.54	47.1	2.6
Meb16	15.20	9.1	6.48	50.9	2.7
Meb17	25.10	17.6	6.52	50.9	2.0
Meb18	36.72	11.3	6.50	50.9	2.7
Meb19	20.31	20.1	6.55	48.7	1.9
Meb20	18.60	72.7	6.54	51.7	0.3
Aroclor 1016	—	—	3.04	100.0	100.0
Aroclor 1232	—	—	2.41	100.0	94.1
Aroclor 1242	—	—	3.31	100.0	92.3
Aroclor 1248	—	—	3.97	100.0	82.2
Aroclor 1254	—	—	5.15	96.0	18.9
Aroclor 1260	—	—	6.39	56.7	0.6

3. Results

3.1. Physical and Chemical Characterization of Soil Samples.

Soil samples from 19 locations were collected in the center of the storm water drainage ditch bed and on each of the flanking banks at incremental distances from the inlet to approximately 365 m downstream in addition to five locations between 365 and 730 m downstream from the inlet (Figure 1). Soil samples collected from the banks were all moist, whereas both dry and moist soil samples were collected from the center of the ditch. Soil was not collected from the center of the ditch at the inlet because only 1–2 cm of soil was present above the bedrock due to erosion from heavy storm water events, but this layer increased in depth with distance from the inlet. Soil samples collected more than 365 m downstream were submerged in water at the time of sampling. The total PCB concentration at the 19 sampling sites ranged from 2.3–265 ppm with an average of 32.8 ppm (± 58.9 ppm), (Table 1). The highest concentration was detected at the east bank nearest to the inlet (Meb2), whereas the lowest concentration was detected at the west bank sampling location Meb11. The average number of chlorines at each site ranged from 6.36–6.61, with a total

average of 6.50 (± 0.053), which is similar to the average of 6.4 observed for Aroclor 1260 (Table 1). The distribution of PCB homologs was similar to A1260 and in prior studies A1260 was reported to be the only Aroclor detected at this site [42]. In some of the samples, higher concentrations of tetra chlorinated congeners were observed (Table 1). The elevated levels of tetrachlorinated congeners detected did not coelute with any known chlorinated non-PCB compounds including pesticides and sulfur indicating that they were likely PCBs. The results suggest that *in situ* PCB dechlorination occurred to some extent in these samples.

3.2. Characterization of Indigenous Microbial Communities.

Bacteria were detected in 18 of 19 samples using universal 16S rRNA bacterial primers (Table 2). In contrast, putative anaerobic dechlorinating and putative aerobic degrading bacteria were detected in only ten samples using primers specific for dechlorinating *Chloroflexi* and *bphA/bphC* genes, respectively (Table 2). At seven locations, both putative aerobic and anaerobic PCB transforming bacteria were detected in the same samples. The numbers of putative anaerobic dechlorinating bacteria ranged from $5 \cdot 10^3$ to

TABLE 2: Analyses of total bacteria, putative PCB dechlorinating and PCB degrading bacteria and the number of putative dechlorinating bacteria in soil samples. Sample Meb9 was not collected.

Location	Presence/absence of				No. of dechlorinating bacteria (16S copies·g soil ⁻¹)
	Bacteria	Dechlorinating bacteria	Aerobic PCB degraders		
Meb1	+	–	–	–	—
Meb2	+	–	–	–	—
Meb3	+	+	+	+	<DL ^a
Meb4	+	+	+	+	$3 \cdot 10^4 \pm 2.8 \cdot 10^4$
Meb5	+	+	+	+	<DL
Meb6	+	+	+	+	$3 \cdot 10^6 \pm 2.8 \cdot 10^6$
Meb7	+	+	+	+	$4 \cdot 10^5 \pm 2.3 \cdot 10^5$
Meb8	+	+	–	–	$3 \cdot 10^5 \pm 2.8 \cdot 10^5$
Meb10	+	–	+	+	—
Meb11	+	–	–	–	—
Meb12	+	–	–	–	—
Meb13	+	–	+	+	—
Meb14	+	+	+	+	<DL
Meb15	+	+	+	+	<DL
Meb16	+	–	+	+	—
Meb17	+	+	–	–	<DL
Meb18	+	+	–	–	$3 \cdot 10^3 \pm 2.8 \cdot 10^3$
Meb19	+	–	–	–	—
Meb20	+	–	–	–	—

Mean values and standard deviations \pm are given ($n = 3$).

^aDL: detection limit $\geq 10^2$ 16S copies/g wet soil.

$5 \cdot 10^6$ dechlorinating bacteria g⁻¹ soil, whereas the numbers at several locations were below the detection limit for enumeration, but presence was detected. The number of putative aerobic degrading bacteria was not determined for this phylogenetically diverse group of bacteria.

The population of putative dechlorinating bacteria was examined by DHPLC in three samples where 16S rRNA gene copies were most abundant (Meb6, Meb7, and Meb8) and compared to the sequences of known PCB dechlorinating bacteria within the *Chloroflexi* group (Figure 2). Five of the 13 identified phylotypes grouped closely together within the clade of known *Dehalococcoides* sp. and the remaining eight phylotypes grouped within the broader o-17/DF-1 *Chloroflexi* group. No trend was observed between the location of the samples and the identified phylotypes, since phylotypes from the three locations were located throughout the phylogenetic tree. This observation indicates the population of indigenous phylotypes was heterogeneous throughout the site.

3.3. Dechlorination Activities by Indigenous Communities.

The indigenous dechlorination potential was examined in soil samples using 2,3,4,5,6-CB as a surrogate, which is saturated with chlorines on one biphenyl ring. Activity was detected in six of the 19 samples (Table 3). The highest dechlorination rate ($6.93 \pm 5.33 \cdot 10^{-3}$ mol% 2,3,4,5,6-CB \times day⁻¹) was measured in Meb16 and the lowest (0.93

$\pm 1.70 \cdot 10^{-3}$ mol% 2,3,4,5,6-CB \times day⁻¹) in Meb17. The observed products of the dechlorination of 2,3,4,5,6-CB were predominantly 2,3,4,5-CB/2,3,5,6-CB, but 2,3,6-CB, and 2,6-CB were detected in sample Meb10. The remaining 13 samples did not show any dechlorination activity after 200 days of incubation despite detection of putative dechlorinating bacteria by cPCR in some of the inactive samples.

3.4. Effects of Indigenous Contaminants on Activity.

The absence of detectable dechlorination activity in some of the samples despite the detection of putative dechlorinating phylotypes was further examined to determine whether high concentrations of PCBs or other contaminants inhibited activity. Soil from inactive samples was mixed with biocatalytically active sediment from Baltimore Harbor, MD, which transforms 2,3,4,5,6-CB to 2,4,6-CB via reductive dechlorination at the two *meta* positions. For all tested samples including sediment from Baltimore Harbor, the lag phase was less than 50 days and the terminal plateau ranged between 6 and 46 mol% 2,3,4,5,6-CB (Table 4). The dechlorination rates varied from 7–12 $\cdot 10^{-3}$ mol% 2,3,4,5,6-CB day⁻¹, where the most active site had approximately 25% greater activity than observed in Baltimore Harbor sediment alone. This soil sample (Meb7) was collected furthest away from the inlet of the drainage ditch, had the highest number of putative dechlorinating bacteria, and had

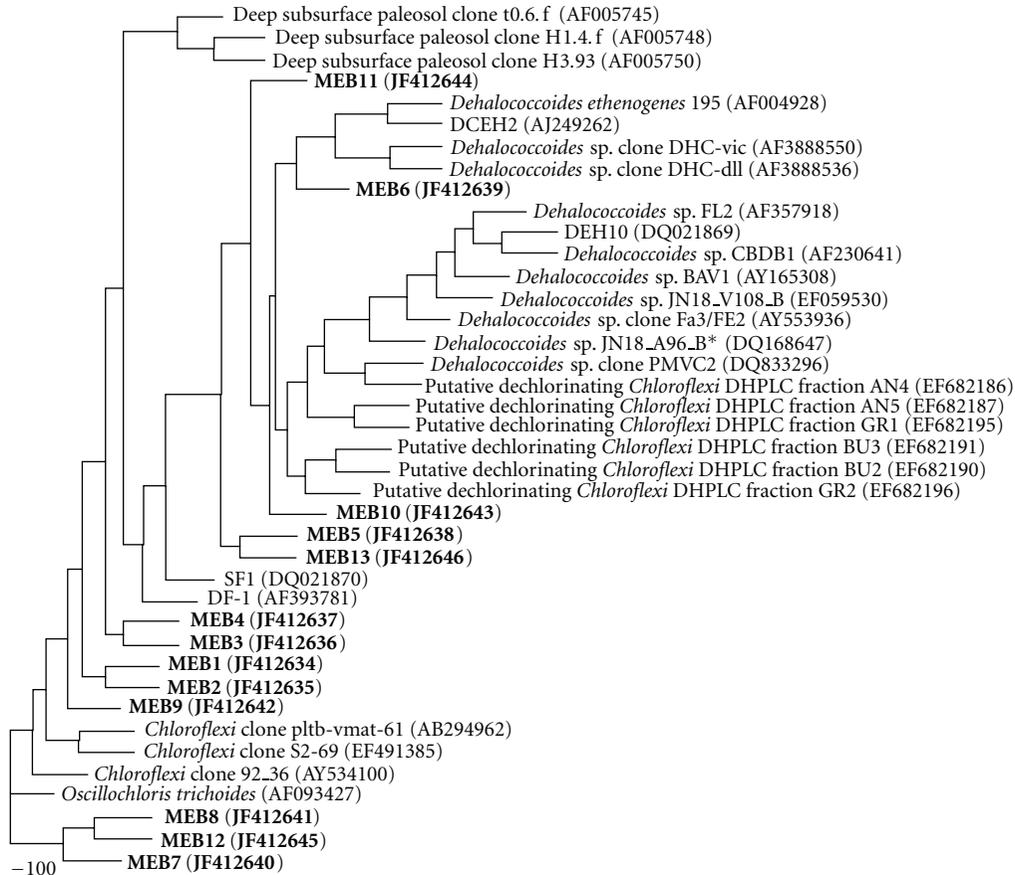


FIGURE 2: Phylogenetic tree showing the relationships between the dominant phylotypes identified in sediment samples Meb6, Meb7, and Meb8 (bold) and the closest dechlorinating species within the dechlorinating *Chloroflexi* group. Phylotypes from sample Meb6, Meb7 and Meb8 were located throughout the phylogenetic tree and did not show any relationship between the sample origins their phylogenetic relationship. Accession numbers are indicated in parentheses. The tree was calculated by the neighbor joining method and supported by FITCH [43]. The scale bar indicates 10 substitutions per 100 nucleotide positions.

a high water content compared to the rest of the samples. The dechlorination rates for the mixed samples were all higher or at levels similar to those observed in the activity assays (Table 3). In all samples tested, the end product of dechlorination was 2,4,6-CB, which is characteristic of BH activity rather than the tetrachlorinated congener products detected predominantly in indigenous ditch microcosms, indicating that the indigenous BH dechlorinating activities were dominant. A statistical evaluation (Student's *t*-test, $P < 0.05$) showed that statistically significant inhibition was found for only samples Meb6 and Meb18 (dechlorination plateau) and Meb17 (dechlorination rate and plateau).

3.5. Effect of Bioaugmentation. Bioaugmentation with a pure culture of the PCB dechlorinating bacterium DF-1 was tested in microcosms with soil from three locations containing approximately 5 ppm (Meb10), 73 ppm (Meb20), and 265 ppm (Meb2) total weathered PCBs. These locations were selected based on the different concentrations of PCBs. The specific dechlorination of only doubly flanked chlorines by this organism could be readily distinguished from the dechlorination of single flanked and unflanked

chlorines by the indigenous populations. Controls with indigenous populations without DF-1 and autoclaved controls showed no significant dechlorination. In the bioaugmented microcosms, significant dechlorination was observed for Meb10 (5 ppm), (Table 5). In addition to DF-1, a highly enriched culture of indigenous PCB dechlorinating microorganisms from MEB10 enriched with 2,3,4,5,6-PCB was also used to bioaugment Meb2, but no significant effect on dechlorination was observed. Evaluation of the changes in homolog distributions indicated that DF-1 was active in Meb10 (Figure 3), with significant reductions of hepta- and octachlorinated congeners and significant increases of tetra- and pentachlorinated congeners. The congeners that were significantly reduced were 22'33'45'6 (PCB-175), 22'33'55'66'/22'33'44'6/233'44'5 (PCB-202/171/156), 22'33'455' (PCB-172), 22'344'55' (PCB-180), 22'33'44'5/233'44'56 (PCB-170/190), and 22'344'55'6/22'33'44'56' (PCB-203/196). All congeners that were dechlorinated significantly more than the control contained both double-flanked *meta* and *para* positioned congeners with the exception of PCB-175 and PCB-171 that only contained a double-flanked *meta* positioned chlorine

TABLE 3: The potential dechlorination activity of the soil samples in microcosms. Locations not listed here did not show any dechlorination activity.

Location	Max rate · 10 ^{-3a} (mol% 2,3,4,5,6-CB × day ⁻¹)	Lag phase (d)	PCB 116 remaining after 200 days (mol% 2,3,4,5,6-CB)	Dechlorination products
Meb10	4.2 (0.7)	0–50	46.0 (7.0)	2,3,4,6-CB/2,3,5,6-CB ^b 2,3,6-CB, 2,6-CB ^c
Meb12	4.2 (4.1)	0–130	82.3 (5.8)	2,3,4,6-CB/2,3,5,6-CB
Meb13	3.4 (3.8)	0–150	78.0 (15.6)	2,3,4,6-CB/2,3,5,6-CB
Meb16	6.9 (5.3)	0–105	58.7 (27.6)	2,3,4,6-CB/2,3,5,6-CB
Meb17	0.9 (1.7)	0–200	89.7 (18.2)	2,3,4,6-CB/2,3,5,6-CB
Meb19	3.0 (5.0)	0–200	68.3 (49.7)	2,3,4,6-CB/2,3,5,6-CB
Negative control	0.1	—	98	—

^a Mean values are given and figures in brackets are standard deviations ($n = 3$).

^b The products 2,3,4,6-CB/2,3,5,6-CB coeluted during GC analysis.

^c This product was detected in one of three replicate cultures <5 mol%.

TABLE 4: Effect of inhibition on the dechlorination activity in microcosms using selected soil and sediment samples mixed 1 : 1 with actively dechlorinating sediment from Baltimore Harbor. Inhibition was determined ($P < 0.05$) for the dechlorination rate and terminal plateau. Lag phase is defined as not showing activity that is different from the autoclaved negative control.

Location	Lag phase (d)	PCB 116 remaining after 200 days (mol% 2,3,4,5,6-CB)	Dechlorination rate · 10 ⁻³ (% 2,3,4,5,6-CB day ⁻¹)	End product	Inhibition ($P < 0.05$)	
					Rate	Plateau
Baltimore Harbor	0–50	5.7 ± 4.5	9.1 ± 2.2 ^b	2,4,6-CB ^a	—	—
Meb6	0–50	31.3 ± 15.9	7.3 ± 1.0	2,4,6-CB	No	Yes
Meb7	0–50	10.7 ± 3.5	12.4 ± 1.1	2,4,6-CB	No	No
Meb8	0–50	21.0 ± 19.2	8.4 ± 3.7	2,4,6-CB	No	No
Meb17	0–50	46.3 ± 6.5	7.5 ± 2.2	2,4,6-CB	Yes	Yes
Meb18	0–50	45.7 ± 10.1	10.5 ± 2.2	2,4,6-CB	No	Yes

^a One tube showed additional terminal products such as 2,4-CB, 2,5-CB, and 2,6-CB.

^b Data are means ± standard deviation ($n = 3$).

TABLE 5: Change in the number of chlorines per biphenyl over the five month incubation period for the bioaugmentation experiments with DF-1.

Location	Chlorines per biphenyl		
	Day 0	Day 145	
		No DF1	With DF1
Meb10	6.34 ± 0.02	6.30 ± 0.19	5.94 ± 0.10
Meb20	6.42 ± 0.02	6.29 ± 0.09	6.30 ± 0.04
Meb2	6.43 ± 0.01	6.42 ± 0.01	6.42 ± 0.01

and PCB-202 that did not contain any double-flanked *meta* or *para* positioned chlorines. However, the latter congener coeluted with PCB-156 (50%-50% split) that contains both double-flanked *meta* and *para* positioned chlorines. In the samples Meb20 and Meb2 containing higher concentrations of total PCB, bioaugmentation with DF-1 did not show any significant effects (data not shown).

4. Discussion

Bacteria capable of aerobic PCB degradation are found ubiquitously in the soil environment and several have been isolated and identified that belong to genera such

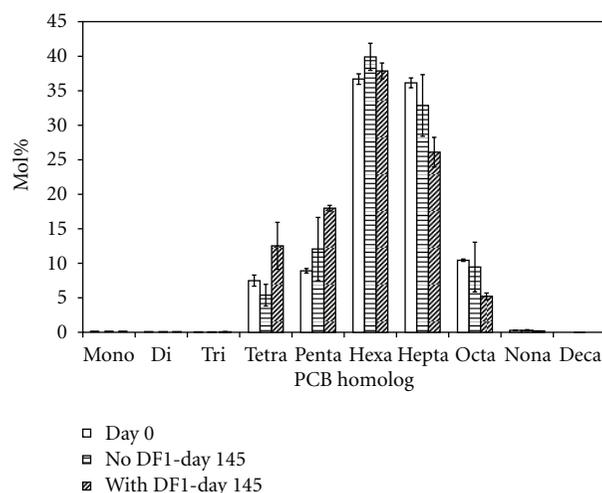


FIGURE 3: Homolog distribution showing the effect of DF-1 in the bioaugmentation experiment compared to effect of the indigenous population for Meb10.

as *Pseudomonas*, *Burkholderia*, *Ralstonia*, *Achromobacter*, *Comamonas*, *Bacillus* and *Rhodococcus* [25, 44]. Although anaerobic dechlorination activity has been detected in soils by activity based methods at several Canadian military installations such as Saglek, Labrador [45], Resolution Island,

Nunavut [30], and Fort Albany, Ontario [46], anaerobic PCB dechlorinating bacteria have not been identified previously from soils. This is the first study to combine microbial community analysis and activity-based techniques on PCB impacted soils. The presence of active dechlorinating bacteria is an essential first step of the sequential degradation process that requires reduction of extensively chlorinated PCB congeners to less chlorinated congeners that can be subject to aerobic ring cleavage by microbial 2,3- and 3,4-dioxygenase activity and can subsequently be mineralized by other aerobic microorganisms.

In the drainage ditch soil examined in this study, the PCB contamination originated predominantly from A1260 contamination [42]. The presence of tetra chlorinated homologs atypical of Aroclor 1260 combined with identification of the putative PCB dechlorinating phylotypes and detection of PCB dechlorination activity suggests that *in situ* dechlorination occurs at this site. The 13 identified phylotypes clustered within the dechlorinating *Chloroflexi* group, either within the the *Dehalococcoides* clade [47–49] within the broader *Chloroflexi* clade, which includes other confirmed PCB dechlorinating bacteria such as *Dehalobium chloro-coercia* DF-1 and strain o-17-group [50]. In two previous reports on bacterial communities in PCB contaminated soil, bacteria closely related to *Proteobacteria*, the *Holophaga-Acidobacterium* phylum, *Actinobacteria*, and *Planctomycetales* and *Cytophagales* were identified [51, 52]. Interestingly, the dominant species included the genera *Burkholderia* and *Variovorax* together with *Sphingomonas* species, *Rhodophila globiformis* group members, and *Acidobacterium capsulatum* that aerobically degrade a variety of organic pollutants including PCBs. However, neither anaerobic dechlorinating bacteria nor any phylotypes related to the dechlorinating *Chloroflexi* were identified.

Prior to the current study, PCB dechlorinating bacteria have only been identified in sediments. Thus, detection of putative anaerobic dechlorinating bacteria and reductive dechlorination activity in the examined soil samples indicates that microbial dehalorespiration of PCBs also occurs in soil. Putative aerobic PCB degrading bacteria were also detected at several locations in the drainage ditch, which suggests that natural attenuation of PCBs could occur in soils by sequential anaerobic dechlorination followed by aerobic degradation. However, the large spatial heterogeneity of both anaerobic and aerobic phylotypes associated with PCB transformation could be an obstacle to bioremediation. Similar heterogeneity observations have been reported in studies of soils contaminated with heavy metals [9] and polycyclic aromatic hydrocarbons [11]. In the first study, it was found that samples collected within 1 cm distance could have a 10,000 fold difference in metabolic potential and that metal concentrations did not correspond with the metabolic potential [9]. In another report, microscale heterogeneities observed in PAH contaminated soil were attributed to the particle size of sand and silt fractions, which impacted the PAH concentrations and availability [11]. This corresponds with observations of the behavior between bacteria and clay minerals in soils contaminated with PCBs [12]. Here, it was observed that soil aggregates called “clay hutches” housed the bacteria

creating a microhabitat, which would limit the PCB availability and constitute the main carbon source in an otherwise carbon limited environment. These observations of spatial heterogeneity on both a macro- and microscale are consistent with the observations of the spatial variability of bacteria involved in PCB transformation in soil in the current study.

The numbers of putative dechlorinating bacteria were 2 to 5 orders of magnitude lower in soils compared with sediments, where up to approximately 10^8 bacteria per g sediment have been reported [33]. Dechlorination rates were lower in the soil compared to sediment indicating that the overall potential for dechlorination is reduced. The lack of correlation between the number of dechlorinating bacteria and the dechlorination rates in the soil samples might have been caused by localized environmental conditions. For example, most of the soil samples at the time of sampling were moist but were not submerged in water as sediment samples. In a study of simulated dredged sediment that was spiked with 300 ppm A1248, it was reported that the dechlorination activities were lowered when the water content was reduced, which caused a lag in the dechlorination of A1248 [53]. Degradation studies of other contaminants in soil such as petroleum hydrocarbons also show that moisture content in soil influences the degradation rate [54].

A possible consequence of the reduced moisture content in the soil samples could be exposure to oxygen that negatively influences anaerobic dechlorination. In a study of methanogenic granules capable of A1254 dechlorination, exposure to oxygen did not significantly influence the dechlorination activity [55]. In six months, 80% of the initial concentration of A1254 had been dechlorinated in samples that had been exposed to oxygen for one week. The reason for this rapid dechlorination could be the formation of biofilms in microniches that stay anaerobic despite the exposure to oxygen at the surface of the granule. Microniches in soil can result in spatial and temporal heterogeneity for parameters such as oxygen, pH, redox, and nutrients as well as PCBs [12, 54]. Thus, another factor affecting the spatial variation in dechlorination rates observed in the soil samples could be the heterogeneous moisture content affecting formation of biofilms and anaerobic microniches that support communities of dechlorinating bacteria.

Successful bioaugmentation of weathered Aroclor 1242 in soil with an enrichment culture from the Hudson River has been observed previously [56]. In the report, *meta*-dechlorination was observed after 19 weeks of incubation resulting in a reduction of the average chlorine content by 0.7 chlorines per biphenyl. Another report that evaluated the effect of sequential anaerobic-aerobic treatment of A1260 contaminated soil from Saglek, Labrador, Canada, [45] initiated with a dechlorinating enrichment, showed a decrease in the average chlorine content of 1.2 chlorines per biphenyl together with a significant homolog shift after three months of anaerobic bioaugmentation [45]. In the current study, bioaugmentation of A1260 contaminated soil with a pure culture of *D. chloro-coercia* has resulted in only 0.4 chlorines per biphenyl after 20 weeks. As expected, dechlorination of doubly flanked *meta*- and *para*-positioned chlorines was observed, but at least one product was

observed that could not have been formed due to DF-1 activity, which suggests that *D. chlororcoercia* might have stimulated dechlorination of single-flanked chlorines by the indigenous dechlorinating population, possibly as a result of priming of the indigenous populations by intermediates products. Stimulation of dechlorinating activity after bioaugmentation with *D. chlororcoercia* has been reported previously [57]. The relatively low rate of dechlorination in soil from Meb10 and lack of enhanced dechlorination at the two other bioaugmented sites might have been caused by inhibition by other contaminants such as metals and/or other organic contaminants [58].

In this study, the spatial distribution of bacteria involved in PCB transformation was investigated in Aroclor 1260 contaminated soil containing heavy metals and organic cocontaminants. The results showed that anaerobic PCB dehalorespiring bacteria were present and active. Detection of genes encoding biphenyl dioxygenase genes suggests that the potential exists for natural attenuation by sequential anaerobic dehalorespiration and aerobic degradation. Although a recent study demonstrated the potential for *in situ* treatment of PCB impacted sediment by bioaugmentation [57], the spatial heterogeneity inherent in soil in the current study had a profound effect on the ability to transform PCBs as only one of the bioaugmented samples showed positive effect. Strategies for effective *in situ* bioremediation of PCB-impacted soils may require additional remedial treatments such as flooding the soil prior to bioaugmentation to provide homologous water content, addition of a carbon source to create an adequately reduced environment for dehalorespiration, and precipitation of potentially inhibitory heavy metals or concurrent or sequential bioremediation with biocatalysts specifically target potentially inhibitory organic co-pollutants. The results illustrate some of the challenges associated with the development of *in situ* bioremediation strategies for PCB impacted soils.

Acknowledgments

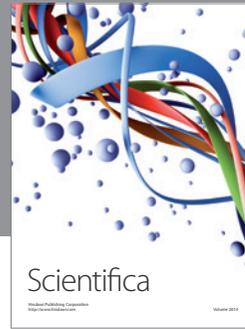
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