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Data Article

Microbial communities in polychlorinated biphenyl (PCB)-contaminated wastewater lagoon sediments: PCB congener, quantitative PCR, and 16S rRNA gene amplicon sequencing datasets

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ABSTRACT

The potential for aerobic and anaerobic microbial natural attenuation of PCBs in freshwater sediments is described by PCB congener, quantitative PCR, and 16S rRNA gene amplicon sequencing datasets generated, in duplicate, from 27 sediment samples collected from a PCB-contaminated freshwater lagoon (54 samples total). Sediment samples were subjected to a hexane PCB extraction protocol and the concentrations of 209 PCB congeners were determined in hexane extracts by gas chromatography with a tandem mass spectrometry detection. DNA was extracted from sediments sediment samples and used for qPCR and 16S rRNA amplicon sequencing. The abundance of 16S rRNA genes (i.e., *Dehalococcoides* and putative dechlorinating Chloroflexi) and functional genes (i.e., reductive dehalogenase (*rdhA*) and biphenyl dioxygenase (*bphA*)) associated with aerobic and anaerobic PCB biodegradation, along with the total 16S rRNA genes abundance, was determined by SYBR green qPCR. The

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microbial community composition and structure in all sediment samples was obtained by 16S rRNA gene amplicon sequencing. Primers targeting the 16S rRNA gene V4 region were used to produce 16S rRNA gene amplicons that were sequencing with the high-throughput Illumina MiSeq platform and sequencing chemistry. The 16S rRNA gene sequencing dataset along with PCB congener and qPCR datasets included as metadata, could be reused in meta-analyses that aim to determine microbial community interactions in contaminated environments, and uncover relationships between microbial community structure and environmental variable (e.g., PCB congener concentrations).

Specifications Table

Subject	Environmental Science
Specific subject area	Aerobic and anaerobic microbial natural attenuation of PCBs in freshwater sediments
Type of data	Dataset Table Raw fastq files Filtered and merged fastq files
How data were acquired	Instruments for PCB analysis: Automated Solvent Extractor (ASE), Gas chromatograph with a tandem mass spectrometer (GC/MS/MS) in multiple reaction monitoring mode Make and model of the instruments used for PCB analysis: Dionex ASE-300, Agilent 7000 triple quadrupole GC/MS/MS Instruments for qPCR analysis: real-time PCR instrument Make and model of the instruments used for qPCR analysis: Applied Biosystems ABI QuantStudio Flex 7 Real-Time PCR System Instruments for 16S rRNA gene amplicon sequencing: Illumina MiSeq benchtop sequencer Make and model of the instruments used for 16S rRNA gene amplicon sequencing: Illumina MiSeq benchtop sequencer
Data format	Raw and analysed
Parameters for data collection	Sediment samples (27) were obtained from a PCB-contaminated freshwater lagoon according to a grid pattern. The concentrations of 209 PCB congeners, and the relative abundance of 16S rRNA genes and other functional genes associated with PCB biodegradation were determined in each sample in duplicate.
Description of data collection	Sediments were mixed with hexane and diatomaceous earth and PCBs were extracted with an ASE. All 209 PCB congeners were resolved, identified, and quantified by GC/MS/MS. DNA was extracted from sediments with a commercial extraction kit. The qPCR data was collected for five different 16S rRNA or functional gene targets with SYBR green as the reporter dye. Standard curves were constructed with known target gene amounts for quantification. Primers targeting the 16S rRNA gene V4 region were used to produce 16S rRNA gene amplicons for sequencing with the Illumina MiSeq chemistry and platform.
Data source location	Institution: University of Iowa City/Town/Region: Iowa City, IA Country: USA Latitude and longitude for PCB-contaminated emergency overflow lagoon located at a wastewater treatment plant in Altavista, VA: 37°06′47″N, 79°16′23″W.

(*continued on next page*)

Data accessibility	Repository for PCB dataset: Pangaea Data identification number: 0.1594/PANGAEA.933459 Direct URL to data: https://doi.org/10.1594/PANGAEA.933459 Repository for qPCR data: Iowa Research Online Data identification number: 10.25820/data.006142 Direct URL to data: https://doi.org/10.25820/data.006142 Raw 16S rRNA gene sequencing reads are deposited in the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA382682 Direct URL to data: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA382682/ Analysed 16S rRNA gene sequences generated by the MG-RAST automated processing pipeline and associated metadata are in MG-RAST under project number mgp92147 Direct URL to data: https://www.mg-rast.org/linkin.cgi?project=mgp92147
Related research article	Mattes, T.E., Ewald, J.M., Liang, Y., Martinez, A., Awad, A., Richards, P.M., Hornbuckle, K.C., and J.L. Schnoor. PCB dechlorination hotspots and reductive dehalogenase genes in sediments from a contaminated wastewater lagoon. *Env Sci Poll Res* 25 (2018) 16376-16388. DOI: https://doi.org/10.1007/s11356-017-9872-x

Value of the Data

- The overall dataset captures spatial variability of sediment PCB contamination and microbial ecology within a freshwater lagoon sampled at 27 different locations. It includes high resolution PCB data (209 congeners as 171 co-eluting peaks), qPCR abundance estimates of specific 16S rRNA gene, reductive dehalogenase gene and biphenyl dioxygenase gene targets, and 16S rRNA gene amplicon sequencing.
- Researchers or engineers who seek to improve PCB remediation decision-making processes will benefit from the dataset as it contains multiple lines of evidence for aerobic and anaerobic microbial natural attenuation of PCBs in aquatic sediment environments.
- Studies that incorporate meta-analysis of microbial community structure in contaminated sediment environments, machine learning approaches to microbial ecology, or investigate microbially mediated sediment PCB dechlorination patterns will benefit from the combined chemical and microbiological data.
- The qPCR dataset, deposited into the Iowa Research Online repository, reveals the potential for anaerobic reductive dechlorination and the aerobic oxidation of PCB congeners in the the sediments.
- The 16S rRNA gene sequencing dataset, deposited into the SRA and MG-RAST repositories, along with the PCB congener and qPCR metadata, reveals insights into the relationship between microbial community structure and individual PCB congener data, as well as interactions among microbial community members in contaminated environments.

1. Data Description

The PCB congener, 16S rRNA gene amplicon sequencing, and qPCR datasets were obtained, in duplicate, from 27 sediment sample locations (for a total of 54 sediment samples) within a PCB-contaminated emergency overflow lagoon [1]. The latitude and longitude coordinates for the PCB-contaminated emergency overflow lagoon located are 37°06′47″N, 79°16′23″W. Approximate GPS coordinates for each of the 27 sediment sample locations within the lagoon are available in MG-RAST under project number mgp92147.

The PCB congener dataset consists of concentrations for 209 PCB congeners from each of the 54 sediment samples. PCB congener concentrations were obtained by extracting PCBs from

sediment samples with hexane and quantifying by gas chromatography with tandem mass spectrometry detection. The PCB congener dataset is provided in a tabular format on the Pangea repository under DOI: 0.1594/PANGAEA.933459 [2].

The qPCR dataset consists of abundance estimates for five different gene targets: total 16S rRNA genes, putative dechlorinating Chloroflexi 16S rRNA genes, *Dehalococcoides*-like 16S rRNA genes, reductive dehalogenase gene RD14 from *D. mccartyi* strain CG5, and biphenyl dioxygenase gene *bphA* in each of the 54 sediment samples.

The names, oligonucleotide sequences, expected product sizes and references for the PCR and qPCR primers used are provide in Table S1. Table S2 summarizes the primer and template concentrations in qPCRs, qPCR standard curve linear ranges, qPCR efficiencies, and the Y-intercepts of the standard curves for each of the five gene targets. This information is provided to conform with MIQE guidelines [3].

Data spreadsheets are provided for each of the five gene targets. Each of the data spreadsheets contain seven tabs: Data Dictionary, Sample setup, Amplification Data, Results, Melt Curve raw data, Calibration curve and analysis, and Results summary. Tabs labelled "Data Dictionary" in each data spreadsheet define all column headings, abbreviations, and variables used. A 'ReadMe' text file that describes the contents of each sheet in the data spreadsheets and pertinent methdological information (e.g., qPCR primer sets used) is also provided. The qPCR dataset has been deposited in the Iowa Research Online (IRO) institutional data repository under the following DOI: 10.25820/data.006142 [4].

The 16S rRNA gene amplicon sequencing dataset consists of 16S rRNA gene sequence reads in raw fastq files and merged fastq files for each of the 54 sediment samples. DNA was extracted from the sediment samples and used as template in PCRs to generate partial 16S rRNA gene amplicons for sequencing. Amplicon sequencing was performed on an Illumina MiSeq benchtop sequencer with paired-end runs. The raw sequencing data output files are demultiplexed, resulting in 2 paired-end read fastq files per sample. For example, for sample A1a, the raw paired-end fastq files are named A1a_L001_R1_001.fastq.gz and A1a_L001_R2_001.fastq.gz. The raw 16S rRNA gene paired-end read sequence files for all 54 samples are deposited in the GenBank Sequence Read Archive under BioProject number PRJNA382682.

The raw paired-end read fastq files were also submitted to MG-RAST, where merging, filtering, and additional sequencing pipeline processing steps were performed. The raw, demultiplexed paired-end read fastq files were merged to form a single fastq file for each of the 54 samples. For example, for sample A1a, the merged fastq file is named A1a_ATTACGTATCAT_L001_R.fastq. The names of each merged fastq file, along with the pre-QC basepair and sequence count are shown in Table S3. The processed data is available under MG-RAST project number mgp92147.

The PCB congener and qPCR data are also included as metadata in the Sequence Read Archive (BioProject number PRJNA382682) and MG-RAST (mgp92147) depositions for the 16S rRNA gene sequencing data.

2. Experimental Design, Materials and Methods

2.1. Sediment sample collection

A defunct emergency wastewater overflow lagoon located at a wastewater treatment plant in Virginia has been known to be contaminated with PCBs for at least 45 years. No wastewater has been discharged to the lagoon for at least 20 years and the overflow is blocked. Water only enters the former lagoon by precipitation and leaves by evaporation. The lagoon sediments have never been dredged. The approximate area of the lagoon is 28,500 m^2 with an approximate depth of 1.8 m.

In September 2015, sediment samples were collected from 27 locations within the lagoon in an approximate grid pattern [1]. This was achieved by navigating to the desired sampling loca-

tion in a small boat and using a hand auger to collect sediment samples. Each sediment sample (approx. 50–100 g) was homogenized briefly by hand before being placed into clean amber glass containers. Interstitial liquid obtained during the sampling procedure was not decanted. Sediment samples were stored at 4°C prior to further processing, as described below.

2.2. PCB extraction and congener analysis

PCB extraction from sediments with hexane was performed as described previously [5]. Approximately 0.5 g of wet sediment was mixed with around 10 g of combusted diatomaceous earth, and then spiked with 50 ng each of the following surrogate standards [PCB14 (3,5-dichlorobiphenyl), PCB65-d5 (2,3,5,6-tetrachlorobiphenyl-d5, deuterated) and PCB166 (2,3,4,4′,5,6-hexachlorobiphenyl)]. PCBs were extracted from the sediment-diatomaceous earth mixture with an Accelerated Solvent Extractor (ASE, Dionex ASE-300). The ASE parameters were as follows: Extraction solvent: Acetone/n-hexane (1:1, v/v), CH_3COCH_3/CH_2Cl_2; Pressure: 1,500 psi; Temperature: 100°C; Static time: 5 min; Static cycles: 1; Flush: 60%; Purge: 90 s; Cell size: 100 mL.

The initial 100 mL hexane extracts were concentrated using a TurboVap until the volume reached approximately 5 mL. The extracts were then placed in clean glass test tubes, and an additional 15 mL of fresh hexane were added to rinse the ASE bottles. This 15 mL of hexane was added to the 5 mL concentrated extracts and further concentrated until the volume reached 1 mL. The TurboVap tubes were subsequently cleaned with 2 mL of fresh hexane which was also added to the test tubes. An additional 2 mL of fresh hexane was added to the test tubes, resulting in a final extract volume of 5 mL. A sulfuric acid liquid-liquid extraction was used to remove polar interferences and other compounds. Two mL of sulfuric acid (18 M) were added to the 5 mL of hexane extract, inverted for 2 min, and centrifuged for 5 min (speed 3000 rpm (~1000 xg) and 20°C). The organic layer was transferred to a clean test tube. The acidic phase was re-extracted twice more by adding 3 mL of fresh hexane, inverting and centrifuging. The final extract was again concentrated using the TurboVap until reaching a volume of 0.5 mL. The concentrated sample extracts were passed through a Pasteur pipette filled with 0.1 g of combusted silica gel and 1 g of acidified silica gel (2:1 silica gel:acid by weight) using ~10 mL of fresh hexane. The final extract was concentrated using the TurboVap until reaching a final volume of 0.5 mL. Twenty-five ng of [[PCB30-d5 (2,4,6-trichlorobiphenyl-2′,3′,4′,5′,6′-d5, deuterated) and PCB204 (2,2′,3,4,4′,5,6,6′-octachlorobiphenyl)] was spiked into the final extract for use as internal standards. PCB congeners were identified and quantified with a modified US EPA method 1668C [6] as follows:

All 209 PCB congeners were resolved and quantified as 171 individual or coeluting congener peaks using an Agilent 7000 gas chromatograph with tandem mass spectrometry detection (GC/MS/MS) in multiple reaction monitoring mode. The GC was equipped with a Supelco SBP-Octyl capillary column (Poly(50% n-octyl/50% methyl siloxane, 30 m × 0.25 mm ID, 0.25 µm film thicknesses)) with UHP helium as the carrier gas (0.8 mL/min) and UHP nitrogen as the collision gas (1.5 mL/min). The GC operated in solvent vent injection mode at the following injection conditions: initial temperature 45°C, initial time 0.06 min, ramp 600°C/min to inlet temperature 325°C at 4.4 psi. The GC oven temperature program was 45°C for 2 min, 45 to 75°C at 100°C/min and hold for 5 min, 75 to 150°C at 15°C/min and hold for 1 min, 150 to 280 at 2.5°C/min and final hold 5 min (total run time 70.86 min). The triple quadrupole MS electron ionization source was set to 260°C. The MS-MS operated with the precursor-product transitions as shown in Table 1.

The sediment water content was determined gravimetrically for each sample from a separate aliquot by drying for 12 h at 104°C. the following equation was used to calculate the water content (%):

$$\%\ \text{dry weight} = \frac{\text{g of dry sample}}{\text{g of wet sample}} \times 100$$

Table 1
Precursor-product mass transitions for each PCB homolog group during MS-MS.

Cl Homolog[a]	Precursor Mass	Product Mass
Mono	188	152
Di	222	152
Tri	258	186
Tetra	291.9	222
Penta	325.9	255.9
Hexa	359.8	298.9
Hepta	393.8	323.9
Octa	429.7	359.8
Nona	463.7	393.8
Deca	497.7	427.9
D5 tri	261	191
D5 tetra	296.9	277

[a] Unlabeled standards were from AccuStandard, New Haven, CT, USA. Labeled standards were from C/D/N Isotopes, Pointe-Claire, QC, Canada.

2.3. Quality assurance/quality control (QA/QC) of PCB congener data

Surrogate standards and laboratory blanks were used to evaluate our QA/QC. Laboratory blanks consisted of combusted diatomaceous earth and were extracted, concentrated, and analyzed in tandem with the field samples. Surrogate standard recoveries were 79 ± 19% (PCB14) and 76± 16% (PCB65-d5). The recovery of surrogate PCB166 was high (260 ± 600%) because of the presence of PCB128 in the samples, which coelutes with PCB166. The presence of PCB128, which impacted surrogate PCB166 recoveries was attributed to the high levels of total PCBs in these sediment samples. Therefore, PCB congener masses were corrected according to PCB14 (congeners 1–39) and PCB65-d5 (congeners 40–209) recoveries. The total PCB mass in field and laboratory blanks averaged<1% of the total PCB mass determined in the sediment samples. The upper 95% confidence interval from the mass of the laboratory blanks ($n = 5$) was used to determine the limit of quantification (LOQ) for each PCB congener. Congener-specific LOQs ranged from 0.0001 to 1.8 ng per g, with a cumulative PCB mass of 24 ng per g. The concentration of PCB congeners with a quantified mass value below the LOQ was set to zero.

2.4. DNA extraction from sediment samples

Approximately 0.25 g of wet sediment were used in each DNA extraction. DNA extractions were performed in replicate for each sediment sample with the DNeasy PowerSoil Kit (Qiagen, Germantown, MD) according to the manufacturer's suggested protocol. DNA concentrations in the extracts were measured with a Qubit fluorometer and the Qubit dsDNA HS assay kit according to the manufacturer's instructions and protocols (Thermofisher Scientific, Waltham, MA). DNA extracts were stored at − 80°C prior to further analysis.

2.5. Real-time quantitative PCR (qPCR)

The abundance of total 16S rRNA genes, putative dechlorinating Chloroflexi 16S rRNA genes, *Dehalococcoides*-like 16S rRNA genes, reductive dehalogenase (*rdhA*) gene RD14 from *D. mccartyi* strain CG5 and biphenyl dioxygenase gene *bphA* were estimated with an ABI QuantStudio Flex 7 Real-Time PCR System (Applied Biosystems, Grand Island, NY) in 384 well plate format. The primer sets used for qPCR were 16SUf/16Sur (total 16S rRNA genes), chl3487f/dehal884r (putative dechlorinating Chloroflexi 16S rRNA genes), dhc793f/dhc946r (Dehalococcoides 16S rRNA genes), CG5-14F/CG5-14R (*D. mccartyi* strain CG5 *rdhA* gene), and bph463f/674r (*bphA*

genes)(Table S1). Each 20 µL qPCR contained 10 µL Power SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA), 0.5 µg bovine serum albumin (to alleviate the effects of PCR inhibitors), qPCR primers (concentration range 0.1 – 1 µM) and either standard curve templates, sample DNA templates (mass range 1 – 10 ng)(Table S2), or no template in the case of NTCs.

The qPCR standard curves, prepared for each 384 well plate, were constructed by adding known amounts of standard DNA template over a linear range between 30 – 3×10^9 gene copies, depending on the gene target (Table S2). The standard curve DNA template for total 16S rRNA gene qPCR was a PCR product amplified from *Burkholderia xenovorans* strain LB400 with the universal 16S rRNA gene primer set 8F/1492R (Table S1). The standard curve DNA template for putative dechlorinating Chloroflexi 16S rRNA gene qPCR was a pCR 2.1-TOPO vector containing a target PCR product amplified with primer set chl3487f/dehal884r (Table S2). The standard curve DNA template for *Dehalococcoides* 16S rRNA gene qPCR was a pCR 2.1-TOPO vector containing a target PCR product amplified with primer set dhc793f/dhc946r (Table S1). The standard curve DNA template for *D. mccartyi* strain CG5 rdhA gene qPCR was a pCR 2.1-TOPO vector containing a target PCR product amplified with primer set CG5-14F/ CG5-14R (Table S1). The standard curve DNA template for *bph*A qPCR was a linearized pCR 2.1-TOPO vector (digested with restriction enzyme HindIII) containing a target PCR product amplified with primer set bph463f/674r (Table S1).

Plates for qPCR (384 wells) were loaded with 20 µL reaction mixtures and placed into the qPCR system for thermocycling. The qPCR thermocycling conditions were as follows: 10 min at 95°C, followed by 40 cycles of 95°C (15 s), and 60°C (1 min), concluding with a PCR product melt-curve procedure, where the temperature was raised from 65°C to 95°C in 0.25°C increments. ABI QuantStudio Real-Time PCR Software (Applied Biosystems, Grand Island, NY) was used to obtain amplification data for standards, samples and NTCs, analyse qPCR standard calibration curve parameters, determine gene copy numbers in unknown samples by analysis of amplification data, and perform qPCR quality assurance and quality control procedures (QA/QC) by analysis of NTC amplification data and melt curve data.

2.6. qPCR QA/QC summary

Amplification in the NTCs was not detected (i.e., Ct undetermined) in the *Dehalococcoides* 16S rRNA, the *rdhA*-RD14, and *bphA* qPCR experiments. Amplification was detected in the NTCs for the putative dechlorinating Chloroflexi 16S rRNA gene and total 16S rRNA gene qPCR experiments. The mean Ct for the total 16S rRNA gene qPCR NTCs was 37.865, and the mean Ct for the putative dechlorinating Chloroflexi 16S rRNA gene qPCR NTCs was 32.162. In both cases, the Ct values in the NTCs were higher than the Ct values for lowest standard used for qPCR (mean Ct = 35.051 for total 16S rRNA gene 30 copy standard and mean Ct = 31.766 for the putative dechlorinating Chloroflexi 16S rRNA gene 30 copy standard). Melt-curve analysis of qPCR products from all five gene targets revealed primarily single peaks with melting temperatures > 77°C, indicating that primer dimer formation and non-specific amplification was minimized. Details of the qPCR QA/QC can be found in the qPCR dataset deposited in the Iowa Research Online (IRO) institutional data repository under the following DOI: 10.25820/data.006142.

2.7. High-throughput Illumina 16S rRNA gene amplicon sequencing

Replicate DNA extracts from the 27 sediment samples were subjected to partial 16S rRNA gene PCR amplification using a bar code containing primer set 515f/806r [7] according to a previously described thermocycling protocol [8]. Sequencing of the 16S rRNA gene amplicons was performed on an Illumina MiSeq benchtop sequencer at the Argonne National Laboratory Next Generation Sequencing Core (Argonne, IL) according to a previously described sequencing protocol [8]. The raw 16S rRNA gene paired-end read fastq sequencing output files for all

54 samples are deposited in the GenBank Sequence Read Archive under BioProject number PRJNA382682. The raw 16S rRNA gene paired-end read fastq were also uploaded onto MG-RAST (www.mg-rast.org). Paired-end reads were merged and sequencing artifacts were removed using the MG-RAST version 4 pipeline [9].

Ethics Statement

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

CRediT Author Statement

Timothy E. Mattes: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Data curation, Supervision, Visualization, Writing – original draft, Writing – review & editing; **Jessica M. Ewald:** Conceptualization, Methodology, Investigation, Visualization, Writing – review & editing; **Yi Liang:** Conceptualization, Methodology, Investigation, Visualization; **Andres Martinez:** Conceptualization, Data curation, Writing – original draft; **Andrew M. Awad:** Methodology, Investigation; **Keri C. Hornbuckle:** Funding acquisition, Project administration, Resources, Methodology, Writing – review & editing; **Jerald L. Schnoor:** Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2021.107546.

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