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Assessment of TCE and chiral PCB Dechlorination Rate, Congener Diversity, and Enantioselectivity in Town Creek, SC, USA Sediment Microcosms

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ASSESMENT OF TCE AND CHIRAL PCB DECHLORINATION RATE, CONGENER DIVERSITY, AND ENANTIOSELECTIVITY IN TOWN CREEK, SC, USA SEDIMENT MICROCOSMS

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ABSTRACT

Polychlorinated biphenyls (PCBs) and trichloroethene (TCE) are ubiquitous contaminants and are recognized as persistent organic pollutants due to their extreme chemical stability. PCBs were manufactured by chlorinating biphenyls that created 209 congeners with various structures, of which 19 are chiral and can exist as a pair of stable atropisomers. PCBs have been known to cause developmental and neurological toxicity in humans and wildlife; they can act as endocrine disrupters, carcinogens, and teratogens. Sangamo Weston Inc. was an industrial plant located near Town Creek in Pickens Country, South Carolina, that manufactured capacitors and used Aroclors 1016 and 1254 as dialectic fluids for the capacitors. Before the plant's closure in 1987, around 220 tons of PCBs were dispensed into Town Creek, which eventually feeds into Lake Hartwell, now recognized as a Superfund site.

My research was conducted with four chiral PCB congeners (149, 136, 95, and 91) found in the Aroclor 1254 mixture. They were selected due to their diverse enantiomeric concentrations measured in the sediment, water column, and organic matter present in the Lake Hartwell watershed. I used sediment from Town Creek in my microcosms due to the high concentrations of PCBs recorded in that area of the watershed. Town Creek is a unique natural system for PCB research since PCBs and chlorinated solvents (such as tetrachloroethene and TCE) are the main contaminants with no hazardous metal or pesticide influence, making it an excellent natural system to evaluate elevated concentrations of Zn^{2+} and Cu^{2+} and their effect on microbial dechlorination of PCBs.

My research objectives also involved using TCE as a halo-primer for sediment and enriched cultures to compare dechlorination rates and to determine the dechlorination products

when exposing the sediment culture to single enantiomers of PCB 95, separated using HPLC methods.

Elevated zinc and copper concentrations had an effect on the diversity of PCB product congeners, their concentrations, and the enantioselectivity of parent chiral PCBs. Treatments with higher concentrations of these metals resulted in a decrease in PCB product diversity and concentrations. Additionally, the treatments with elevated zinc and copper showed statistically significant changes to the enantiomeric fractions of the parent chiral PCBs, indicating that the metals influenced enantioselective degradation. DNA analysis, amplifying the 16s rRNA gene, showed that zinc and copper concentrations also influenced microbial diversity. The presence of the essential metals decreased overall microbial diversity in the treatments.

The use of TCE as a halo-primer reduced the lag time before PCB dechlorination was observed in both an enriched culture and the sediment microcosms. The enriched culture had a faster dechlorination rate than the sediment microcosms. However, the sediment microcosms had more dechlorination products and terminal products, indicating that there may be fastidious bacterial strains responsible for further dechlorination of PCBs that were absent in the enriched culture that had been grown on only tetrachloroethylene (PCE) for generations prior to my study.

In the individual enantiomer experiment with PCB 95, only the racemic treatments displayed dechlorination products. However, the dechlorination of PCB 95 in the racemic treatments was enantioselective suggesting that the presence of both enantiomers upregulates enzymatic functions or encourages bacterial growth responsible for dechlorination.

My studies served to increase the scientific knowledge on halo-priming as an effective use for stimulating PCB dechlorination, how essential metals influence anaerobic PCB dechlorination, and how single PCB enantiomers biodegrade.

DEDICATION

I would like to dedicate my work to my mother, who has provided me with endless support and wisdom beyond my years. It is an honor to follow in your footsteps and achieve a PhD.

ACKNOWLEDGEMENTS

I would like to thank Dr. Lee for her continued support, guidance, wisdom, and patience with me during the five years I worked on this research. Along with my co-chairs, Dr. Finneran and Dr. Bain, providing me with expertise, encouragement, and valuable questions to further explore areas of my work. And of course, my committee, Dr. Bridges, who has patiently explained statistical analysis to me time and time again. A special shoutout to Dr. Brian Powell who not only helped me with ICP-MS but shared with me the ins and outs of the delicate instrument.

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1. INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Introduction

Polychlorinated biphenyls (PCBs) are a worldwide contaminant that were mass produced primarily in the 1930s and banned in the 1970s. They are recognized as persistent organic pollutants (POPs) due to their extreme chemical stability. PCBs were manufactured by chlorinating biphenyls to create up to 209 congeners with various structures. Of the 209 congeners, 19 are chiral and can exist as a pair of stable atropisomers. Atropisomeric PCBs have restricted rotation around the biphenyl bond that results in non-superimposable mirror images known as enantiomers. Chiral molecules contain a pair of enantiomers which have identical physio-chemical properties and structure but are non-superimposable with their mirror image. Enantiomers are identified by the direction in which they rotate plane-polarized light and can have different biological interactions with macromolecules such as enzymes.

Before being banned in the United States in 1979, PCBs were used in electric and hydraulic fluids, capacitors, transformers, and additives in pesticides, paints, and adhesives (EPA, 2021). PCBs have been known to cause developmental and neurological toxicity in humans and wildlife; they can act as endocrine disrupters, carcinogens, and teratogens (Tam et al., 2023; Andersson et al., 1999; Chu et al., 1998). They especially pose a threat to aquatic ecosystems and are known to bioaccumulate and biomagnify in food chains.

Several natural systems have been co-polluted with PCBs and hazardous metals. In the United States, silver, cadmium, lead, zinc, and copper were found along with PCBs in the Hudson River sediment (Feng et al., 1998). The zinc and copper were determined to come from anthropogenic sources (Feng et al., 1998). Aside from elevated contamination due to anthropogenic sources, zinc and copper are both naturally occurring metals that are found in

most sediment or soils (Huang et al. 2020). Torch Lake, MI, is another location in the US that is co-polluted with heavy metals and PCBs (EPA, 2022). One of the main sources of metal contaminants in the lake was mining of native copper in the area. However, various industrial waste products were also disposed of in or near the lake adding other pollutants such as PCBs, lead, arsenic, and PAHs to the lake sediment (EPA, 2022). New Bedford Harbor is heavily contaminated with PCBs and hazardous metals such as Pb, Cd, Cr, and Cu. Additionally, the copper concentration found in New Bedford Harbor's sediment was significantly higher than average background levels (Shine et al. 1995). Other US sites such as Baltimore and New York Harbor have been extensively analyzed for their hazardous metal concentrations and PCB source distributions (Feng et al. 1998). Since PCBs are a global contaminant, there are several other sites around the world that are co-polluted with metals, such as the Baltic and Mediterranean Sea, Taihu Lake and Guangzhou, China (EEA, 2024; Lang et al., 2018; Xu et al., 2022; Xu et al., 2019). However, little research has been conducted to examine the effects of metal contaminants on PCB in situ degradation.

Many enzymatic reactions rely on essential metals to act as electron donors or acceptors. Additionally, metals can form ligands and react as cofactors for many enzymatic functions. Metals often catalyze reactions that lead to the cleaving of DNA, a crucial role for nucleic acid reactions (Mordasini et al., 2003). Trivalent cobalt is in the center of vitamin B12, which is a known cofactor for reductive dehalogenases (RDases) (Schipp et al., 2013).

The proposed remediation efforts for PCBs are typically dredge-and-treat methods where the contaminated sediment or soil is removed and treated off-site, though most of the contaminated extractions are incinerated or contained in a landfill rather than treated. *In situ* remediation for PCBs remains a challenge given the physio-chemical properties of the molecule

that render it difficult to degrade. However, the bioremediation by fastidious microbial communities provides an opportunity for reductive dechlorination of PCBs *in situ*. Extensive research has been conducted on the microbial communities responsible for the dechlorination of chlorinated contaminants, yet there are still gaps in the literature on enzymatic functions utilized by the bacteria responsible for dechlorination as well as the surrounding microbial community that contributes to the growth of the dechlorinating bacteria.

Among the gaps in the literature, there is less research conducted on the enantiomeric degradation of PCBs *in situ* or with enriched cultures. PCBs were commercially produced as racemic mixtures, meaning equal amounts of both enantiomers are present. However, research has shown that enantiomer fractions of chiral PCBs *in situ* deviate from the racemic mixture, suggesting that the microbial community responsible for dechlorination degrades the PCBs enantiomerically. Along with enantiomeric degradation gaps in the literature, it is unknown whether elevated essential metal concentrations affect dechlorination rates or products.

The goal of my research study was to obtain further understanding of essential metals present in sediment that may affect the microbial community responsible for dechlorinating PCBs. Likely, *Dehalococcoides* are the dominant species among the organohalide-respiring bacteria (OHRB) present in the anaerobic sediment microcosms. *Dehalococcoides* are a fastidious group of anaerobic respiring bacteria in the *Chloroflexi* phylum (Wang et al., 2014). These bacteria have been identified as mainly responsible for the dehalogenation of PCBs and chlorinated organic solvents under anerobic conditions.

However, due to low bioavailability and solubility of PCBs, the biomass of OHRB in natural systems is low. Therefore, halo-priming can be employed as a method to stimulate OHRB growth prior to spiking with PCBs in lab settings. Halo-priming is a method that uses

smaller, more bioavailable compounds with the goal to upregulate enzymes used to degrade a target chemical. The result of the technique is the stimulation of microbial growth that can typically decrease the lag time before any PCB degradation is observed. The chlorinated solvents used for halo-priming are typically tetrachloroethene (PCE) or trichloroethene (TCE). In 2014, a study conducted by Wang et al. successfully isolated three unique reductive dehalogenase genes responsible for dechlorinating PCBs and TCE, which was used as a halo-primer, in *Dehalococcoides mccartyi* strains CG1, CG4, and CG5. Three RDases were discovered from these assays, designated *pcbA1* (found from strain CG1), *pcbA4* (found from strain CG4), and *pcbA5* (found from strain CG5) with *pcbA4* and *pcbA5* sharing 97% amino acid identity. The three strains were observed to remove chlorines predominantly from the *meta*- and *para*-positions but with different specifications with respect to the strain. However, each RDase was upregulated when cultures were spiked with PCE prior to spiking with PCBs. The culture halo-primed with PCE displayed identical PCB dechlorination pathways, indicating there are RDases with dual functions that dechlorinate both PCBs and PCE. Their study supported the methodology behind halo-priming and presented evidence for its effectiveness in reducing the lag time for PCB dechlorination. These specific RDase genes can serve as potential biomarkers for assessing PCB dechlorination activities (Wang et al., 2014).

Sangamo Weston Incorporation was an industrial plant located near Town Creek in Pickens Country, South Carolina, that manufactured capacitors and used Aroclors 1016 and 1254 as dialectic fluids for the capacitors (EPA, 2021). The last two numbers of the Aroclors indicate the chlorine weight percentage composition (i.e. the 1254 mixture contains 54% chlorines by weight). Before the plant's closure in 1987, around 220 tons of PCBs were dispensed into Town Creek, which feeds into Twelve Mile Creek, eventually leading into Lake Hartwell (EPA, 2021).

In 1990, the EPA placed Twelve Mile Creek on a National Priorities List and recognized Lake Hartwell as a Superfund site in 1991 (EPA, 2021).

My research was conducted with four chiral PCB congeners that are found in the Aroclor 1254 mixture, which was the dominant mixture used by Sangamo Weston. PCBs 149, 136, 95, and 91 have been measured in diverse enantiomeric concentrations in the sediment, water column, and organic matter present in Lake Hartwell (Dang et al., 2013; Wong et al., 2001). Throughout my dissertation, the IUPAC names will be used to identify the PCB congeners. I used sediment from Town Creek, which was the receiving water for waste from the Sangamo Weston plant, for my microcosm study due to the high concentrations of PCBs recorded in that area (Dang et al., 2013). Town Creek and the Lake Hartwell watershed are also a unique natural system for PCB research since PCBs and chlorinated solvents (such as PCE and TCE) are the main contaminants with no hazardous metal or other contamination. Most environmental systems with PCB contamination also contain elevated hazardous metals, pesticides, PAHs, or other various halogenated organic compounds. Thus, Town Creek was a model natural system to evaluate various essential metal concentrations and their effect on microbial dechlorination of PCBs. Therefore, I manipulated the concentration of two essential metals, Cu^{2+} and Zn^{2+} (as $CuCl_2$ and $ZnSO_4$), to determine whether there was an effect on the dechlorination rate or products of my target PCBs. Since PCBs often occur as co-contaminants with metals determining the effects of essential, naturally occurring metals such as zinc and copper on PCB dechlorination can provide insight to modifying remediation efforts in locations with the co-pollutants. To reduce the lag time of PCB dechlorination in microcosm studies, I included TCE as a halo-primer.

For another one of my objectives, I compared the dechlorination rates of TCE and PCBs in my sediment microcosms from Town Creek with an enriched culture of *Dehalococcoides* cultivated from the Savannah River Site, referred to as the SRNL culture. The SRNL culture has been growing on PCE for generations and had never been previously exposed to PCBs. Comparing the dechlorination rates between the sediment microbial community and an enriched culture can provide insight on how halo-priming affects natural systems. Additionally, using the SRNL culture that has not been exposed to PCBs prior to this study, can help determine if there are bifunctional enzymes or bacterial strains responsible for TCE and PCB dechlorination.

My final research objective quantified the dechlorination products when exposing the anaerobic sediment culture to single enantiomers of PCB 95. The separation of enantiomers using HPLC methods is rare in the literature since the process is tedious. Spiking sediment microcosms with individual enantiomers of PCB 95 can provide insight into the enantioselectivity of the environmental microbial community present at Town Creek.

1.2 Research Objectives

The overall goal of my study was to determine if essential metal concentrations affect the dechlorination rate of TCE and PCBs in the mixed microbial community found in Town Creek, SC, USA sediment. Additionally, I determined an enantioselective pattern for the dechlorination of PCB 95. To accomplish my goals, I had three main objectives that build on one another to further understanding of PCB dechlorination in sediment microbial cultures.

Objective 1: Halo-priming microcosm study with enriched *Dehalococcoides* culture compared to sediment culture

The intention behind this design was to contrast the dechlorination rate of PCBs in a halo-primed enriched culture (named SRNL throughout my dissertation) to that of a mixed

microbial community collected from contaminated sediment. The aim was to illustrate how environmental systems differ from controlled laboratory microcosms. My hypothesis was that the enriched culture would dechlorinate both TCE and PCBs at a faster rate than the sediment treatments.

Objective 2: Testing the effects of elevated essential metal concentrations on the dechlorination products and enantioselectivity of chiral PCBs

The goal was to use an anoxic media combined with sediment collected from Town Creek to observe the dechlorination rate of TCE and PCBs. Halo-priming from the first objective was deployed in this study. Another goal was to manipulate essential metal conditions of Cu^{2+} and Zn^{2+} in the media designed to improve the growth of *Dehalococcoides* anerobic respiring bacteria (Swamy, 2021). For this objective, I did not inoculate any enriched culture into the media. Only the microbial community collected from the Town Creek sediment was used in the microcosms. My hypothesis was that elevated metal concentrations would inhibit dechlorination activity for both TCE and PCBs.

Objective 3: Sediment microcosm study with PCB enantiomers

The goal was to use enrichments from my previous sediment microcosms collected from Town Creek to observe the effects that separated enantiomers had on dechlorination rates. The focus was on identifying the changes in enantiomeric fractions (EFs) from dechlorination of the separated enantiomers. Specifically, PCB congener 95 was used given a prior study that developed HPLC methods to separate the enantiomers from the commercial, racemic product. I hypothesized that the (-) enantiomers would exhibit a faster dechlorination rate than the (+) enantiomers similar to a previous study by Yu et al. (2018).

2. LITERATURE REVIEW

2.1 Prior PCB Studies in Lake Hartwell Watershed

Using passive water samplers at Town Creek, upstream of Lake Hartwell, Dang et al. (2013) collected enantiomeric data that demonstrated PCB 95 and PCB 91 had contrasting behaviors due to their differences in the water column. PCB 91 was non-racemic in the low-density polyethylene (PE) passive samplers, but PCB 95 was almost entirely racemic (Dang et al., 2013). Figure 2.1 displays the various locations sampled in Town Creek by Dang et al. (2013) and their proximity to Lake Hartwell. Locations D6-D8 contained the highest concentrations of PCBs at around 3000 ng/g of the PE samplers (Dang et al., 2013); therefore, these locations were ideal to sample for my projects.

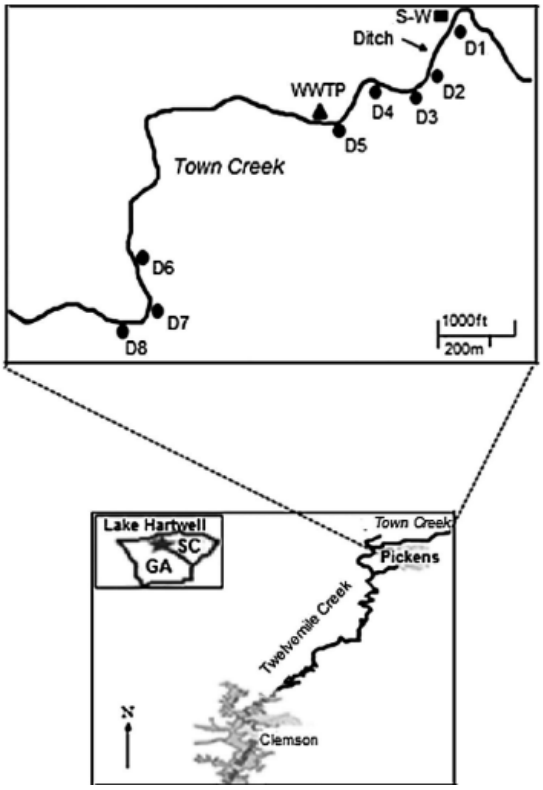

Figure 2.1. Town Creek locations that were previously sampled and analyzed for enantiomeric fractions. Not all locations were sampled, but higher concentrations of PCBs were detected in downstream locations D6-8 so those locations were sampled for this study. Dang, et al. (2013).

Another study conducted by Dang et al. (2010) collected enantiomeric data from various types of organic matter present in Twelve Mile Creek, the receiving stream for Town Creek. Asian clam, mayflies, yellowfin shiner, fine benthic organic matter, coarse particulate organic matter, periphyton, and semipermeable membrane devices were collected to determine the enantiomeric fractions (EF) of six chiral PCB congeners (84, 91, 95, 136, 149, and 174) (Dang et al., 2010). Non-racemic enantiomeric fractions were recorded for congeners 91, 136, and 149 in the water column as well as for all the

organic matter sampled (Dang et al., 2010). Contrastingly, PCB 95 was once again found to be racemic in the water column but was in non-racemic enantiomeric fractions in the organic matter (Dang et al., 2010). Non-racemic composition of the PCBs observed in the study in the basal (organic matter types) food web of Twelve Mile Creek suggested evidence of microbial processes.

Another study previously explored the enantiomeric composition of chiral PCBs present in Lake Hartwell sediment (Wong et al., 2001). Sediment cores as well as surficial samples were taken from the Twelve Mile Creek arm of Lake Hartwell (Wong et al., 2001). Results showed a range of enantiomeric ratios, which were used rather than enantiomeric fractions, for PCBs 149, 136, 91, and 95, depending on the location along the arm of the lake that was sampled. The (-) enantiomer eluted first for PCBs 149 and 136. At the time of the study, the elution order for PCBs 95 and 91 was unknown.

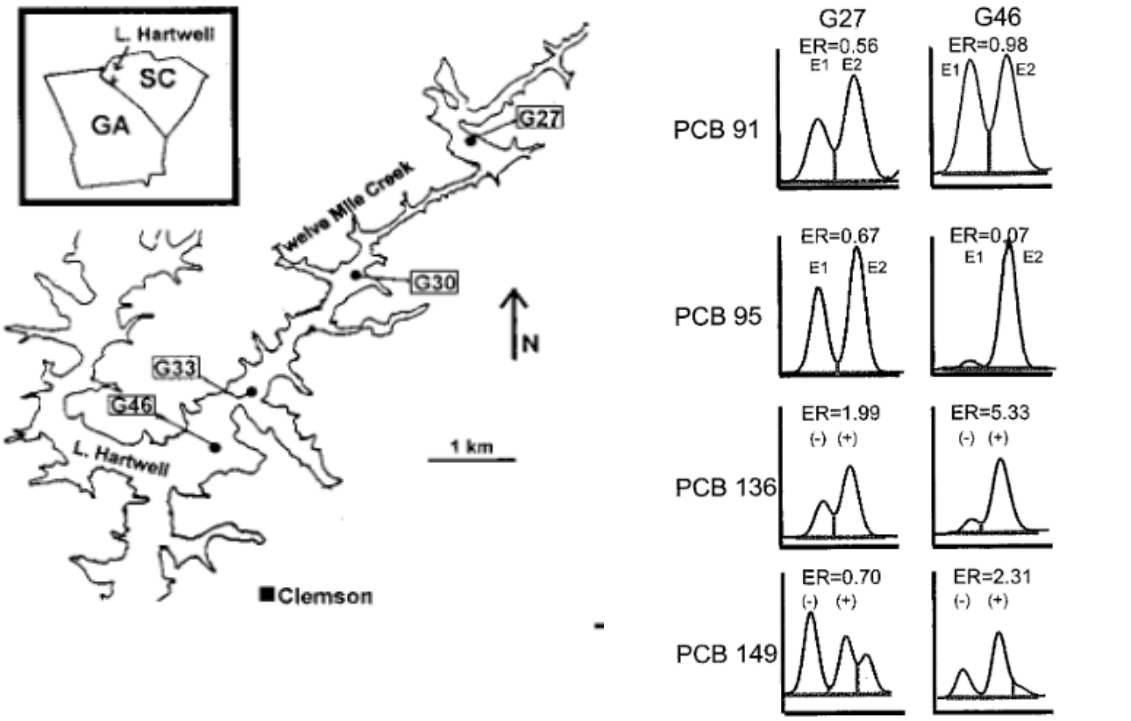

Figure 2.2. Locations along the Twelve Mile Creek arm of Lake Hartwell where core sediment samples were taken and analyzed for the enantiomeric ratios displayed by the chromatographs on the right (Wong et al., 2001).

The variation in enantiomeric ratios and fractions throughout the Twelve Mile Creek implies that in situ enantioselective reductive dechlorination was transpiring. Likely, these bioprocesses were occurring due to diverse, anaerobic, sediment microorganisms.

A modeling study conducted in 2006 examined core sediment samples collected from Lake Hartwell in 1987 and 1998 to compare PCB congener dechlorination and distribution (Bzdusek et al., 2006). The authors applied positive matrix factorization to the data collected to determine the profile of PCBs sources. Results suggested that the dechlorination congener profiles did not change from 1987 to 1998, but the dechlorinated factor increased from 73% in 1987 to 87% in 1998. The increased dechlorination factor indicated that over time, more PCBs were dechlorinated, but the congeners that resulted from the dechlorination in 1998 were no different from the congeners collected from the sediment in 1987. The study also explored

anaerobic dechlorination profiles to help determine the pathway in which the chlorines were removed from the biphenyl ring (Bzdusek et al., 2006). They found that process M + Q was most preferred by the microbial community responsible for dechlorinating the PCBs. Process M is either a flanked or unflanked meta dechlorination and process Q is flanked or unflanked para dechlorination (Bedard et al., 2006). However, process LP was found to be needed to dechlorinate higher chlorinated congeners such as hexa-chlorinated biphenyls. Process LP indicated that the para position is flanked and dechlorinated. This process resulted in dechlorination of PCB 149 to PCB 95, which was the favored dechlorination pathway used by the microbial community in Lake Hartwell (Bzdusek et al., 2006).

Pakdeesusuk et al. (2003) used sediment from the Twelve Mile Creek arm of Lake Hartwell for a microcosm study. The focus of their study was to determine if the microbial community present in Lake Hartwell sediment were capable of reductive dechlorination and whether that degradation was enantioselective. Therefore, the PCB congeners used in this study were 132 and 149, two chiral congeners. Triplicate microcosms were prepared for each of the congeners. Sediment slurries were prepared in an anerobic chamber containing an atmosphere of 98% N_2 and 2% H_2. The sediments were homogenized and mixed thoroughly with a sulfide-reduced mineral media before being dispensed into amber serum bottles and purged with nitrogen/carbon dioxide gas mixture (70/30). The microcosms were spiked with either a concentrated solution of PCB 132 or PCB 149 dissolved in acetone to reach a final concentration of 500µg/g (Pakdeesusuk et al., 2003). Killed controls were prepared the same way but were autoclaved for three consecutive days prior to spiking with PCBs. Headspace samples were taken to observe any methane production and H_2 concentration. Extractions (3-5g) were taken periodically to analyze PCB degradation.

Achiral gas chromatography was used to determine the concentration of PCB 149, 132, and their dechlorination products (Pakdeesusuk et al., 2003). Chiral gas chromatography was used to determine enantiomeric fractions of PCBs 149, 132, 91, and 95. Results showed a decrease in PCB 132 (2,2',3,3',4,6'-hexachlorobiphenyl) and an increase in its dechlorination products, PCB 91 (2,2',3,4',5-pentachlorobiphenyl) and PCB 51 (2,2',4,6-tetrachlorobiphenyl), which was a dechlorination product of PCB 91. These products indicated a preference for meta dechlorination (Pakdeesusuk et al., 2003). After a lag period of around 60 days, dechlorination was seen in microcosms containing PCB 149 (2,2',3,4',5',6-hexachlorobiphenyl). PCB 95 (2,2',3,5',6-pentachlorobiphenyl) was formed from para dechlorination to PCB 53 (2,2',5,6'-tetrachlorobiphenyl) and then to PCB 19 (2,2'6-trichlorobiphenyl). PCB 19 is the terminal product since there are only ortho chlorines present, which is unlikely to be further dechlorinated from anaerobic reductive dehalogenation. Meta dechlorination for PCB 149, which would have produced PCB 91, was not detected indicating that the meta pathway for PCB 149 was not favorable for the microbial community observed in the sediment from the Twelve Mile Creek arm (Pakdeesusuk et al., 2003). These results were similar to the later modeling conducted by Bzdusek et al. (2006) that showed that Process LP (flanked para dechlorination) was most favorable in Lake Hartwell.

Throughout the duration of the experiment by Pakdeesusuk et al. (2003), racemic concentrations were observed for parent congeners, PCBs 149 and 132, implying that their dechlorination did not occur in an enantioselective manner. However, dechlorination products from PCB 149, PCB 91 and 95, were observed to be in non-racemic concentrations. As the experiment progressed, the enantiomeric fractions of both product congeners, PCB 91 and 95, deviated further from 0.5, indicating that the enzymes responsible for dechlorinating the two

product PCBs behaved in an enantioselective manner (Pakdeesusuk et al., 2003). The gas chromatography (GC) elution order for PCBs 91 and 95 were unknown at the time, so no conclusions were drawn on the favorability of the (+) versus the (-) enantiomers for dechlorination. The work by Pakdeesusuk et al. (2003) suggested that the enzymes responsible for PCB dechlorination can have enantioselective properties depending on the substitution pattern and number of chlorines present on the biphenyl rings.

2.2 Synergistic Microbial Communities for *Dehalococcoides*

Dehalococcoides are known as primitive bacteria since they lack various genetic codes for synthesizing enzymes used in their metabolism. Instead, they contain mechanisms to absorb the required nutrients from the surrounding environment. Therefore, *Dehalococcoides* rely on a synergistic microbial community network to supply them with important co-factors or nutrients they cannot synthesize themselves.

In past research, methanogens were seen as antagonists for reductive dechlorination of organic pollutants since they use available hydrogen sources for their own metabolism (Yang et al., 2000; Heimann et al., 2007; Antoniou et al., 2019). However, recent studies have shown that methanogens are vital for maintaining a functional microenvironment balance and may supply *Dehalococcoides* with imperative co-factors for their metabolism (Yuan et al., 2021; Wang et al., 2019). A study focused on *Methanosarcina barkeri* determined that these methanogens may release the co-factor F430, which significantly reduced the activation barrier and lag time of dechlorination, indicating that methanogens may be vital for reductive dechlorination of organohalide contaminants (Yuan et al., 2021). Additionally, the importance of *Methanosarcina* was evaluated using enriched cultures of *Dehaloccocoides mccartyi* strains CG3 and SG1 with or without the presence of *Methanosarcina.* With lactate as the electron donor, *Methanosarcina*

played an important role in converting lactate to acetate to serve as a carbon source, and provided H_2 for the dechlorinators (Wang et al., 2019).

Fermentative bacteria are vital for TCE and PCB dechlorination since they produce the hydrogen needed for reductive dechlorination. In natural systems, hydrogen is typically 'locked' in an organic molecule such as lactate, acetate, or glucose, and fermenters convert these molecules into hydrogen that the dechlorinating bacteria use for reductive dechlorination (Delgado et al., 2016). Previous research also suggested that fermenters can provide *Dehalococcoides* with a carbon source such as acetate, and enzymatic cofactors, such as cobalamins (Fang et al., 2017). Fermentative bacteria are largely responsible for producing vitamin B12, which is a known imperative cofactor for *Dehalococcoides* (Fang et al., 2017).

Sulfate-reducing bacteria (SRB) are often seen as competitors to dechlorinating bacteria since they also use hydrogen as an electron donor. However, SRBs can be beneficial for the dechlorination of TCE or PCBs since the bacteria can supply a carbon source in the form of acetate to the dechlorinators (Ding et al., 2018). Prior studies indicated a link between reductive dechlorination and sulfate reduction because Matturo et al. (2019), for example, noted inhibition of PCB dechlorination once all sulfate was consumed. The inhibition for PCB dechlorination was likely due to the formation of hydrogen sulfide, which can be toxic to dechlorinators. Prior research indicated that *Desulfovibrio*, a SRB, may play an important role for supporting OHRB by supplying them with acetate as a carbon source and cobalamin cofactors (Wang et al., 2019).

Metal reducers are important for *Dehalococcoides* since they can provide vitamin B12 and iron-sulfur clusters have been identified as vital cofactors for reductive dechlorination (Schipp et al., 2013). Since *Dehalococcoides* lack genes to synthesize cytochromes or quinones, they use the iron-sulfur cluster to transfer electrons from hydrogenase to RDase, the terminal

electron sink (Löffler et al., 2013). In the natural environment, iron often exists as Fe_2O_3 with an oxidation state of Fe^{3+}, but to form the iron-sulfur cluster, iron must be in an oxidation state of Fe^{2+} (Ayala-Castro et al., 2008). Iron-reducing bacteria can enhance the ability of dechlorinating microorganisms by driving the Fenton reaction to produce Fe^{2+} (Zhong et al., 2024). *Firmicutes*, a phylum containing metal reducing bacteria, can tolerate higher concentrations of hazardous metals and may be vital for reducing Fe^{3+} in contaminated systems (Burkhardt et al., 2011). Additionally, there have been some metal reducers such as *Geobacter* and *Shewanella* that have been identified to also deahlogenate chlorinated solvents, PCE and TCE (Sung et al., 2006; Zhong et al., 2024).

2.3 Halopriming PCB Cultures

Chen and He (2018) explored the idea of halopriming a culture of *Dehalococcoides* using chloroethenes such as tetrachloroethene (PCE) and trichloroethene (TCE). *Dehalococcoides* is a genus of bacteria under the phylum *Chloroflexi* that has been identified as one genus of the OHRB mainly responsible for the dehalogenation of organic compounds under anerobic conditions (Chen and He, 2018). Halopriming is a technique used to stimulate microbial growth to decrease the lag time before any PCB degradation is observed.

Treatment groups were composed of cultures containing specific strains of *Dehalococcoides*, either CG1, CG4 or CG5, and were pre-grown using PCE or TCE at concentrations ranging from 0.1 mM to 0.7 mM (Chen and He, 2018). Cultures were prepared in 160 mL serum bottles amended with an anaerobic salt media and supplemented with acetate as the carbon source and H_2 as the electron donor (Chen and He, 2018). After extensive bacterial growth was observed, cultures were spiked with Aroclor 1260 to reach a final concentration of 8.1 µM in the serum bottles.

Headspace samples were taken to observe the degradation of PCE and TCE using gas chromatography with a flame-ionization detector (FID) for TCE detection and an electron-capture detector (ECD) for PCB detection. Rapid dechlorination of PCB 180 (hepta-chlorinated biphenyl) was observed within one week in treatments halo-primed with at least 0.3 mM of either PCE or TCE, although, PCB dechlorination was not observed until concentrations of TCE or PCE were below 0.3 mM (Chen and He, 2018). Likely, it was more energy efficient for the microbial community to dechlorinate TCE and PCE, so PCB dechlorination was not favored until the concentration of PCE and TCE were below the 0.3 mM threshold. In previous studies with sediment cultures, such as Pakdeesusuk et al. (2003), PCB dechlorination was not observed till after 60 days of incubation. With Chen and He (2018), PCB dechlorination was recorded within 3 hours after spiking the treatment groups with Aroclor 1260. The average number of chlorines per biphenyl for each treatment group dropped from 6.38 to 5.8 after one week (Chen and He, 2018). Their work suggests pre-cultivation with TCE or PCE accelerated the onset of PCB dechlorination activity.

In addition to observing the decrease in chlorines, Chen and He (2018) also analyzed reductive dehalogenase (RDase) gene expression for one of the known RDase, *pcbA4*. Real time q-PCR analysis showed *pcbA4* gene expression in CG4 cultures pre-grown with PCE or TCE. Peak concentrations of *pcbA4* occurred 48 hours after dosing with PCE or TCE. The amendment of Aroclor 1260 did not show any significant increase in *pcbA4* gene expression as compared to spiking with PCE or TCE (Chen and He, 2018). The lack of increase could explain why halopriming is effective in stimulating PCB dechlorination since amending cultures only with PCBs did not have much effect on increasing *pcbA4* gene expression.

A previous study conducted in 2006 developed sediment-free enriched anaerobic cultures containing *Dehalococcoides* with the goal to observe the dechlorination processes of Aroclor 1260 (Bedard et al., 2006). Aroclor 1260 was a commercial mixture that was the most highly chlorinated, containing 60% of chlorines by weight. Sediment samples containing Aroclor 1260 were sampled from the Housatonic River in Lenox, MA. The authors transferred sediment slurries around five to eight times into a sulfide-free medium containing bicarbonate buffer to achieve sediment-free microcosms and maintain a pH around 7.0. The microcosms were prepared in an anerobic chamber in an atmosphere containing 95-97% N_2 and 3-5% H_2. Acetate and formate were used as carbon sources as well as electron donors. The researchers found that the media including formate were no faster at dechlorinating PCBs; therefore, after subsequent transfers to obtain sediment-free cultures, only acetate was used with H_2 to serve as the sole carbon source and electron donor, respectively (Bedard et al., 2006). They used bromobiphenyl as a halo-primer at 350µM. The medium was amended with Aroclor 1260 at various concentrations ranging from 5 µg/mL to 500 µg/mL. Treatments amended with higher concentrations of Aroclor 1260 showed signs of 4-5 times faster dechlorination rates than those with lower Aroclor 1260 concentrations. Since the media was sediment-free, Bedard et al. (2006) created a PCB-acetone-silica mixture to increase the surface area for deposition of PCBs and make these compounds more readily available to the bacteria. PCBs were first dissolved in acetone, then 300mg of ~240 mesh silica was added. The mixture was rotated to coat the walls and bottom of the serum bottles before the acetone was evaporated using a steady stream of N_2 gas, leaving behind a PCB-silica compound (Bedard et al., 2006).

The researchers used GC with mass spectrometry (MS) to quantify dehalogenation of PCBs and bromobiphenyl. They observed Process N, primarily flanked meta dechlorination, to

be the dominant dechlorination process used by the enriched culture. Very few para dechlorinations were observed, and no ortho dechlorinations were recorded (Bedard et al., 2006). DNA was extracted and amplified using PCR, specifically with the 16s rRNA gene. Analysis showed that the most abundant bacteria in the PCB-dechlorinating culture were members of the *Bacteriodales* (27-32%) order and *Dehalococcoides* only constituted about 13-18% of the anaerobic culture (Bedard et al., 2006). Since *Dehalococcoides* rely heavily on their environment and other microbial communities to supply them with nutrients, such as vitamin B12, it is not surprising that their percentage was lower. Obtaining an entirely pure culture of *Dehalococcoides* is unrealistic due to their metabolic needs. After 110 days of incubation at 22 to 24°C, around 76% of the hexa- through nonachlorobiphenyls in Aroclor 1260 were dechlorinated to tri- through pentachlorobiphenyls (Bedard et al., 2006). Data showed that there was well over 50% dechlorination of PCB 136. With more than 50% transformed to a product congener, the evidence provided suggested that both enantiomers were dechlorinated (Bedard et al., 2006). The researchers did not separate the enantiomers, so it is unknown if they dechlorinate at the same rate. The researchers suggested that steric hinderance from the four ortho chlorines would block access of the dehalogenase enzyme response for dechlorination (Bedard et al., 2006). However, the mole percentage of PCB 136 dropped from 1.56% to 0.14% indicating that there was no steric hinderance, and that it is unlikely there is an enantiomeric selectivity of the dehalogenase responsible for dechlorinating PCB 136.

2.4 Enantioselective PCB Dechlorination

A study conducted by Yu et al. (2018) observed the enantioselective dechlorination of chiral PCBs by *Dehalococcoides mccartyi* strain CG1. Researchers used both *in vivo* and *in vitro* experiments with living cells and crude cell extracts to draw comparisons and conclusions (Yu et

al., 2018). PCBs 174 (2,2',3,3',4,5,6'-heptachlorobiphenyl), 149, and 132 are all chiral congeners that are present in Aroclor 1260 and were followed to observe the enantioselectivity of *D. mccartyi* CG1, which mainly removed flanked meta chlorines (Process N). In living cells, the researchers found that meta chlorides were preferably removed from the (-) enantiomers of two of the spiked, parent congeners, PCBs 174 and 149 to form the (-) enantiomer of the product congener, PCB 91. Contrastingly, meta chlorides were removed from the (-) enantiomer of the parent congener, PCB 132, to form the (+) enantiomer of the product congener, PCB 91. Overall, the (-) parent enantiomer was favored for dechlorination, but the product enantiomers differed depending on the parent compound (Yu et al., 2018).

Analyzing the crude cell lysates, chlorides were removed solely from the (-) enantiomer of PCB 174, producing only the (+) enantiomer of PCB 149. After around 24 h of incubation, the enantiomeric fraction for PCB 149 gradually decreased, indicating that over time more of the (-) enantiomer accumulated. Further dechlorination displayed accumulation of the (-) enantiomer of PCB 91 (Yu et al., 2018). For PCB 132 dechlorination, the (-) enantiomer was favored, yet the first 15 minutes of incubation showed that the (+) enantiomer of PCB 91 was produced. Steadily the enantiomeric fraction decreased, indicating that more of the (-)-PCB 91 enantiomer was produced over time. Contrastingly the Pakdeesusuk et al. (2003) study found racemic concentrations of PCB 132. Yu et al. (2018) theorized that the reductive dehalogenase enzyme, *pcbA1*, had greater affinity for the (-) enantiomers of PCBs 174, 149, and 132 since *pcbA1* had been identified as a predominant enzyme responsible for dechlorination in *D. mccartyi* CG1 (Yu et al., 2018).

2.5 Identification of Reductive Dechlorinase Enzymes

In 2014, a study conducted by Wang et al. successfully isolated three unique reductive dehalogenase genes responsible for dechlorinating PCBs in *Dehalococcoides mccartyi* strains CG1, CG4, and CG5 (Figure 2.3). The three strains were observed to remove chlorines predominantly from the meta and para positions but with different specifications with respect to the strain. Strain CG1 dechlorinated the 33' meta positions more favorably while strain CG4 was found to favor the 44' para position before dechlorinating the 55' meta. Strain CG5 showed the most extensive dechlorination removing primarily from both the 33' and 55' meta positions (Wang et al., 2014).

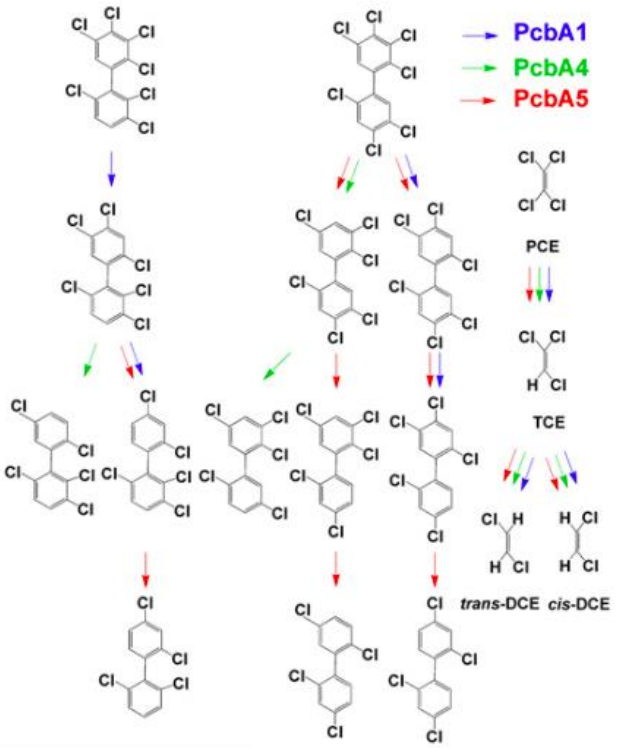

Figure 2.3. The three RDase genes characterized from Wang et. al. 2014's experimental study and the positions they favor for chlorine removal. Bedard, D. (2014). *PNAS*, *111*, 11919-20.

To identify the genes responsible for PCB and PCE dechlorination, the researchers used transcriptomic (PCE-fed enriched cultures) and meta-transcriptomic (PCB-fed mixed cultures) analyses as well as qPCR (PCE- and PCB-fed enriched cultures) using specific primers for each *rdhA* gene. In vitro assays were conducted with crude cell lysates from PCE fed cultures to help further confirm PCB dechlorination occurred from the same reductive dehalogenase enzymes. Three RDases were discovered from these assays, designated *pcbA1* (found from strain CG1), *pcbA4* (found from strain CG4), and *pcbA5* (found from strain CG5) with *pcbA4* and *pcbA5*

sharing 97% amino acid identity (Figure 2.3). These specific RDase genes can serve as potential biomarkers for assessing PCB dechlorination activities (Wang et al., 2014).

2.6 PCB Dechlorination with Hazardous Metals

Lu et al. (2020) used an enriched culture of *Dehalococcoides mccartyi* strain CG1 to understand the detailed impact of metals on organohalide respiration. They tested the effects of four metal ions (i.e., $Cu^{2+,}$ Cd^{2+}, Cr^{3+} and Pb^{2+}) as well as their mixtures on reductive dechlorination of PCE and PCBs. Essential metals, especially divalent ones, are essential to many enzymatic reactions. Metals can act as electron donors or acceptors, and they play a critical role in nucleic acids by catalyzing reactions leading to the cleavage of DNA. Additionally, metals can bind to ligands that can then react as cofactors for many enzymes. For example, trivalent cobalt is in the center of vitamin B12, which is a known cofactor for RDases. PCB 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) a non-chiral congener present in Aroclor 1260, as well as PCE were used as electron acceptors by Lu et al. (2020). Results displayed in Figure 2.4 show that the inhibitive impact on organohalide respiration depended on both the type and concentration of metal ions (Lu et al., 2020).

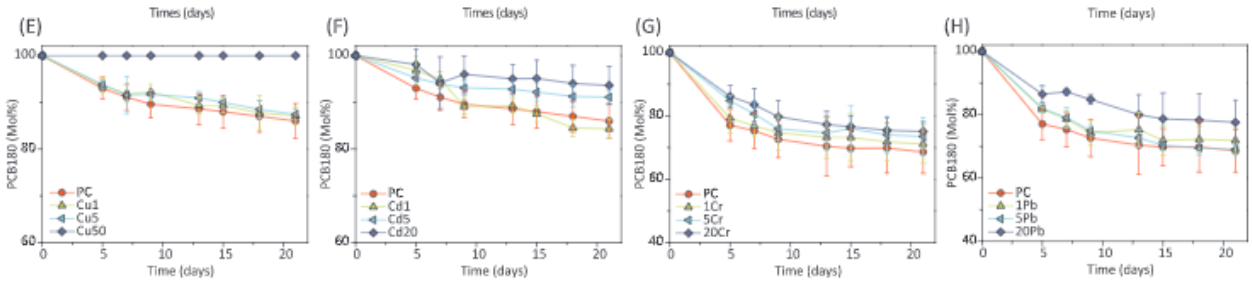

Figure 2.4. The mole percentage decrease of PCB 180 over the course of 20 days at different concentrations (mg/L) of the metal ions. From left to right: Cu^{2+}, Cd^{2+}, Cr^{3+}, and Pb^{2+}.

2.7 Identification of Imperative Co-factors for RDases

Schipp et al. (2013) ran a series of cultivation experiments testing for the vitamin and cofactor requirements for reductive dehalogenase enzymes present in the enriched culture of *Dehalococcoides* strain CBDB1. The bacteria strain genome was analyzed for encoded proteins annotated to contain or interact with organic cofactors and their expression. Additionally, sequencing the genome showed a high number of *rdh* (reductive dehalogenase homologous) genes, which can suggest their pivotal role in the metabolism of these microorganisms. Schipp et al. (2013) noted that the key enzyme responsible for catalyzing reductive dehalogenation reactions in organohalide respiring organisms is a membrane-associated protein, *RdhA* (reductive dehalogenase homologous A). The protein contained a corrinoid in the active center, two iron-sulfur clusters, and a twin-arginine-transport signal peptide. This peptide indicated that the protein is transported through the membrane and is likely attached to the outside of the cell (Schipp et al., 2013). The corrinoid is assumed to be located at the active site of the enzyme due to the ability of cobalamins to catalyze dehalogenation in the presence of electron donors. The authors also discussed prior literature published on *Dehalococcoides* genomes that suggested the use of corrinoids, thiamine, biotin, pyrodoxal-5-phosphate, flavin nucleotides and molybdopterin as enzyme-bound organic cofactors and the soluble cofactors tetrahydrofolate, S-adenosyl methionine and pantothenate/CoA in *Dehalococcoides* strains (Schipp et al., 2013).

One of the cultivation experiments surveying vitamin requirements for *Dehalococcoides* strain CBDB1 growth removed cyanocobalamin (a synthetic form of vitamin B12) from the medium, which resulted in a strong decrease in cell count, confirming that vitamin B12 is an essential vitamin for the growth of strain CBDB1 and likely all other *Dehalococcoides* strains (Schipp et al., 2013). The addition of cobalt chloride to the media did not promote cell growth,

indicating that cobalt alone, present in the center of vitamin B12, was not enough to facilitate bacterial growth. Another cultivation experiment focused on the presence or absence of biotin. Media not containing biotin showed negligible growth of *Dehalococcoides* strain CBDB1, indicating that biotin is an essential vitamin for cell growth (Schipp et al., 2013). A similar experiment was done with thiamine, and the results followed the same pattern. The presence of thiamine was vital for *Dehalococcoides* strain CBDB1 growth. However, comparative genome analysis in *Dehalococcoides* showed that cofactor biosynthesis pathways for corrinoids, the corrin ring system, thiamine, and biotin were incomplete (Schipp et al., 2013). Additionally, no gene in the *Dehalococcoides* genome was identified to encode an enzyme catalyzing cobalt incorporation into the corrin ring. Contrastingly, all proteins needed to uptake corrinoids from the environment and the biosynthesis of cobalamin from cobyrinates or cobinamids were encoded in the *Dehalococcoides* genome. The results imply that *Dehalococcoides* required these essential vitamins to be present in their environment to survive (Schipp et al., 2013). Gathered from prior literature, the authors state that *Dehalococcoides* seem to have specialized genomes to take up different precursors with a corrinoid ring structure, and the bacteria modifies the upper and lower ligands depending on their specific needs. As an example of this specialization, it was shown for strain 195 that dehalogenation of TCE was catalyzed by reductive dehalogenases containing 5,6-dimethyl benzimidazole (DMB) as the lower ligand of the cobamide cofactor and that other benzimidazol derivatives could be modified and incorporated into the cobamide to produce active reductive dehalogenases (Schipp et al., 2013).

2.8 Summary of Literature Review

The literature review provides strong evidence of enantioselective dechlorination of PCBs in the Lake Hartwell watershed and *Dehalococcoides* as one of the main genera of bacteria

responsible for the dechlorination of PCBs in the environment. Along with enantiomeric data collected at Lake Hartwell, many studies using enriched culture of *Dehalococcoides* record diverse enantiomeric fractions of PCBs, indicating that these bacteria degrade PCBs enantioselectively. A few RDases have been identified that degrade both TCE and PCBs, indicating that these enzymes may be bifunctional. Also, RDase *pcbA1* has been recorded to preferentially dechlorinate the (-) enantiomer of chiral PCBs. Prior studies have identified *Dehalococcoides* as primitive bacteria that require a synergistic microbial community to supply them with co-factors, vitamins, and electron donors to facilitate growth. It is evident that vitamin B12 is an imperative co-factor for the enzyme metabolism of *Dehalococcoides*. Therefore, I can hypothesize that trivalent cobalt, which is found in the center of vitamin B12, is a crucial essential metal for their organohalide degradation process.

However, little research has been conducted that explores other essential metals such as zinc and copper and how they may affect the bacteria's metabolism or PCB dechlorination. At this moment, there is only one study that considered hazardous metals that would be co-contaminants with PCBs (Lu et al., 2020), but the metal concentrations were significantly higher than would be expected in environmental systems that are not contaminated with metals. Additionally, little research has explored whether the presence of essential metals can impact enantioselectivity or dechlorination rates of chiral PCBs. There are currently no papers on the impact of individual enantiomers on PCB dechlorination rates in sediment microcosms.

3. METHODS AND MATERIALS

3.1 Chemicals

All PCB congeners were purchased from AccuStandard (New Haven, CT, USA). PCBs 149, 91, 95, 102, 96 arrived as 5 mg solids; PCB 136 as a 20 mg solid; PCB 53 as a 25 mg solid; and PCB 54 as a 50 mg solid. PCB 51 arrived as 5 mg dissolved in isooctane. Aldrin, which served as an internal standard for PCB analytic techniques, was also purchased from AccuStandard as a 10 mg solid. Isooctane was purchased from Fischer Scientific in a 4-liter amber glass bottle. Neat trichloroethene (TCE) and cis-dichloroethene (cis-DCE) were purchased from VWR International. Vinyl chloride and ethene were purchased from AIRGAS. Silica, Floated (200 Mesh and Finer) was purchased from Fisher Scientific. Hexane was purchased from Fischer Scientific in a 2.5-liter amber glass bottle. Acetone was purchased from Fischer Scientific in a 4-liter amber glass bottle. For information regarding the chemicals used in the anaerobic media designed to facilitate growth of dechlorinating microorganisms, consult Appendix A in Swamy (2021).

3.2 Stock Solutions

PCBs 102, 96, and 51 were each dissolved in 50 mL of isooctane to create individual stock solutions of 100 mg/L. Stock solutions of PCBs 149, 136, 95, 91, and 19 were created at 1 mM each. Some remaining PCB 19 in the lab was determined to be 0.0024 g, and therefore was dissolved in 9.32 mL of isooctane to get a concentration of 1 mM (257.5 mg/L) (See Appendix for details). Five milligrams of PCBs 149 and 136 were dissolved in 13.85 mL of isooctane to create a 1 mM (360.9 mg/L) solution. The 5 mg of PCBs 95 and 91 were each dissolved in 15.32 mL of isooctane to create a 1 mM (326.4 mg/L) solution. The 10 mg of Aldrin was dissolved in

166 mL of isooctane to create a stock solution of 60.2 mg/L. Twenty-five milligrams of PCBs 53 and 54 were each dissolved in 50 mL of isooctane to create individual stock solutions of 50 mg/L.

Nitrogen was used to concentrate each PCB standard stock solution to 27 mg/L. The contents were diluted by half five times to create a standard curve with concentrations of 27 mg/L, 13.5 mg/L, 6.75 mg/L, 3.38 mg/L, 1.69 mg/L, and 0.844 mg/L. Lastly, 16.6 µL of 60.2 mg/L Aldrin was added to each of the standards using a microliter syringe. This brought the final concentration of Aldrin to 0.04 mg/L in each of the standards. PCB congeners in each solution were 149, 136, 95, 91, 102, 54, 53, 51, and 19. For detailed information on preparing the standard curve solutions, see Appendix I.

Stock solutions of elevated [Zn^{2+}] and [Cu^{2+}] for the Essential Metals Experiment (Objective 2) were created by dissolving 0.038 g of $ZnSO_4*7H_2O$ in 100 mL of Milli-Q water to reach 1.32 mM and dissolving 0.018 g of $CuCl_2*2H_2O$ in 100 mL of Milli-Q water to reach 1.06mM.

A 50mL stock solution of lactate at 237.4 mM was created by removing 1 mL of a 60% lactate syrup purchased from VWR International and diluting it with Milli-Q H_2O to the 50 mL mark on a volumetric flask. A 50 mL stock solution of sodium acetate at 400 mM was created by dissolving 1.64 g of sodium acetate (from VWR International) in Milli-Q H_2O. A 50 mL stock solution of sodium formate at 400 mM was created by dissolving 1.36 g of sodium formate (from VWR International) in Milli-Q H_2O.

Chlorinated solvents and their products were analyzed in the gas phase. Four standard stock solution bottles of trichloroethene (TCE), *cis*-dichloroethene (*cis*-DCE), vinyl chloride (VC), ethene, and methane were created to run a calibration curve on the GC-FID to monitor

dechlorination. Using neat TCE, 0.09 µL were added to a sealed 100 mL Milli-Q H_2O serum bottle using an 18-gauge needle and microliter syringe for a total concentration of 1 µmol of TCE per bottle. To this same bottle, 0.075 µL of neat *cis*-DCE was added using the same methods. Using a gas syringe, 0.05 mL of pure VC, methane, and ethene were added to that serum bottle so all chemicals had a final concentration of 2.09 µmol. It was not possible to obtain smaller than 0.05 mL of pure gas, so 1 µmol for VC, methane, and ethene was unattainable. For the 5 µmol standard bottle sealed with 100 mL of Milli-Q H_2O, 0.45 µL of neat TCE, 0.375 µL of neat cis-DCE, and 0.125 mL of pure VC, methane, and ethene were added. For the 10 µmol standard bottle, 0.9 µL of neat TCE was added along with 0.75 µL neat *cis*-DCE, and 0.25 mL of pure VC, methane, and ethene. For the final standard bottle at 20 µmol, 1.8 µL of neat TCE, 1.5 µL of neat *cis*-DCE, and 0.5 mL of pure VC, methane, and ethene were added.

Stock solutions of individual chiral PCBs for microcosm spiking (10 µL) were created with the intention of having 1.25 mg of PCBs per serum bottle (100 mL of media). Therefore, 20 mg of PCBs 149, 136, 95, and 91 were purchased from AccuStandard and dissolved separately in 160 µL of isooctane for a stock solution concentration of 125,000 mg/L. However, PCBs 136 and 95 would not dissolve after 5 hours of vortexing and were diluted using another 160 µL of isooctane. This brought the final stock solution concentration of PCB 136 to 62,436 mg/L and PCB 95 to 62,669 mg/L.

For the Enantiomer Experiment (Objective 3), a working solution of 100 mg/L of PCB 95 was made by dissolving 5 mg into 60 mL of hexane. HPLC was used to separate the enantiomers with a Chiralcel-OJ column (2.1x150mm, 3 micron, coated polysaccharide phases) and by following the method previously developed by Ranasinghe et al. (2019). Methods were slightly modified to 10-min total runs rather than 8 min due to down shifting of the retention time of each

eluent, see 3.10.3 HPLC Separation of PCB 95 for more information. The final amount of (-)-95 collected was 0.634 mg while the final amount of (+)-95 was 0.589 mg. Both enantiomers were dried using nitrogen gas and re-constituted with 120 µL of isooctane for a stock solution with a concentration of 5,283 mg/L and 4,908 mg/L, respectively. A stock solution of 5,050 mg/L of racemic 95 was created by dissolving 5 mg into 990 µL of isooctane.

3.3 Sediment Collection

Sediment was collected from Town Creek, SC, USA in 2022 at 34°55’58’’N 82°44’8’’W using a 5 grid plot sampling method. The weather was sunny at 63°F with 35% humidity, wind 4 mph NE, and the creek water level was particularly high with a fast current. The coordinates given were taken at the center of the plot, and each of the other four corner samples were taken 8 inches from the center. This particular location was chosen based on previous research conducted by Dang et al. (2013) with the highest concentrations of PCBs at around 3000 ng/g of low-density polyethylene samplers recorded at the location. It was assumed if the water column in this region contained a high concentration of PCBs, then the clay sediment would also have high concentrations of PCBs. Thus, I located an area with minimal rocks and more clay or silt sediment for sampling since PCBs are more likely to absorb onto organic matter than mineral surfaces. Clay and silt are more likely to have organic matter than sand. Additionally, the size of clay and silt particles result in more surface area than sand particles. If there are high PCB concentrations in those sediments, I can be more certain that the fastidious microbial community responsible for dechlorination would be at a greater abundance. Samples were taken using a metal spoon to completely fill up an 8 oz glass jar with a two-part metal lid for sealing. Five total jars were used, once for each plot on the grid. Samples were taken back to the lab and homogenized by thoroughly mixing the sediment with a metal stirrer.

3.4 Media Preparation for Sediment Microcosms

Media for the sediment microcosms was prepared using an anoxic media recipe and dispensed into 30 160 mL serum bottles (Swamy 2021; Appendix A). Instead of 90 mL of media added to each serum bottle, 98 mL was added since no culture was going to be added. No yeast extract, ferrous chloride, sodium sulfide, TCE, or lactate were added to the media prior to autoclaving and sparging. The bottles were sealed with a blue rubber butyl stopper and aluminum crimp cap before autoclaving on a 20-minute liquid cycle, then stored at 4°C till sediment was collected and homogenized. Serum bottles were opened and around 10.1-10.3 g of wet sediment were dispensed into each bottle using a powder funnel. Each serum bottle was then sparged using a gas mixture of 80:20 N_2:CO_2 for 30 minutes with the canula placed at the bottom of the bottles. Afterward each serum bottle was sealed with the blue rubber butyl stopper with the canula still inside, and the canula was brought to the headspace to sparge the headspace for 6 minutes before removing and fully sealing the serum bottles with aluminum crimp caps.

Once fully sealed, yeast extract, ferrous chloride, sodium sulfide, and lactate were all added to the bottles in the concentrations given by the media recipe (Swamy 2021; Appendix A). Neat TCE was spiked into the treatment bottles using a microliter syringe with 1.8 µL to reach a concentration of 20 µmol per bottle. A milliliter of 1 M sodium lactate was added, bringing the final volume of the treatment bottles to 100 mL with the final lactate concentration of 10 mM per bottle. Each bottle was vortexed for 10 sec to help distribute the final components added, then left stagnant at 25°C for 48 h to allow TCE to partition between the liquid and gas phase prior to sampling Day 0 concentrations.

For killed controls, bottles were autoclaved again on a 20-minute liquid cycle three consecutive times, over the course of three days, to destroy any possible spore production prior

to spiking with TCE and lactate. TCE was added after the three autoclaving events to prevent the bottles bursting in the autoclave and releasing toxic TCE gas. Lactate was added after TCE to prevent any growth of the microbes prior to autoclaving.

3.5 SRNL Culture Preparation

An enriched culture of *Dehalococcoides* was obtained from Dr. David Freedman's lab group (Eaddy, 2008). The culture originated from samples previously taken from a site at the Savannah River National Laboratory in Aiken, SC. Over the years this culture has been growing on PCE or TCE as the electron acceptor with lactate as the donor and has successfully dechlorinated PCE/TCE to ethene. For purpose of my dissertation, the culture will be referred to as the SRNL enrichment culture (Eaddy, 2008).

Six treatment bottles were set up using the anoxic media recipe (Swamy 2021; Appendix A). Each treatment was spiked with 30-50 µmols of neat TCE. The neat TCE was allowed to partition throughout the media and headspace for 24 hours prior to amending the media bottles with culture or lactate. Seven milliliters of the SNRL enrichment culture were added to each of the six serum bottles after 24 hours and day 0 TCE concentrations were recorded on a GC-FID.

These six treatments were monitored for 14 days prior to the addition of PCBs. Since the SRNL enriched culture is sediment-free, a PCB-silica compound was created to make the PCBs more bioavailable by providing a surface for the microorganisms to attach to (Bedard et al., 2006). PCB 136 was spiked into the microcosms because it was one of the hexa-chlorinated chiral PCBs observed in the Town Creek sediment microcosm experiments. Acetone was added directly into a vial containing 14.15 mg of PCB 136 to dissolve it before transferring the solution to a 50 mL volumetric flask. Using more acetone, the solution diluted to the 50 mL line on the flask. To make the solution easy to distribute to six serum bottles, a 10 mL volumetric flask was

used to measure 10 mL of acetone and the solution in the 50 mL volumetric flask was transferred to a beaker and diluted with 10 mL of acetone. This brought the final concentration of the PCB 136 acetone stock solution to 235.8 mg/L.

Using six clean, autoclaved serum bottles, 300 mg of 200 mesh silica were weighed and dispensed into the serum bottles. From the PCB 136 acetone stock solution, 10 mL were pipetted into each of the serum bottles. Unfortunately, due to the lack of speed or the heavy draft of the fume hood, 6 mL of acetone evaporated before all the stock solution was dispensed. Therefore, 14.15 mg of PCB 136 was dissolved in 54 mL of acetone not 60 mL, so the calculated concentration was 262 mg/L. Ten milliliters were dispensed into five of the six serum bottles, while the last bottle received 4 mL. For the 5 bottles there was 2.62 mg of PCB 136 added, and for the one bottle with 4 mL there was 1.05 mg of PCB 136 added. The serum bottles were gently swirled to mix the acetone solution and the silica mesh. Nitrogen gas evaporated the acetone leaving behind a PCB-silica compound. Each bottle was sealed with a rubber butyl stopper and aluminum crimp cap. To ensure the sealed bottle was anoxic, a steady stream of nitrogen gas was injected into the bottles for 6 minutes using a needle while another needle served as a vent.

The six treatment bottles containing the SRNL enriched culture growing on TCE were slowly added to each of the six bottles containing the PCB-silica compound. This was done by using a 10 mL syringe equipped with a needle, first filling it with nitrogen gas and injecting it into the SRNL treatment bottles and then extracting 10 mL of the culture to inject into the sealed serum bottled with the PCB-silica compound. The SRNL treatment bottles were gently shaken prior to each extraction. These steps were repeated until all the culture was transferred into the six serum bottles containing the PCB-silica compound. The bottles were gently rotated before

letting them sit for 30 minutes prior to extracting 1 mL for the day 0 PCB analysis. The calculated final concentration of PCB 136 in the new treatment bottles containing 100 mL of culture was 26.2 mg/L or 72.6 µM in five of the bottles, while the last one (that had 4 mL of the acetone solution) had a final concentration of 10.5 mg/L or 29.0 µM. Three of the bottles were kept stagnant at room temperature (23°C) while the other three were kept in a rotating incubator at 30°C and 100 RPM.

To analyze PCBs in the SRNL enriched culture, each bottle was first inverted twice before extracting 1 mL of media with the silica mesh. The 1 mL was then combined with 1 mL of isooctane in a 2 mL centrifuge tube, vortexed for 5 minutes and centrifuged at 16,000 rpm for 10 minutes. The top, organic layer was removed and dried using a Pasteur glass pipette packed with glass wool and sodium sulfate prior to running on the GC-ECD to determine PCB concentrations. The sample had to be diluted in the GC vial by adding 10 µL of sample and 90µL of isooctane into the GC insert to avoid contaminating the instrument. PCB analysis of the SRNL culture was conducted for 80 days.

3.6 Preparation of Sediment Microcosms for Halo-priming Experiment

The goal of this experiment was to compare the dechlorination rate of chiral PCB 136 in halo-primed treatments of the SNRL enriched culture and the microbial community in the sediment collected from Town Creek. Only three replicates of the sediment microcosms (Section 3.4 Media Preparation for Sediment Microcosms) were used in the halo-priming experiment since they contained PCB 136 and had only the anoxic media with no metal amendments. Table 3.1 displays the treatments created for the Halo-priming Experiment.

Table 3.1. Set-up for Halo-priming Experiment treatments.

	PCB Content	**Replicates**
SRNL Culture	136	6
Sediment Microcosm Culture	136	3

TCE dechlorination was monitored for 179 days in the treatments prior to spiking with 10 µL of the PCB stock solutions. For the final concentrations, see section 3.2 Stock Solutions. TCE analysis was done every two weeks using manual 0.2 mL headspace injections on a 2014 Shimadzu GC-FID with a 30m GS-Q column (Swamy, 2021).

For PCB analysis in the sediment microcosms, the serum bottles were vortexed for 10 sec prior to extracting 0.5 mL and adding it to a 2 mL centrifuge tube with 0.5 mL of isooctane. The centrifuge tubes were then vortexed for 5 min and centrifuged at 16,000 rpm for 10 min. The top, organic layer was removed and dried using a Pasteur glass pipette packed with glass wool and sodium sulfate prior to running on the GC-ECD. The sample was diluted in the GC vial by adding 50 µL of sample and 50 µL of isooctane into the GC insert to avoid contaminating the instrument. PCB analysis of the sediment microcosms was conducted for 389 days.

3.7 Essential Metal Experiment

The purpose of this experimental design was to determine if elevated [Cu^{2+}] or [Zn^{2+}] influenced the dechlorination rate of PCBs or the product congeners produced. Additionally, the enantiomeric fractions (EFs) were determined to see if the dechlorination was enantioselective given the treatment conditions. EFs were calculated using the equations derived from previous work conducted at the Lake Hartwell watershed (Dang et al., 2010). The preparation of the treatment bottles in this experiment was described in Section 3.4 Media Preparation for Sediment

Microcosms. There were three replicate bottles for each treatment and the killed controls if applicable, see Table 3.2.

Table 3.2. Microcosms treatment setup for Essential Metals Experiment.

	PCB Content	**Metal Content***	**Killed Controls**
Treatment 1	Racemic PCB 149 + 136	No metals	N/A
Treatment 2	Racemic PCB 95 + 91	No metals	N/A
Treatment 3	Racemic PCB 149 + 136	10x [Cu^{2+}]	Yes
Treatment 4	Racemic PCB 149 + 136	10x [Zn^{2+}]	Yes
Treatment 5	Racemic PCB 95 + 91	10x [Cu^{2+}]	Yes
Treatment 6	Racemic PCB 95 + 91	10x [Zn^{2+}]	Yes

*The metal content column is for additional concentrations of metals. Trace metals were used in every treatment as per the media recipe (Swamy 2021; Appendix A).

To determine how much [Zn^{2+}] and [Cu^{2+}] were present in the anoxic media, the molarity of each compound, $ZnSO_4*7H_2O$ or $CuCl_2*2H_2O$, in each treatment bottle was calculated to be 1.32×10^{-6} M and 1.06×10^{-6} M, respectively. Therefore, for [Zn^{2+}] and [Cu^{2+}] the molar concentration was 1.32×10^{-7} M and 1.06×10^{-7} M, respectively. To achieve 10x this concentration of either zinc or copper in the metal treatments, 1 mL of the stock solution (see 3.2 Stock Solutions) were added to the treatments.

Halo-priming in this experiment was used as a technique rather than a treatment to help facilitate growth of the microbial community responsible for dechlorination. TCE dechlorination was monitored for 179 days in the treatments prior to spiking with 10 µL of the PCB stock solutions. For the final concentration calculations, see 3.2 Stock Solutions. TCE analysis was conducted every two weeks using manual 0.2mL headspace injections on a Shimadzu GC-FID with a 30m GS-Q column (Swamy, 2021).

Each treatment was vortexed for 10 sec after spiking with PCBs to encourage even distribution of PCBs throughout the media and sediment. Treatment bottles sat stagnant at 25°C for 24 h before sampling for Day 0 PCB concentration to allow PCBs to adsorb onto the sediment and partition throughout the media. Half a milliliter samples were taken from the treatments after vortexing for 10 sec and added to 0.5 mL of isooctane for liquid-liquid extraction in 2 mL centrifuge tubes. Centrifuge tubes were then vortexed for 5 min before centrifuging at 16,000 rpm for 10 min. The top, organic layer was removed and dried using a glass Pasteur pipette packed with glass wool and sodium sulfate to remove any remaining aqueous components. Samples were then diluted by one-half before adding to GC vials for analysis. A GC insert was used and 50 µL of sample was added along with 50 µL of isooctane for dilution. A low concentration of 0.04 mg/L Aldrin was added to each GC vial to serve as an internal standard to measure the response factor of the GC. The internal standard served to accurately quantify the concentration of the PCB congener present in the GC vial, which can then be used to calculate the amount in the original sample. For further GC-ECD analyses of achiral and chiral PCB concentrations see 3.10.1 GC-ECD Analysis of PCBs.

For the killed controls, individual PCB congener stock solutions had to be diluted again to re-dissolve the PCBs. Each of the PCB standards was diluted with 160 µL of isooctane before spiking with 10 µL to each killed control serum bottle. For PCBs 91 and 149, the total volume was 230 µL since previously 90 µL was removed from the 160 µL stock solution for the treatments. For PCBs 95 and 136, the total volume was 390 µL since previously 90 µL was removed from the 320 µL stock solution for their respective treatments. Each killed control was spiked with 10 µL of this new stock solution, bringing the final concentration to 3.8 mg/L for

PCBs 91 and 149 and 3.7 mg/L for PCBs 95 and 136 in the 100 mL serum bottles. See Table 3.2 for the microcosm treatments.

Killed controls were not created for treatments without metal amendments due to prior research conducted on microcosms in the Lake Hartwell watershed showing that dechlorination does not occur without a microbial community present (Pakdeesusuk et al., 2003). Killed controls created for the metal amended treatments were analyzed using the same techniques as the treatments; however, controls were monitored less frequently as samples were taken once every three to four weeks since no activity was observed. TCE dechlorination was monitored for the killed controls for 246 days before spiking with PCBs.

3.8 Enrichment of Town Creek Microcosms

After 160 days of monitoring PCB dechlorination in the Town Creek sediment microcosms for the Essential Metals Experiment, it was determined that one bottle with no metal amendment, T17, was exhibiting extensive PCB dechlorination indicative of growth of microbial dechlorinators. Thus, I chose to further enrich the sediment culture in bottle T17, which contained penta-chlorinated biphenyls (95 and 91). Nine bottles containing 20 mL of anoxic media were created and autoclaved in preparation for enrichments (Swamy 2021; Appendix A). After autoclaving, roughly 2 g of wet sediment from Town Creek (Section 3.3 Sediment Collection) was added to each bottle to serve as a sorbent for PCBs, making it easier for the microbial community to interact with the PCBs. The media plus sediment was then sparged for 30 min with 80:20 N_2:CO_2. Then the headspace was sparged for 25 min instead of the usual 6 since the headspace was 4x the normal amount. Then, 20 µL of yeast extract was added to each bottle after sparging. A mixture of TCE only, PCB only, or TCE + PCBs along with various

electron donors (either lactate, acetate, formate, or a combination of them) were used to stimulate further growth, as displayed in Table 3.3.

Table 3.3. The enrichment bottles with the various electron donor or electron acceptor combinations for the Enantiomer Experiment.

	PCB spike only	**TCE spike only**	**TCE + PCBs**
Lactate only		1 enrichment bottle	
Lactate:acetate		1 enrichment bottle	1 enrichment bottle
Lactate:formate			1 enrichment bottle
All three	3 enrichment bottles	2 enrichment bottles	

All electron donors were spiked in a 1:1 format at 10 mM each as the final concentration in the bottles by spiking with either 0.84 mL or 0.5 mL of acetate and formate, respectively, from their respective stock solutions. For the bottles containing TCE, 1.8 µL of neat TCE was spiked for a final concentration of 20 µmol per bottle. For the bottles containing PCBs, 2 µL stock solutions of PCBs 95 and 91 were spiked for a final concentration of 11.6 µM of PCB 91 in the enrichments and 19.2 µM of PCB 95. Electron donors and acceptors were given three days to partition throughout the serum bottles before 0.5 mL of culture from the active sediment microcosm (T17) was extracted and spiked into the bottles. Before each extraction, the treatment bottle was vortexed for at least 10 seconds to ensure the sample would be homogenized. Enrichments were monitored for 70 days using either GC-FID or GC-ECD methods depending if they contained TCE or PCBs, respectively. After 70 days, all enrichments were combined into a 1-liter glass bottle using the Hungate Technique (Hungate, 1969) to serve as the culture needed for the Enantiomer Experiment. After combining, the 1-liter bottle was vortexed for 10 sec prior to dispensing 10 mL of culture into serum bottles for the Enantiomer Experiment.

3.9 Enantiomer Experiment

This study was designed to observe the dechlorination of single PCB 95 enantiomers spiked in the sediment microcosms. The Town Creek Enrichment Microcosms (Section 3.8) were used as the culture in this experiment. Treatment bottles were prepared using an anoxic media recipe; however, the recipe was cut in half since the total volume in each serum bottle was 50 mL instead of the original 100 mL (Swamy, 2021; Appendix A). Treatment bottles were autoclaved after media preparation. From the new combined enrichments, 10 mL of the sediment slurry was used to spike into the treatment and control serum bottles for a total volume of 50 mL. Control bottles were autoclaved three times consecutively to ensure any spore production was sterilized. Serum bottles were then spiked with 5 µL of the PCB 95 stock solutions to bring the final concentration in the treatment bottles to 0.49-0.53 mg/L. Concentrations in this experiment were lower than my previous projects since separating the enantiomers using HPLC was tedious. Microcosms were also amended with 10 mM of lactate to serve as the electron donor. Table 3.4 displays the experimental set-up for each treatment.

Table 3.4. Treatment and control set-up for Enantiomer Experiment. Each treatment and control consisted of three replicates.

	PCB Congener (mg/L per bottle)	**Killed Controls**
Treatment 1	PCB (-)-95 (0.53)	Yes
Treatment 2	PCB (+)-95 (0.49)	Yes
Treatment 3	Racemic PCB 95 (0.51)	Yes

To distribute the spiked PCBs evenly, each treatment was vortexed for 10 sec. Treatment bottles were left at 25°C for 48 h before sampling for Day 0 PCB concentration to allow PCBs to reach a quasi-equilibrium between the sediment and media. After vortexing for 10 min, 0.5 mL samples were taken from each treatment. The collected sample was added to 0.5 mL of isooctane

for liquid-liquid extraction in 2 mL centrifuge tubes. Centrifuge tubes were then vortexed for 5 min before centrifuging at 16,000 rpm for 10 min. The organic layer at the top of the tube was removed and eluted through a glass Pasteur pipette packed with glass wool and sodium sulfate to remove any remaining aqueous media. A GC vial insert was used and 100 µL of sample was added to the GC vials along with 0.04 mg/L of Aldrin to serve as an internal standard. PCB dechlorination was monitored every 2 weeks. Killed controls were monitored less frequently, every 3-4 weeks since no PCB dechlorination was detected.

3.10 Analytical Techniques

3.10.1 GC-ECD Analysis of PCBs

PCB achiral analysis was done using 1µL auto-injections on a 6890 Agilent GC-ECD equipped with a 60 m Rtx-5 column with a 0.25 µm coating and a 1.0 µm diameter. The temperature program started at 160°C then ramped 5°C/min until reaching 185°C then held for 8 min then ramped 2°C until 225°C then held for 2 min before ending the run. Total runtime was 37 min, modified from methods previously created (Sivey and Lee, 2007). Injector temperature was 250°C while detector temperature was 350°C. Hydrogen was used as the carrier gas.

PCB chiral analysis was done using a 6850 Agilent GC-ECD equipped with a 25 m Chirasil-Dex column (25 m × 0.25 mm × 0.25 µm, Chrompack). Previous temperature ramps, carrier gas, and runtimes that were established by Pakdeesusuk et al. (2013) were also used in these studies for chiral PCB analysis. The column temperature ramped from 100 to 150°C at 10°C/min and then from 150 to 200°C at 0.5°C/min with a total runtime of 64 min. The injector temperature was set at 250°C with the detector at 325°C. Enantiomeric fractions were determined using the equation from previous work conducted in the Lake Hartwell watershed (Dang et al., 2010).

$$EF = \frac{Peak\ area\ of\ (+)\ enantiomer}{(Peak\ area\ of (+)\ enantiomer + peak\ area\ of\ (-)\ enantiomer)} \quad (3.1)$$

In both the achiral and chiral GC runs, a low concentration of 0.04 mg/L Aldrin was added to each GC vial to serve as an internal standard to measure the response factor of the GC. The internal standard serves to accurately quantify the concentration of the PCB congener present in the GC vial, which can then be used to calculate the amount in the original sample. The relative response factor (RRF) is used to determine sample concentrations by using the formula:

$$RRF = \left(\frac{Area\ of\ Analyte}{Mass\ of\ Analyte}\right) * \left(\frac{Mass\ of\ Internal\ Standard}{Area\ of\ Internal\ Standard}\right) \quad (3.2)$$

The same concentration of Aldrin was used in each PCB standard and sample to determine the RRFs (3.2 Stock Solutions). The average RRF for each PCB in the five PCB standards created was used as the RRF for calculating the unknown PCB mass in a sample. The middle PCB standard concentration was run once every time with samples to track the GC response and validate using the RRFs calculated from the beginning of the experiment.

The calibration process of the 6890 Agilent GC for the PCB 136 parent and anticipated product congeners for the SRNL culture encountered an issue due to contamination of the electron-capture detector (ECD). The issue was eventually solved; the details of the trouble-solving can be found in the Appendix.

3.10.2 GC-FID Analysis of TCE

TCE dechlorination analysis was done using manual 0.2 mL headspace injections from a Luer Lock gas syringe with a bevel open end purchased from VICI Precision Sampling. Headspace injections were run on a 2014 Shimadzu GC-FID with a 30 m GS-Q column with temperature ramps, runtimes, and carrier gas already established by Swamy (2021). The column

temperature was held at 40 ºC for 1.5 minutes, then increased to 200 ºC at the rate of 40ºC/min, then held at the final temperature for 2 min. The injector temperature was 200ºC with the detector temperature of 300ºC. Total runtime was 7.5 min. Methane was also analyzed using these parameters and was included in the TCE standards (3.2 Stock Solutions). A calibration curve using the peak area was created every time the treatments or controls were sampled using the TCE standards to analyze unknown concentrations. TCE standards were stored upside down to avoid gases leaking from the butyl stopper.

3.10.3 HPLC Separation of PCB 95 Enantiomers

A working solution of 100 mg/L of PCB 95 was made by dissolving 5 mg into 60 mL of hexane. Thermo Ultimate 3000 high-performance liquid chromatograph coupled to a single wavelength UV detector (HPLC-UV) (Waltham, MA, USA) was used to separate the enantiomers with a Chiralcel-OJ column by following the method previously developed by Ranasinghe et al. (2019). Chromeleon 6.8 was used to record and integrate peak areas. Column temperature was kept constant at 20ºC with a flowrate of 1mL/min. Wavelength was set at 280 nm for PCB 95 detection. Methods were slightly modified to a 10-min total run rather than 8 min due to down shifting of the retention time of each eluent. The new retention times for each eluent were at 5.8 min and 7.5 min. Eluents were collected then dried using a steady stream of nitrogen gas and re-constituted with isooctane to make the stock solutions. The first eluent on both the Chiralcel-OJ and the Chiralsil-Dex column was determined to be (-)-95 (Ranasinghe et al., 2019). Concentrations of the eluents were obtained by using a regression equation created from the areas for each enantiomer to plot the calibration curve. Eluents were run on a 6850 Agilent GC-ECD equipped with a Chirasil-Dex column to check for purity and concentration of the individual enantiomers.

3.10.4 ICP-MS Analysis of Sediment Composition

After 389 days of sampling, treatments were sacrificed for further analysis of sediment composition and DNA (see section 3.11). Metals in the Town Creek microcosm sediments were determined by inductively coupled plasma mass spectrometry (ICP-MS). Serum bottles were unsealed, and the sediment was removed and added to weigh boats to dry overnight prior to the extraction process. The liquid media was poured back into the serum bottles and lightly capped.

After sediment had dried, about 1g from each treatment was placed into a beaker with 5mL of Milli-Q water and 5mL of 70% $HNO_{3,}$ bringing the final concentration of HNO_3 to 8 M. A watch glass was placed on top of the beakers and the solution was refluxed for 30 min. Beakers were allowed to cool before adding 10 mL of 0.01 M H_2SO_4. Solutions were covered with aluminum foil and allowed to react at room temperature for 2 d prior to dilution for ICP-MS analysis. Twenty milliliters of Milli-Q water were added to each beaker, swirled, then 2 mL was removed and placed into a centrifuge tube. Samples were centrifuged at 14,000 rpm for 5 min to ensure no particles were transferred into the samples run by the instrument. The top 1 mL of the supernatant was removed and placed into a 15 mL plastic centrifuge tube with 9 mL of Milli-Q water, ready for ICP-MS analysis (Thermo X-Series II, Clemson USA).

3.11 Molecular Techniques

3.11.1 DNA Extraction

The DNA extraction kit used was FastDNA^{TM} SPIN Kit for Soil purchased from MP Biomedicals. The protocol was followed using 500 mg of dried sediment from each microcosm in the Essential Metals Experiment. A total of 1300 µL was recovered in the Binding Matrix step and transferred to the SPIN filter tube three times since the filter tube can only hold a maximum volume of 600µL. In the final step to elute the DNA, 100µL of DNA Elution Solution (DES) was

used. The purity of DNA was checked using a NanoDrop™ 2000 from Thermo Scientific. Only 2 µL were used from each sample and 2 µL of DNA Elution Solution (DES) was used as a blank. All microcosms reported no less than 5 ng/µL of DNA with no less than 1.44 purity. The purity value is determined by calculating ABS_{260}/ABS_{280}.

3.11.2 16s rRNA Gene Analysis

DNA extractions were stored in 2 mL plastic centrifuge tubes at -80°C until they were ready to ship to Mr. DNA (www.mrdnalab.com, Shallowater, TX, USA) for sequencing. The forward 341F 5'-CCTACGGGNGGCWGCAG-3' and reverse 806R 3'-GGACTACNVGGGTWTCTAAT-5' primers were used to amplify the V4 variable region of 16S rRNA gene in a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit obtained from Qiagen, USA under the following conditions: 95°C for 5 min, followed by 30-35 cycles of 95°C for 30 sec, 53°C for 40 sec and 72°C for 1 min with a final elongation step at 72°C for 10 min (Mr. DNA, Shallowater, TX, USA). After amplification, PCR products were checked in a 2% agarose gel for determining the relative intensity of bands and amplification success. Samples were paired and multiplexed using unique dual. Pooled samples were purified using calibrated Ampure XP beads. Then sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on Illumina MiSeq following the manufacturer's guidelines (Illumina, San Diego, CA, USA). Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). The raw, paired-end demultiplexed FASTQ files obtained from Mr. DNA for each treatment were assembled, denoised, and filtered to remove forward and reserve primers using Quantitative Insights Into Microbial Ecology (QIIME, v2) (Bolyen et al., 2019).

A series of codes were used in QIIME to develop taxonomic, alpha, and beta diversity plots including an amplicon sequence variant (ASV) vs. total sequences plot. Additionally, a metadata table was created to determine diversity based on metal or PCB treatments (i.e. zinc vs. copper and hexa-chlorinated biphenyls vs. penta-chlorinated biphenyls). Diversity plots generated were an overall taxon, Bray Curtis, Faith PD, Jaccard, Shannon, an alpha rarefaction curve using ASV, weighted, and unweighted unifrac.

3.12 Statistical Analysis

Statistical analysis for all projects was determined by running t-tests using *Microsoft Excel*^{TM}, expect for a Kruskal-Wallis ANOVA test run by QIIME when reporting difference among molecular data. Occasionally, JMP was used to confirm p-values obtained from *Microsoft Excel*^{TM}. P-values less than 0.05 were considered evidence of statistical significance. The t-tests were to determine if treatments influenced the average number of days, or increased the lag time required for *cis*-DCE to begin appearing in the microcosms. For example, in the Essential Metal experiment, the mean first day *cis*-DCE appeared in metal treatments were compared to the non-amended treatments. The t-tests were also applied if there was significant change in the mean enantiomeric fraction from day 0 to day 389, the last sampling day for the Essential Metal project. Regression t-tests were run using *Microsoft Excel*^{TM} to determine if the mean rate of the appearance of PCB 96 was significantly different between enriched cultures and sediment cultures in my Halo-priming Experiment. In my Enantiomer Experiment, regression t-tests were also used to determine if the differences in the mean rate of dechlorination of (-) and (+)-95 were significant, as well as changes in EF values for PCB 95 and PCB 91 from day 0 to the final day in two replicates.

4. RESULTS AND DISCUSSION

In this chapter, I will provide figures detailing the results for each of my experiments. At the end of each section, there will be a discussion of all the results for each project. The discussion section will provide detailed analysis of my results and cite literature relevant to the claims I make based on my data.

4.1 Halo-priming Experiment Results

4.1.1 TCE and PCB Dechlorination

Sediment-free microcosms amended with the SRNL culture were compared to sediment microcosms collected from Town Creek, SC, USA after both treatments were halo-primed with TCE and spiked with PCB 136. The enriched *Dehalococcoides* culture (SRNL enrichment culture), rapidly dechlorinated all TCE to *cis*-DCE within 4 days after spiking (Figure 4.1). The SRNL culture had previously been exposed to PCE for generations, so quick dechlorination to ethene was expected. In Figure 4.1, VC is an abbreviation of vinyl chloride.

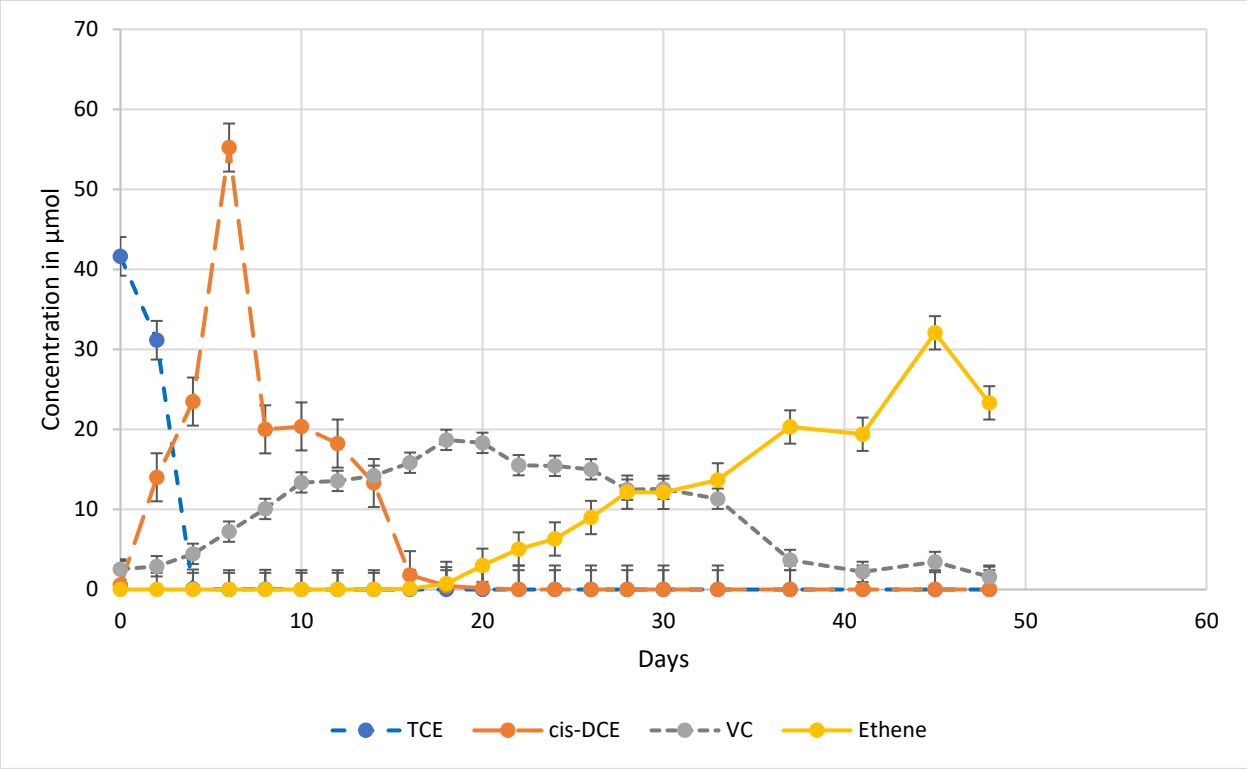

Figure 4.1. TCE dechlorination in all six replicates in the SRNL culture. Rapid dechlorination of TCE to ethene was observed in 5 of 6 replicates. Error bars represent standard error across replicates.

All but one replicate exhibited complete dechlorination to ethene by day 40. Ethene began appearing in the SRNL treatments by day 18, indicating rapid dechlorination of TCE. The small concentration of vinyl chloride (VC) at day 0 was likely due to its presence in the initial culture used to enrich the microcosms, and not from rapid dechlorination of TCE to VC on day 0.

Figure 4.2 illustrates the slower dechlorination of TCE to *cis*-DCE in the Town Creek sediment microcosms. No VC was detected after 70 days of incubation, but all TCE was dechlorinated to *cis*-DCE by day 55.

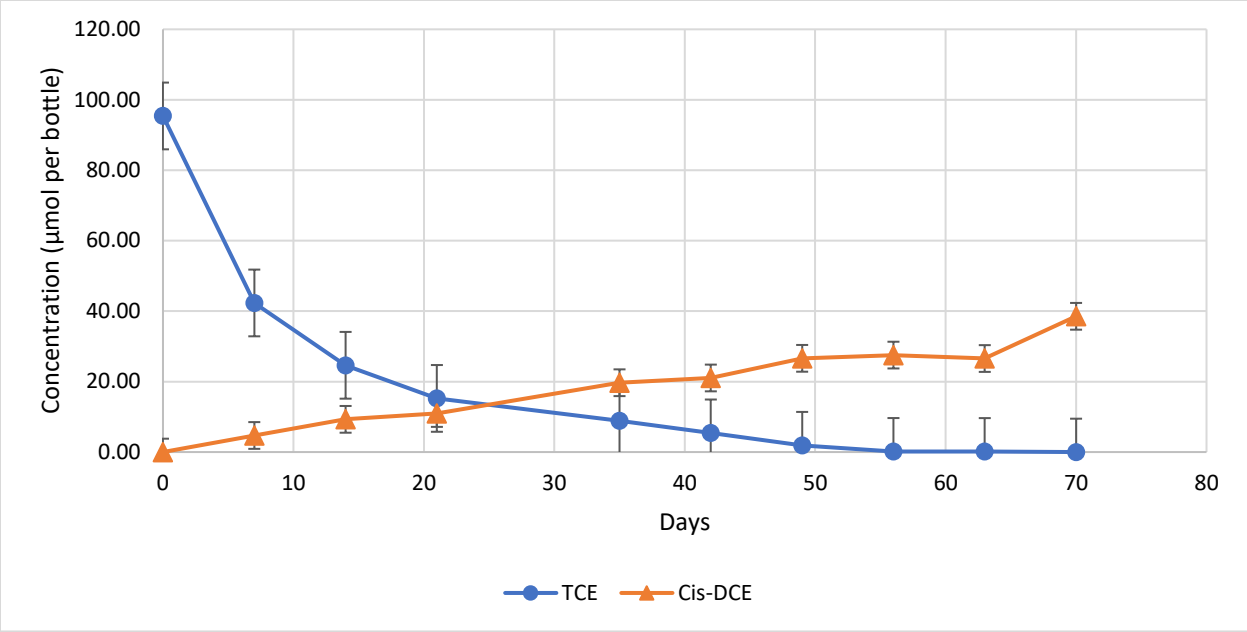

Figure 4.2. In 6 sediment culture replicates halo-primed with TCE prior to spiking with PCB 136, the TCE dechlorination to *cis*-DCE took dramatically longer, with an average of 44.3 days (standard deviation was 18.4 days). Error bars represent standard error across replicas.

As predicted, the SRNL culture that has been grown on PCE for generations dechlorinated TCE to *cis*-DCE more rapidly than the microbial community found in Town Creek sediment. Chlorinated solvents, such as TCE, were contaminants at Town Creek. However, the SRNL culture was enriched with dechlorinating microbes specific for PCE/TCE reduction. Additionally, no VC was detected in the sediment cultures after 70 days of incubation.

Figure 4.3 shows the dechlorination pathway for PCB 136, the parent congener spiked in both the enriched *Dehalococcoides* culture and the sediment culture. Since PCB 54 has only ortho chlorines, it is considered the terminal product in the anaerobic dechlorination pathway for PCB 136. The dechlorination pathway for PCB 136 is not diverse, since only meta dechlorination is possible. Therefore, it is likely that only a select few bacterial strains are responsible for its dechlorination.

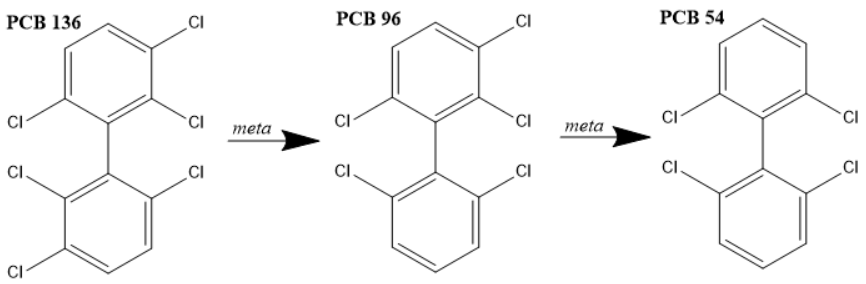

Figure 4.3. Dechlorination pathway for PCB 136.

Figure 4.4 depicts the concentration of PCB 96 as it increased in all the SRNL treatments. The presence of PCB 96 on day 0 indicates rapid dechlorination of PCB 136 within hours of spiking with the parent congener.

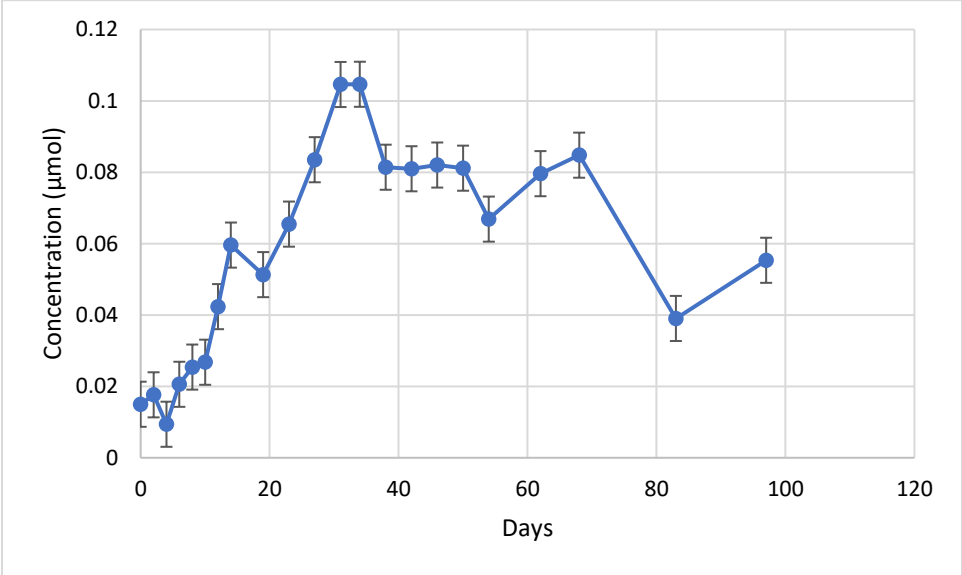

Figure 4.4. The average PCB 96 concentrations plotted over days in the enriched SRNL culture treatments. Error bars represent standard error across all replicates.

The decrease in PCB 96 on day 84 was likely due to one replicate's extraction containing a concentration below the limit of detection in the GC, and not due to PCB 54 accumulating. One replicate, bottle 6, had a smaller concentration of PCB 136 due to a handling error that allowed acetone to evaporate too quickly. Additionally, there was a contamination in the GC with PCB

136, so later samples had to be diluted to avoid contaminating the gold seal and inlet with PCB 136 again. Therefore, the dilution likely caused the presence of PCB 96 to appear as a lower concentration than was present in the microcosms.

Figures 4.5 and 4.6 display the increasing concentrations of PCB products 96 and 54, respectively, in the sediment cultures. Two different figures for each product congener allows for the issue of one sediment culture replicate having a much higher concentration of PCB 54 that would dwarf the appearance of PCB 96.

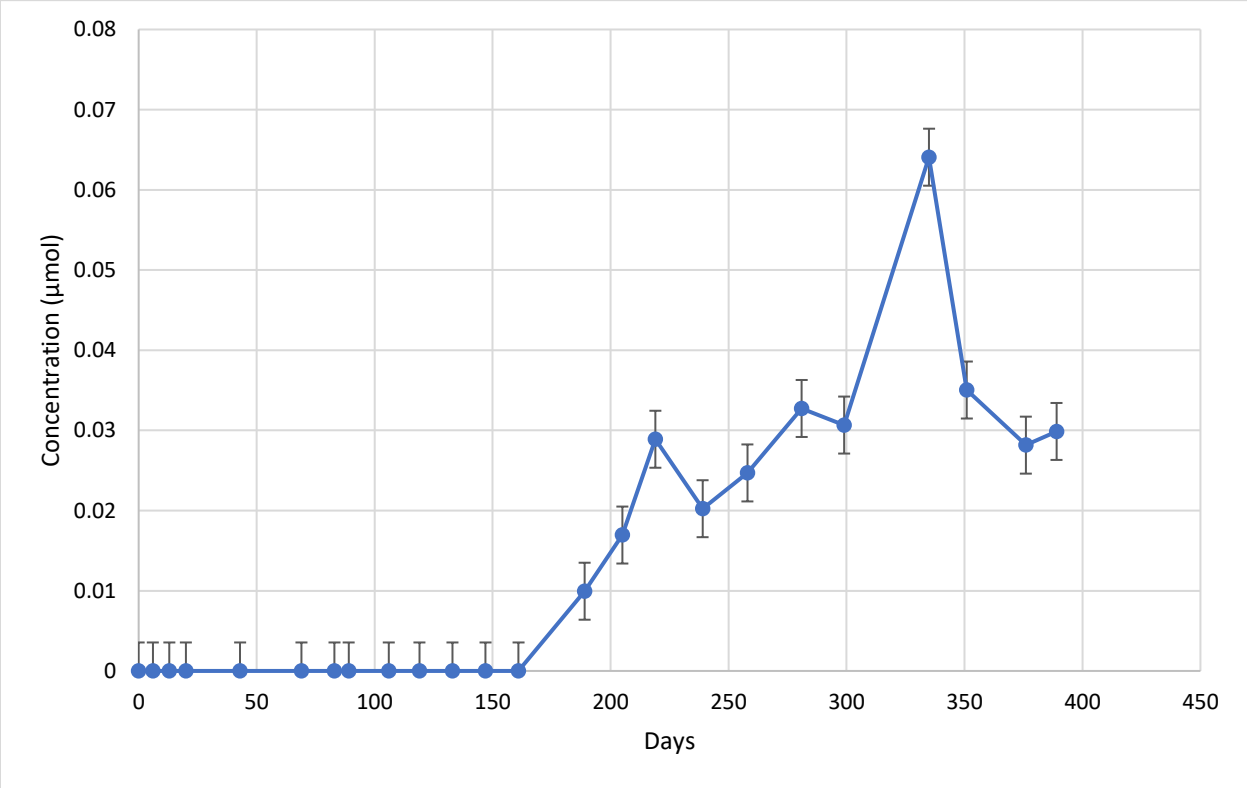

Figure 4.5. The average PCB 96 plotted over days in the sediment culture treatments. Error bars represent standard error across all replicates.

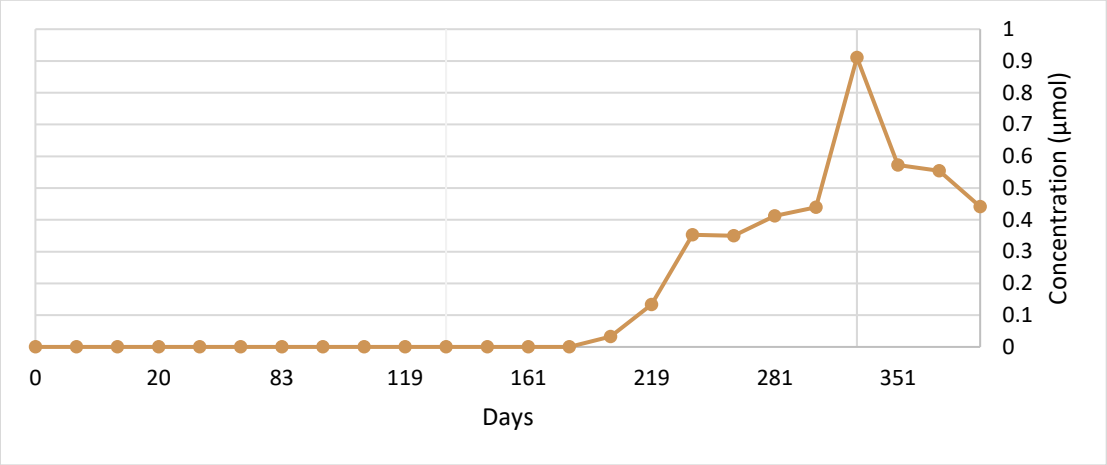

Figure 4.6. Increasing concentration of product PCB 54, from PCB 96. Only one replicate reported the accumulation of PCB 54; therefore, no error bars are shown.

There is a noticeable lag time in the sediment cultures as PCB 96 was not recorded until 189 days whereas the SRNL culture showed PCB 96 appearing within a few hours after spiking. The appearance of PCB 96 was statistically significantly different between the enriched and sediment cultures with a p-value < 0.005. However, 16 days after PCB 96 was recorded in the sediment microcosms, PCB 54, the dechlorination product of 96, began to appear in one replicate. After 84 days of sampling the SRNL cultures, no PCB 54 was recorded.

Figures 4.7-4.9 show the rate of PCB 96 or PCB 54 accumulating in the SRNL and sediment treatments. I decided to show the rate of the products appearing in the treatments because the dechlorination of PCB 136 was inconsistent in the GC chromatograms. The concentration of PCB 136 would decrease and increase each sampling week. As a result, quantifying the rate of the product accumulation was more accurate or appropriate for comparison.

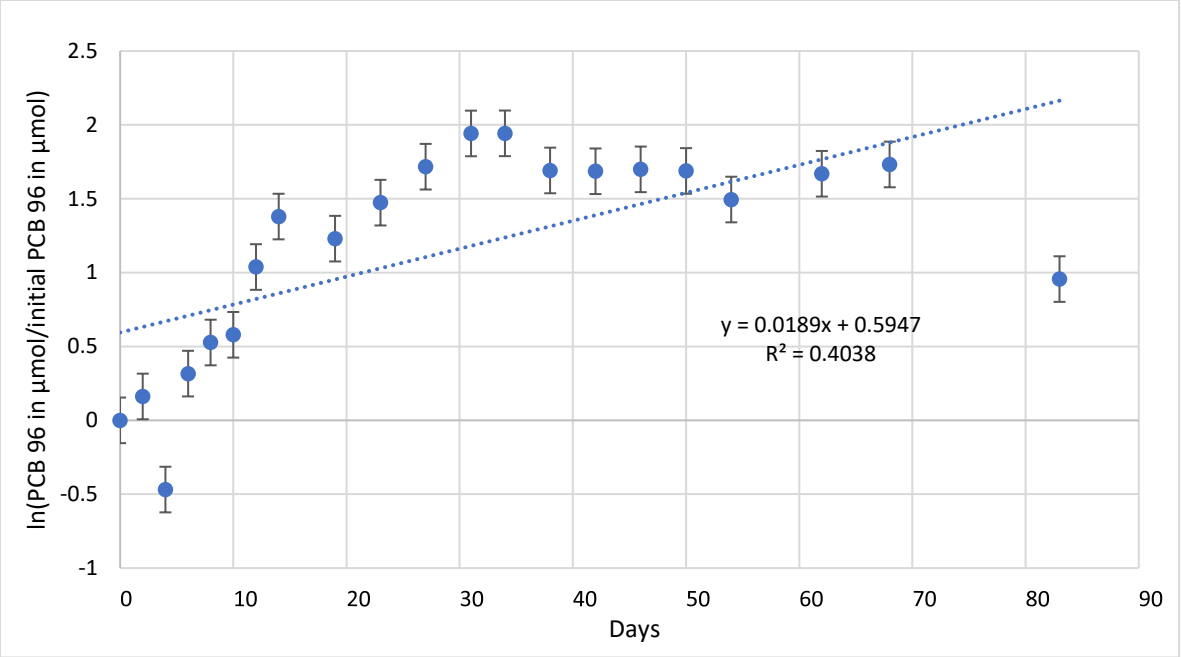

Figure 4.7. The rate of the appearance of PCB 96 in SRNL treatments spiked with PCB 136. Error bars represent standard error across replicates.

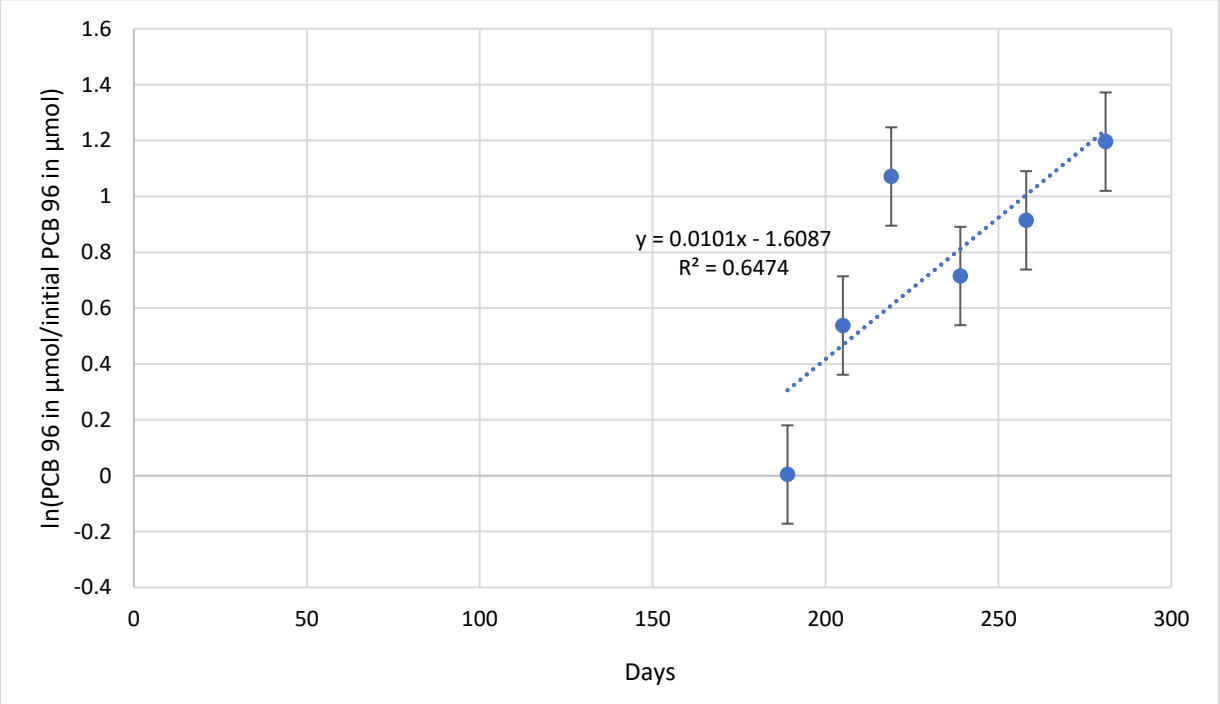

Figure 4.8. The rate of the appearance of PCB 96 in the sediment treatments spiked with PCB 136. Error bars represent standard error across replicates.

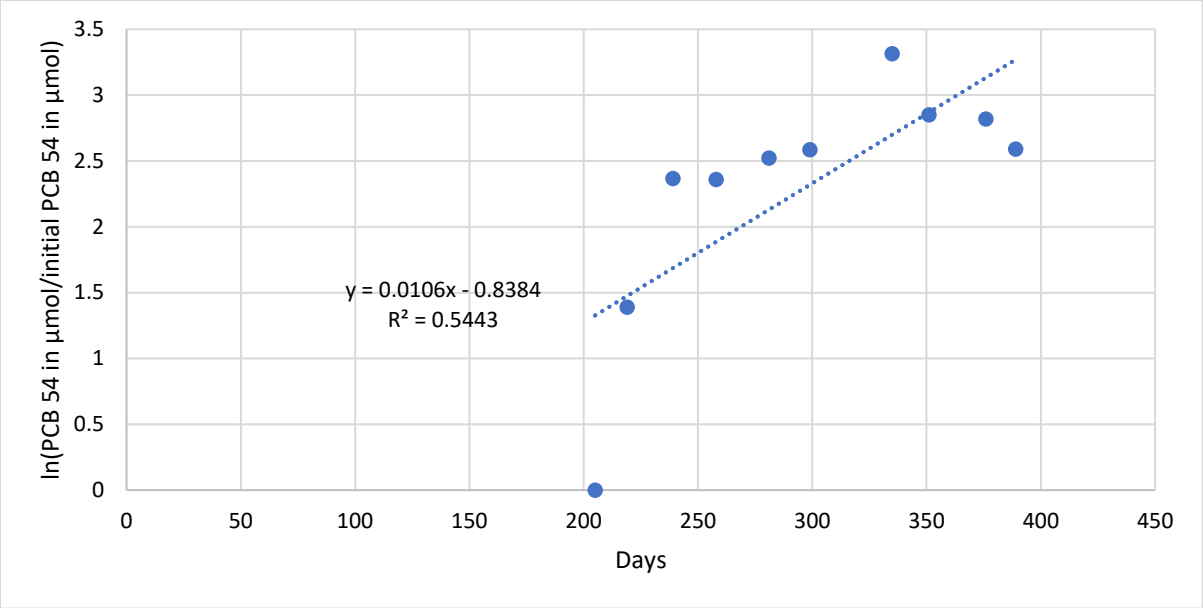

Figure 4.9. The rate of PCB 54 accumulating in the one sediment culture replicate spiked with PCB 136. No error bars are present since the data was from one replicate.

Figure 4.7 compared to Figure 4.8 shows the rate of PCB 96 accumulating in the different treatments. There was a lag time for the first 180 days in the sediment treatments, but once PCB 96 appeared in the microcosmos, the rate of accumulation over 80 days was 0.01 µmols/day. However, this was less than the SRNL culture's rate of 0.018 µmols/day. The results suggest that the SRNL culture produced PCB 96 at a faster rate than the sediment culture. After running a regression t-test, the change in PCB 96 over time in the SRNL culture was statistically significant with a p-value of 0.002. Additionally, the regression t-test for the sediment culture yielded a p-value of 0.05. However, statistical analysis comparing the rate of dechlorination in the SRNL culture to the sediment culture yielded a t-observed value less than 2, indicating the rates were not statistically significant from one another.

PCB 54 never appeared in the SRNL culture, suggesting that the enzyme(s) or microorganism(s) responsible for dechlorinating PCB 96 were not present. The SRNL culture

was not introduced to PCBs prior to this study, so it is likely the culture was missing key microorganisms responsible for the further dechlorination of PCB 96, unlike the sediment culture which was collected from a contaminated creek and had been exposed to PCBs for decades.

I calculated the rate of PCB 54 accumulating in the sediment microcosm to determine if the second PCB product appeared faster than PCB 96. At a rate of 0.0106 µmols/day for PCB 54 compared to 0.0101 µmols/day for PCB 96, PCB 54 did accumulate slightly faster than PCB 96. A regression t-test reported that the line was statistically significant with a p-value of 0.01. However, only one microcosm produced PCB 54, so it is difficult to compare the rates. It is interesting to note the concentration differences in PCB 54 and PCB 96 as well as the rates. The concentration of PCB 54 was almost two times the amount of PCB 96 in the treatment. Perhaps there were more bacterial strains equipped with enzymatic functions to dechlorinate PCB 96 to PCB 54 in the one microcosm that detected PCB 54, or maybe the microcosm had a specific bacterial strain in abundance that was responsible for dechlorinating PCB 96 to PCB 54.

4.1.2 Molecular Analyses for Sediment Culture

A taxabar plot (Figure 4.10) was generated via QIIME for the three replicate sediment treatments spiked with PCB 136. The molecular analysis in this project was minimal, as it only recorded the phylum and was not specific enough to determine the species present in the sediment microcosms. Molecular analysis was mainly used for the Essential Metals Experiment to show how copper or zinc can affect microbial compositions. However, three replicates from the Essential Metals Experiment were used for comparison to the SRNL cultures in this study. No molecular analysis was done for the SRNL culture, since prior research had been conducted on cultivation (Eaddy, 2008).

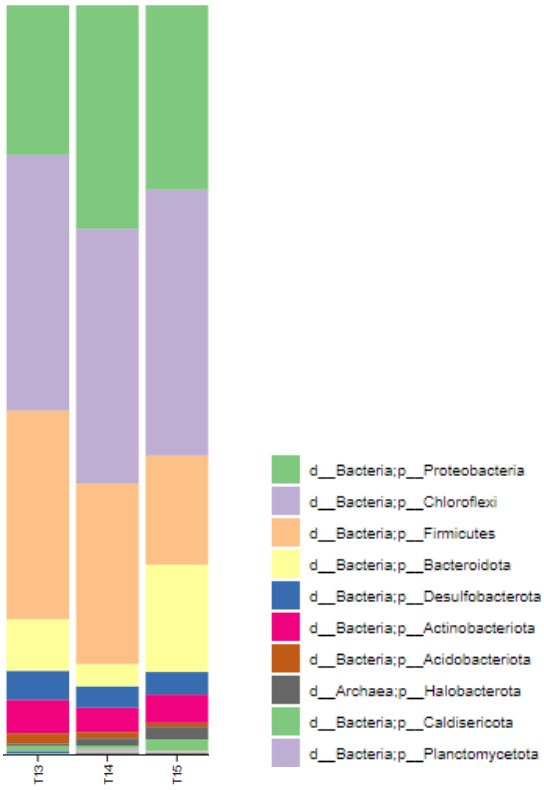

Figure 4.10. Taxa-bar plot generated from QIIME for the three sediment treatments after incubation for 389 days.

The use of TCE as a halo-primer likely proliferated bacterial growth of dechlorinators, since each replicate of the sediment treatments displayed that over 33% of the microbial population was from the *Chloroflexi* phylum. The *Chloroflexi* phylum includes the genera *Dehalococcoides*, *Dehalogenimonas*, and *Dehalobium*, which are known to be involved in dechlorination of PCBs (Wang et al., 2014; Krzmarzick et al., 2012). Additionally, the *Chloroflexi* phylum was at the highest abundance for each of the treatments. The *Proteobacteria* phylum appears above the *Chloroflexi* in the diagram due to ordering based on treatment number, and not from percent abundance. Interestingly, the one microcosm that was able to dechlorinate PCB 96 to PCB 54 was T13 and did not show any significant difference in the microbial community composition. However, it is likely that a particular strain or strains of

bacteria responsible for the dechlorination of PCB 96 were present in T13 which would not be shown when only comparing the phylums of each treatment.

4.1.3 Discussion of Halo-priming Experiment Results

The use of TCE as a halo-primer for the SRNL culture may have aided in upregulating the enzymes used to dechlorinate PCBs. Wang et al. (2014) discovered three reductive dechlorinase enzymes associated with three strains of *Dehalococcoides* that dechlorinated PCE as well as PCBs. Although further dechlorination of PCB 96 to PCB 54 was not observed in the enriched SRNL culture, it is likely that the enzymes or specific strains of bacteria responsible for further dechlorination were absent from the culture. Unpublished work by Brothersen (2011) showed the loss of further dechlorination products when performing continuous enrichment methods. Brothersen (2011) observed the loss of PCB 19, a terminal product with the spiked parent congeners of PCB 132 and PCB 91, when conducting multiple enrichments. Therefore, it is possible that specific strains of bacteria were responsible for various steps in the dechlorination process of PCBs, and further enrichment lost these strains. The SRNL culture had only been grown on PCE for generations, so the culture likely lacked the specific strain of bacteria needed to further dechlorinate PCB 96 to PCB 54.

The sediment culture experienced a longer lag time than the SRNL culture before PCB dechlorination was observed. A prior study conducted using sediment from the Twelve Mile Creek arm of the Lake Hartwell watershed observed a lag time of 100 days before PCB dechlorination, with no halo-priming (Pakdeesukuk et al., 2003). In my study, PCB product congeners appeared around day 180. Pakdeesukuk et al. (2003) used a different sampling location, which may suggest that the microbial communities differ based on the location. Additionally, PCBs 149 and 132 were the parent congeners spiked by the previous researchers

which could perhaps upregulate different enzymes or proliferate specific bacterial growth that may have been absent in my study. PCB 149 was spiked along with PCB 136 in the sediment microcosms in my study but was not compared to the SRNL culture for the halo-priming experiment since only PCB 136 was used in the SRNL culture. However, dechlorination products of PCB 149 were observed around day 50 in my sediment microcosms, which is a dramatic decrease in lag time compared to Pakdeesukuk et al. (2003). PCB 149 has various dechlorination pathways unlike PCB 136, so it is possible that there are multiple strains of bacteria capable of dechlorinating PCB 149, while only a few strains may be able to dechlorinate PCB 136 (Figures 4.3 and 4.11).

The taxonomic plot generated from QIIME (Figure 4.10) indicated that the *Chloroflexi* phylum was around 30% for each sediment treatment, making up a large portion of the microbial community present indicating that the use of chlorinated solvents aided in the growth of the dechlorinating phylum. The *Chloroflexi* phylum encompasses the genera *Dehalococcoides*, *Dehalogenimonas*, and *Dehalobium* which are all known to be involved in dechlorination for either PCBs or TCE (Krzmarzick et al., 2012).

A study designed to determine the variation of RDase genes present in sediment cultures versus sediment-free cultures concluded that some RDase genes were not bifunctional for dechlorinating both PCBs and TCE, indicating that there were PCB-only degrading enzymes that may not be stimulated via halo-priming (Ewald et al., 2022; Qiu et al., 2020). Qiu et al. (2020) determined that 38 out of 164 sediment cultures contained RDases *pcbA4* or *pcbA5* with only one culture containing *pcbA1*. However, 135 of the cultures dechlorinated PCBs, indicating that *pcbA4*, *pcbA5*, and *pcbA1*, may not be the only RDases responsible for PCB dechlorination (Qiu et al., 2020). Additionally, those RDases were bifunctional, meaning they dechlorinate PCE and

PCBs. It is likely that non-identified RDases exist for dechlorinating only PCBs. These results may explain why my sediment microcosms exhibited more dechlorination of PCB 136, and the appearance of PCB 54 in at least one treatment which was not shown in the enriched SNRL culture. It is likely that the sediment culture contained a greater variety of bacteria and more RDase genes that can further dechlorinate PCBs.

Other studies showed that the use of halo-primers, PCE and TCE, can increase the biomass of dechlorinating bacteria such as various *Dehalococcoides* strains (Wang et al., 2015; Wang and He, 2013; Chen and He, 2018). Therefore, halo-priming can be a valuable technique to stimulate dechlorination of more complex contaminants such as PCBs.

A study conducted using contaminated sediment from the Baltimore Harbor estimated the average rate of dechlorination for 91 PCB congeners with 25 different dechlorination pathways using the Anaerobic Dehalogenation Model (ADM) (Karakas et al., 2017). The authors found an average lag time of 100 days and a plateau at 200 days for PCB dechlorination. The median rate for all PCB dechlorination pathways was 0.02 %mole d^{-1} with a range of 0 to 0.133 d^{-1} (Karakas et al., 2017). These results are similar to my study, with a rate of 0.01 d^{-1} for the sediment cultures and 0.018 d^{-1} for the SRNL culture.

Kinetic parameters were calculated for the dechlorination of Aroclor 1248 in sediment collected from the St. Lawrence River, NY (Cho et al., 2003). The initial dechlorination of the parent congeners, hexa-chlorinated biphenyls, occurred at the slowest rate of dechlorination at around 0.006 to 0.018 nmol d^{-1} with a halftime of about 120 days. However, the accumulation of the terminal products, tri-chlorobiphenyls, had a significantly faster rate of about 0.008 to 2.337 d^{-1} with a halftime of about 87 days. Likely, the first dechlorination step of the product congener requires the greatest activation energy, and thus the longest time. In my study, the accumulation

of PCB 96 and PCB 54 observed in the sediment cultures had a rate similar to the initial dechlorination of hexa-chlorinated biphenyls in Aroclor 1248 calculated by Cho et al. (2003). The faster rate observed for the accumulation of PCB products could be due to the diverse congeners spiked with Aroclor 1248 instead of solely PCB 136. The rate of dechlorination could be faster due to the possibility of more product congeners and intermediate dechlorination steps. Additionally, with the presence of more PCBs, specific strains of bacteria could be upregulated leading to quicker or more dechlorination.

The use of TCE as a halo-primer can be an effective method to stimulate bacterial activity and growth prior in sediment cultures to spiking with PCBs. While PCB 136 still experienced a long lag time before dechlorination appeared in sediment microcosms, 180 days, the use of TCE likely induced the enzymes responsible for dechlorination. Using the SRNL culture, it is possible that spiking with TCE helped to upregulate the appropriate enzymes or proliferate bacterial growth to dechlorination PCB 136 to PCB 96. Since the SRNL culture had never been exposed to PCBs prior to this study, it is important to note the relatively fast rate at which the culture was able to dechlorinate 136 to 96. Within hours of spiking, PCB 96 began accumulating in the treatments. Additionally, the SRNL culture had a faster rate than the sediment culture for the appearance of PCB 96.

The ability of the SRNL culture to dechlorinate PCB 136 and the rate at which PCB 96 appeared in the treatments supports the hypothesis that there were bifunctional RDases responsible for the dechlorination of TCE and PCBs. Since Wang et al. (2014) cultivated three RDases that are bifunctional and present in specific *Dehalococcoides* strains (CG1, CG4, CG5), I suspect these strains were also present in the SRNL culture, which is enriched with *Dehalococcoides* (Eaddy, 2008). However, there are likely RDases that are specific for PCB

dechlorination only and have not been identified. In one sediment replicate, further dechlorination of PCB 96 to PCB 54 was observed and may indicate that a specific strain of *Dehalococcoides* exists in the sediment that was not present in the SRNL culture. Compared to various other natural systems, the rate for my sediment microcosms and the SRNL culture was slower. Likely, the microbial and sediment composition influences the rate and which product congeners are produced. In addition, only PCB 136 dechlorination was observed in this study, which has only one dechlorination pathway and not many intermediate products. The presence of more intermediate products may stimulate further bacterial growth and enzymatic functions leading to faster dechlorination rates.

Future research should focus on identifying more RDases that may be responsible for only PCB dechlorination, as this could illuminate which bacterial strains can dechlorinate specific PCB congeners. Additionally, this study could be replicated with more PCB congeners to determine if the SRNL culture can further dechlorinate PCBs. For example, PCB 149 could be used as a parent congener in the SRNL culture since it has more diverse dechlorination pathways than PCB 136 (Figures 4.3 and 4.11). If the SRNL culture shows diversity in its dechlorination, and can further dechlorinate PCB 149 products, then perhaps the *Dehalococcoides* strains present in the enriched culture have more bifunctional RDases for TCE and PCB dechlorination than observed in my study.

4.2 Essential Metal Experiment Results

4.2.1 TCE and PCB Dechlorination

All treatments in the Essential Metal Experiment were collected using contaminated sediment from Town Creek, SC, USA and halo-primed with TCE prior to spiking with either hexa-chlorinated or penta-chlorinated biphenyls. Elevated concentrations of either copper or zinc

were added to treatments prior to TCE or PCB spiking to observe how the higher concentrations impacted dechlorination. A schematic detailing the dechlorination pathways for the chiral PCBs spiked in the various treatments is shown in Figure 4.11. The dechlorination pathway for PCB 136 is displayed in the previous halo-priming experiment (Figure 4.3).

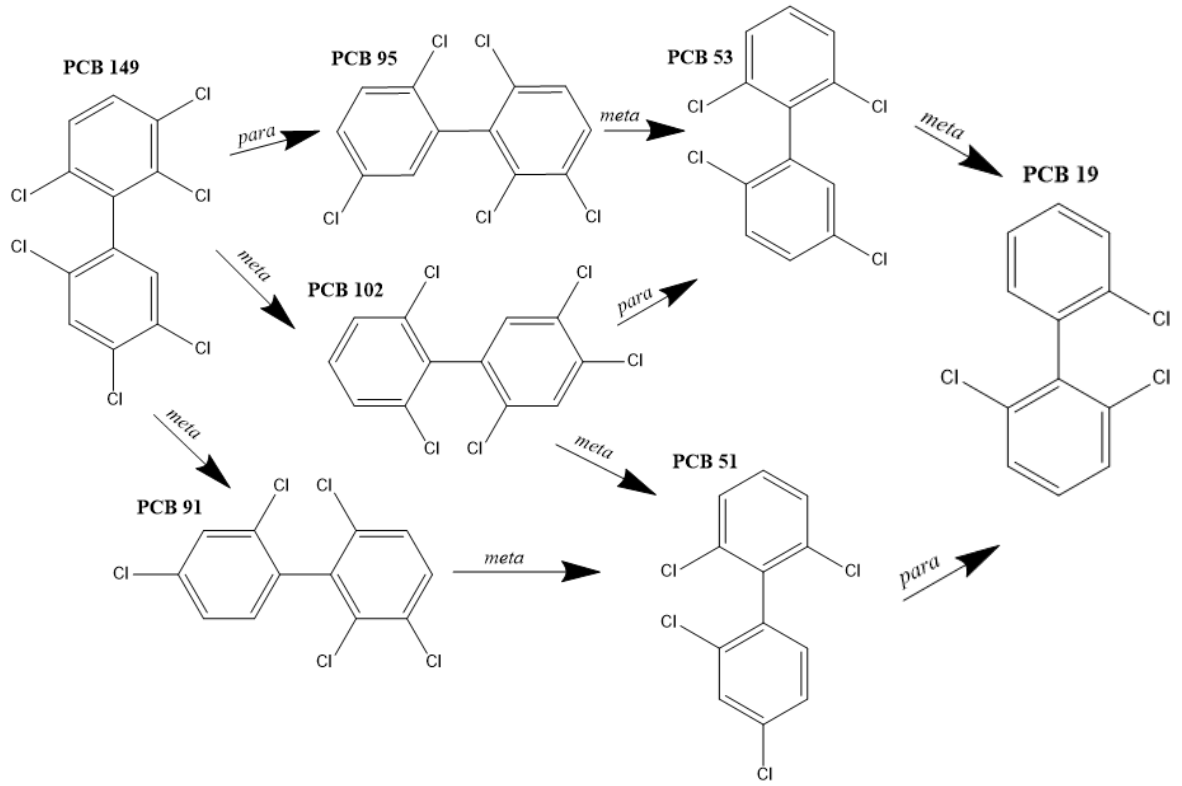

Figure 4.11. Dechlorination pathways for PCBs 149, 95, and 91.

All sediment microcosm treatments were halo-primed with 20 µmols of TCE for 179 days before spiking with PCBs. Microcosms without metal amendments dechlorinated TCE to *cis*-DCE in a shorter timespan than microcosms amended with either 10x $[Zn^{2+}]$ or $[Cu^{2+}]$.

Figure 4.12 shows the average TCE dechlorination to *cis*-DCE for all six of the non-amended metal treatments 179 days prior to spiking with PCBs. The average day *cis*-DCE appeared in all non-amended treatments was 19.8 (standard deviation is 11 days) and all TCE

was dechlorinated to *cis*-DCE by day 56 with an average of 44.3 days (standard deviation is 18.4 days).

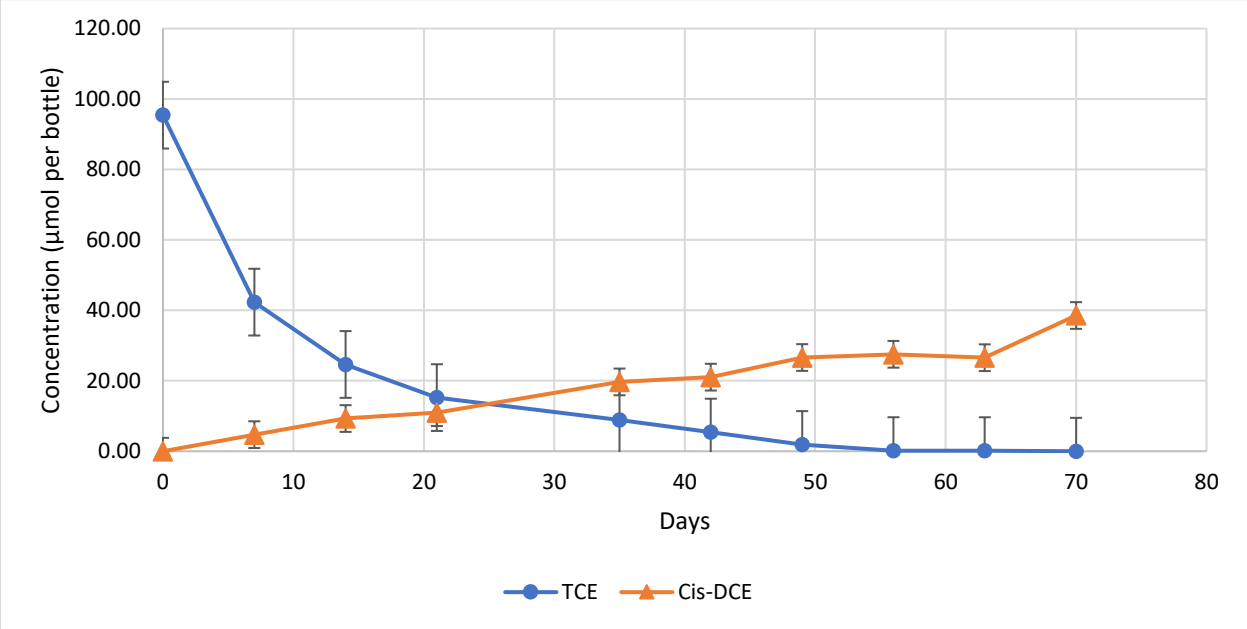

Figure 4.12. TCE dechlorination in microcosms without metal amendments (n=6). Error bars represent standard error across replicas.

Figure 4.13 displays the average TCE dechlorination into *cis*-DCE for all treatments amended with either elevated zinc or copper 179 days prior to spiking with PCBs. The average day *cis*-DCE appeared in all [Zn^{2+}] amended treatments was 36.2 (standard deviation is 11.7 days), and all TCE was dechlorinated to *cis*-DCE by day 97 with an average of 61.7 days (standard deviation is 25.5 days). The average day *cis*-DCE appeared in all [Cu^{2+}] amended treatments was 50.2 (standard deviation is 16.4 days), and all TCE was dechlorinated to *cis*-DCE by day 112 with an average of 79.2 days (standard deviation is 42.4 days).

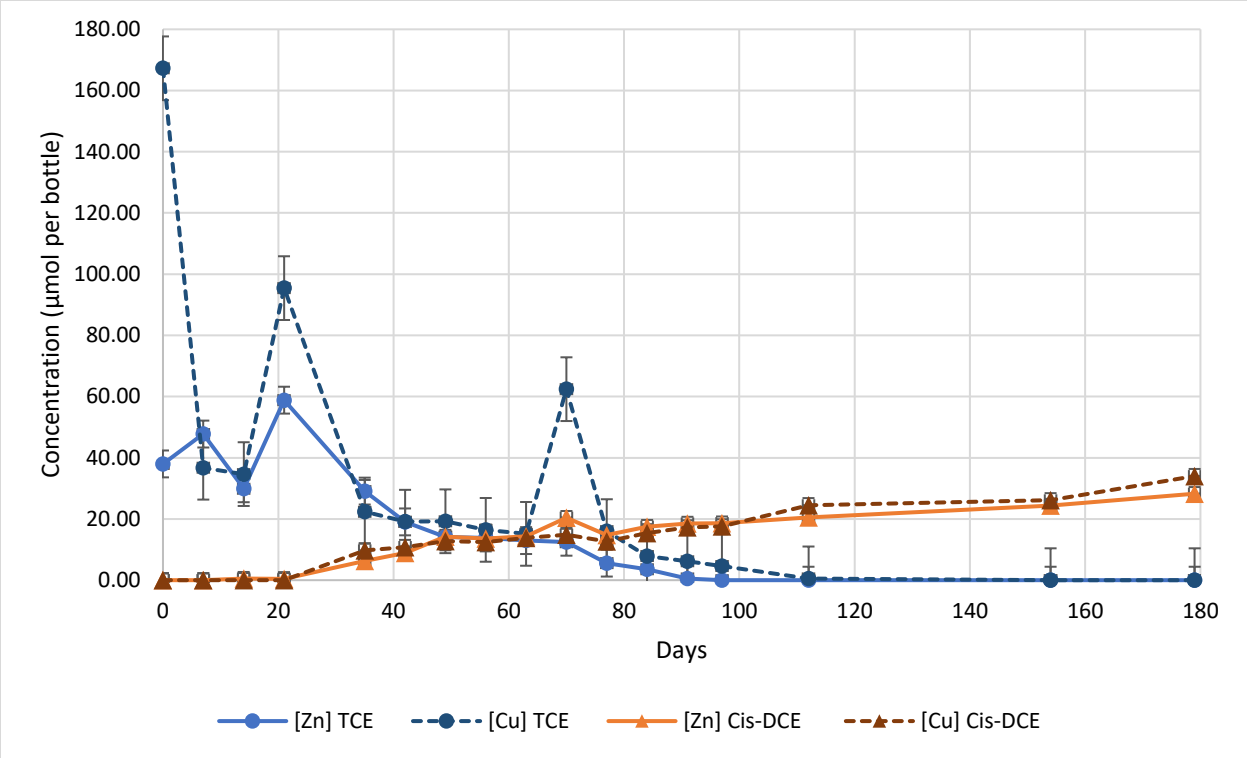

Figure 4.13. TCE Dechlorination in [Zn^{2+}] and [Cu^{2+}] amended treatments. Error bars represent standard error across replicas.

Statistical analysis determined whether metal amendments influenced the average number of days for *cis*-DCE to begin appearing in the treatments. The p-value for [Cu^{2+}] amended treatments compared to the [Zn^{2+}] amendments was insignificant. However, [Zn^{2+}] compared to the non-amended metals proved to be significant with a p-value < 0.05. With the [Cu^{2+}] amended treatments, the p-value was < 0.005 compared to the non-amended metals. These values suggest that the increased concentration of [Cu^{2+}] and [Zn^{2+}] had an inhibition effect regarding dechlorination. Whether this inhibition affected the enzyme directly responsible for the dechlorination of TCE or altered an indirect enzyme involved in the dechlorination process is unknown.

Figure 4.14 displays the product congeners of the treatments containing no metal amendments. Results from two replicates (17 and 18) are shown. PCBs 95 and 91 were the parent congeners spiked into three replicates. PCB 91 undergoes *meta*- dechlorination to PCB 51 while PCB 95 *meta*- dechlorinates to PCB 53. Both PCB 51 and 53 dechlorinate at a *meta*-position to PCB 19 (Figure 4.11). PCB 19 is the terminal product congener as it only contains *orthro*- chlorides, which have not been recorded to dechlorinate under anaerobic conditions with the microbial community present in this sediment. It is worth noting that one replicate never produced any PCB product congeners but did dechlorinate TCE to *cis*-DCE in the halo-priming step. It is assumed there was another microbial community present in the sediment that was responsible for dechlorinating TCE but was unable to dechlorinate PCBs.

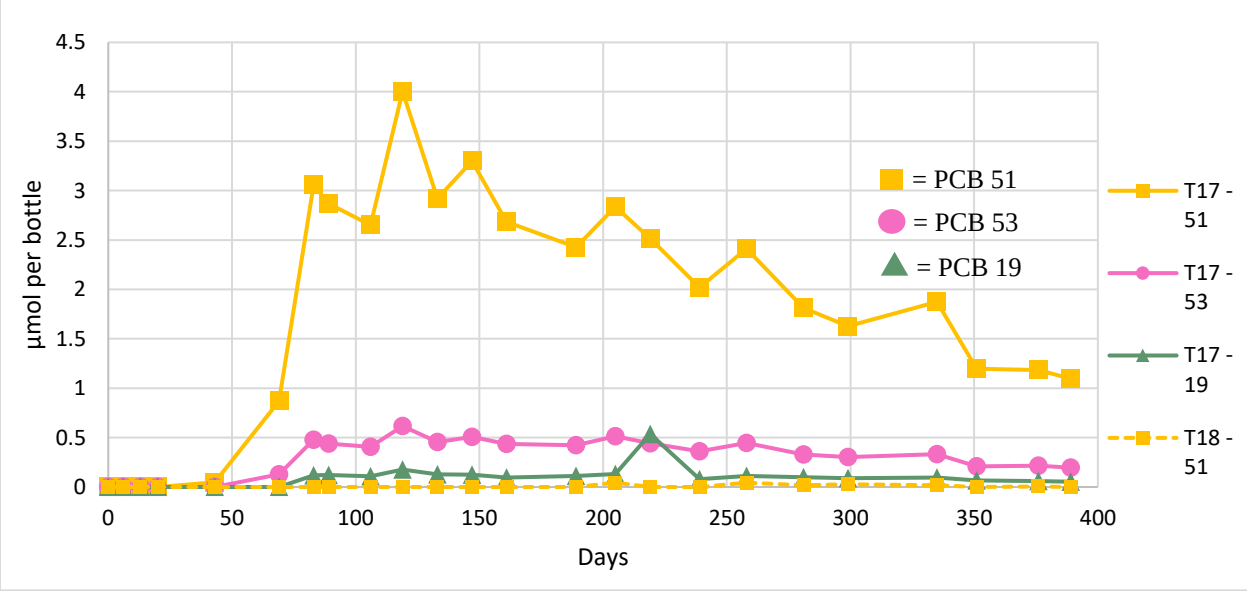

Figure 4.14. Penta-PCB dechlorination in sediment replicates containing no metal amendments. Parent PCB congeners were PCB 91 and PCB 95. No error bars are shown since data is from individual replicates.

Figure 4.15 shows the product congeners of the microcosm treatments spiked with hexachlorobiphenyls and containing no metal amendments. PCB 96 was detected in all three replicates; therefore, the averages are displayed in the graph. The remaining PCB product congeners (PCB 54, PCB 95, PCB 102, PCB 91, PCB 51, PCB 53) were detected in only certain replicates, as indicated by 'T' followed by a number, e.g., T13 is treatment 13. PCBs 149 and 136 were the parent congeners spiked into three replicates. PCB 149 can *meta*- dechlorinate to either PCB 102 or PCB 91. PCB 149 can also *para*- dechlorinate to PCB 95. PCB 102 can either *meta*- dechlorinate to PCB 51 or *para*- dechlorinate to PCB 53. It is unknown which pathway is more favored since both PCB 51 and 53 were present. PCB 136 can only *meta*- dechlorinate to PCB 96 which can only *meta*- dechlorinate into PCB 54 (Figures 4.3 and 4.11). PCB 54 is the terminal product congener since it contains four *ortho*- chlorides. Each replicate displayed a different dechlorination pathway for PCB 149. It is assumed that there were separate and diverse microbial communities present in each replicate that were responsible for the difference in product congeners.

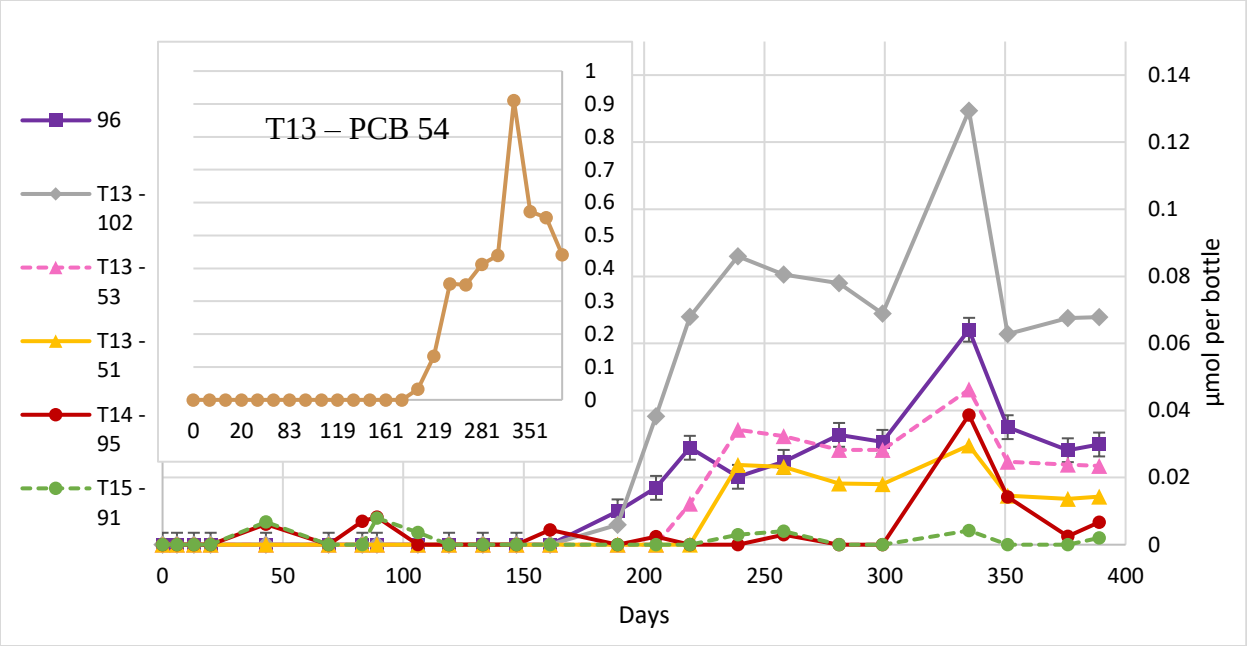

Figure 4.15. Hexa-PCB dechlorination in sediment treatments containing no metal amendments. Parent PCB congeners were PCB 149 and 136. Error bars represent standard error across replicates with PCB 96 dechlorination. All other PCBs were detected in only one replicate; thus, no error bars are shown for these congeners.

Figure 4.16 exhibits the PCB product congener, PCB 51, in the treatment bottles containing either increased [Zn^{2+}] or [Cu^{2+}]. Only PCB 91 undergoes *meta*- dechlorination to PCB 51 which was the only product that was above the detection limit. For PCB 95 dechlorination, PCB 53 is the expected product, so it can be assumed that an enzymatic pathway responsible for PCB 95 dechlorination was inhibited due to the increased essential metal concentrations.

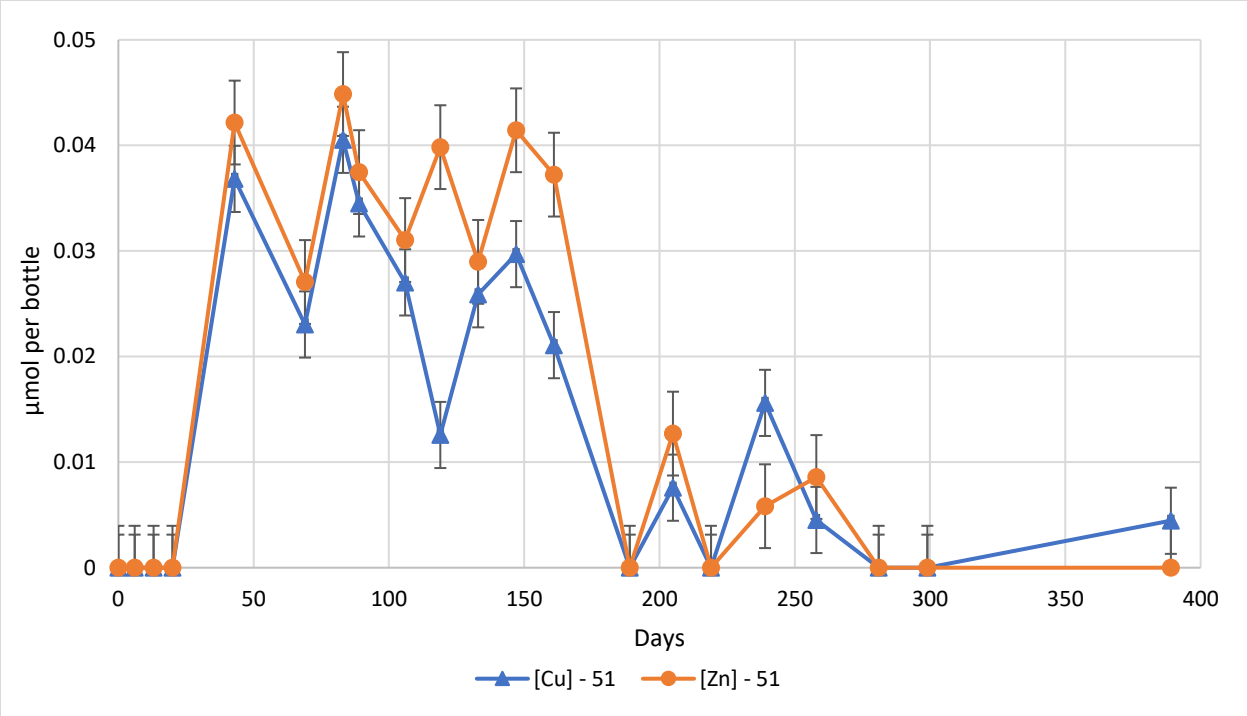

Figure 4.16. Penta-PCB dechlorination in sediment treatments containing either 10x [Zn^{2+}] or [Cu^{2+}]. Parent PCB congeners were either PCB 91 or 95. Error bars represent standard error across replicates.

In Figure 4.17, there is dramatically less variation in the dechlorination products compared to the treatments without elevated zinc or copper concentrations (Figure 4.15). It is worth noting that every metal treatment displayed concentrations of PCB 96, the dechlorination product of PCB 136. Therefore, the PCB 96 values in the graph are averages across the replicates. However, these concentrations were much lower than the ones recorded for the non-amended treatments. Only one replicate from each metal treatment showed PCB 91, a *meta*-dechlorination product from PCB 149. All replicates for the treatments containing no metal amendments displayed different product congeners for PCB 149; therefore, it can be assumed that elevated zinc and/or copper concentrations influence the dechlorination pathway of PCB 149.

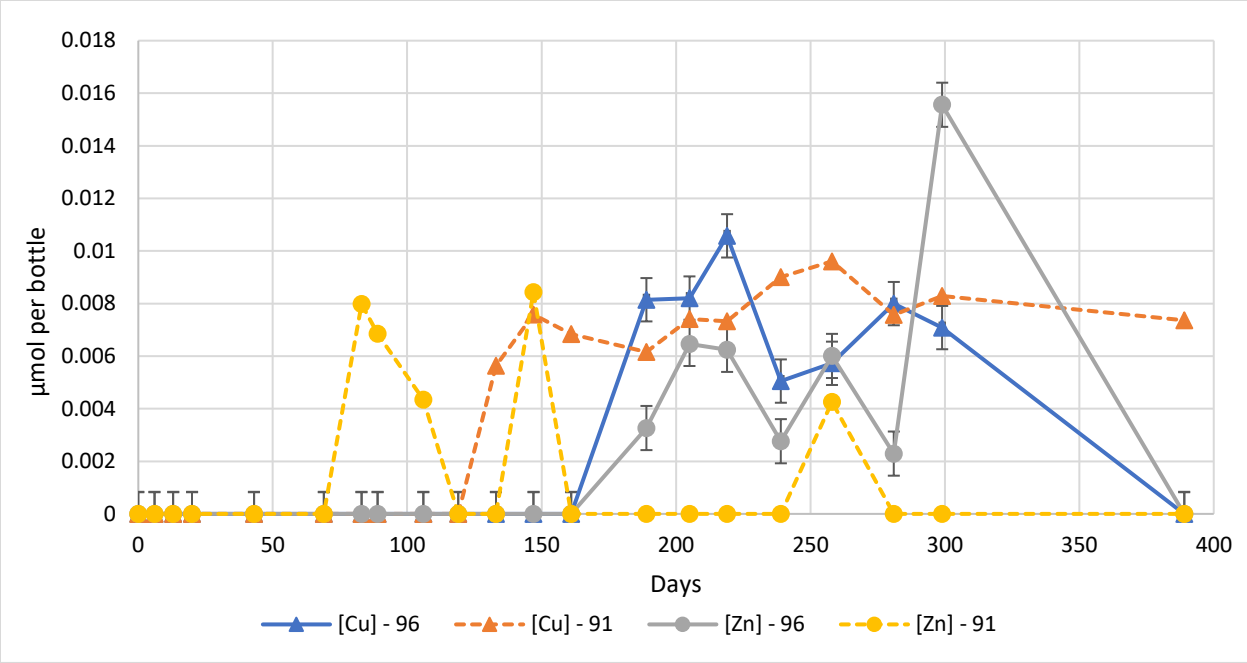

Figure 4.17. Hexa-PCB dechlorination in sediment treatments containing either 10x [Zn^{2+}] or [Cu^{2+}]. Parent PCB congeners were either PCB 149 or 136. Error bars represent standard error across replicates for PCB 96. Only one replicate for each metal treatment displayed PCB 91; thus, no error bars are shown.

Figure 4.18 shows the final EFs of PCBs after day 389 in all treatments compared to day 0 EFs. EF values at 0.5 would indicate a racemic mixture. Only treatments amended with metals displayed statistically significant changes in EFs compared to day one EF values, implying that metals play a role in the enantioselectivity of the enzymes responsible for dechlorination. However, the EF value for PCB 91 in the zinc treatments did not display a significant difference. It is important to note that the EFs are averages from three replicates, and one of the replicates in the non-amended metals treatment, (T17, a highly dechlorinating replicate) showed a statistically significant deviation from day one values with PCBs 91 and 95; however, the other two replicates showed little dechlorination which likely affected the p-value. It is important to note that the commercial PCB products had slightly varied EFs from the expected racemic value (0.5).

The average EF values on day 0 for PCB 136 was 0.52, PCB 149 was 0.506, PCB 95 was 0.51, and PCB 91 was 0.48. During incubation the EF values decreased for all PCB parent congeners except for PCB 91, indicating that the (-) enantiomer accumulated in the media.

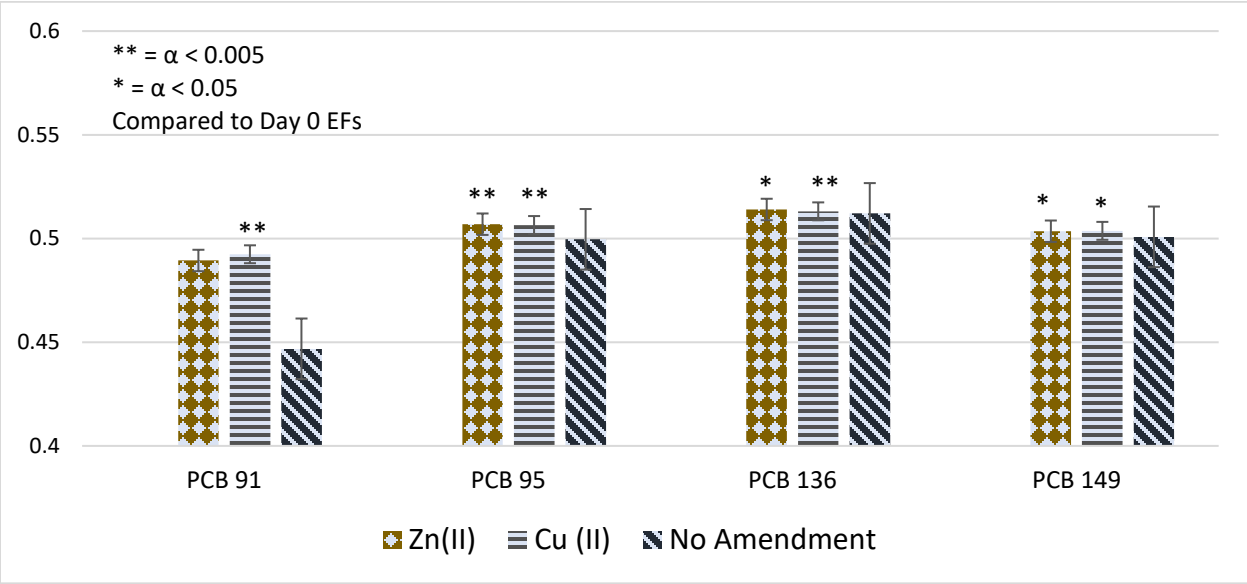

Figure 4.18. Enantiomeric fractions (EF) calculated using peak areas and the formula EF=(+)/[(+) + (-)] for each treatment after incubating for 389 days. Error bars represent standard error across replicates.

4.2.2 ICP-MS Analyses

At the conclusion of the experiment, sediment from each replicate was analyzed for their metal content using ICP-MS. ICP-MS analysis revealed little variation of transition metal components in the sediment from each bottle, indicating that the background elemental composition had a minor effect on dechlorination. The percent abundance of iron isotopes suggested the involvement of microbial processes. The most abundant isotope in each treatment was Fe-57, traditionally in sediment there would be a higher concentration of Fe-56 present (Jochum and Enzweiler, 2014). It is likely that iron reducers were active in each microcosm and have a pivotal role in dechlorination (Li et al., 2021).

Treatment #	54Fe	55Mn	56Fe	57Fe	58Fe	58Ni	59Co	60Ni	61Ni	62Ni	63Cu	64Ni	64Zn	65Cu	66Zn	67Zn	68Zn	69Ga	70Zn
1	176.60	7.72	293.97	315.74	1.27	1.26	0.12	0.16	0.17	0.17	0.24	0.99	1.02	0.23	1.09	1.21	1.19	0.41	0.36
2	187.93	7.83	313.24	335.25	1.33	1.32	0.12	0.15	0.16	0.16	0.23	1.01	1.05	0.22	1.12	1.25	1.21	0.44	0.62
3	156.21	7.25	259.23	279.49	1.14	1.13	0.11	0.15	0.16	0.16	0.21	0.87	0.90	0.20	0.96	1.07	1.04	0.37	0.40
4	127.83	6.58	211.46	227.52	0.93	0.92	0.12	0.12	0.13	0.12	0.40	0.60	0.61	0.39	0.66	0.74	0.73	0.30	0.27
5	107.66	6.02	176.68	189.95	0.80	0.80	0.11	0.13	0.13	0.13	0.32	0.56	0.57	0.30	0.61	0.69	0.67	0.29	0.35
6	92.69	3.94	152.46	164.65	0.69	0.68	0.08	0.10	0.11	0.10	0.29	0.49	0.50	0.28	0.54	0.62	0.60	0.27	0.33
7	143.27	7.82	236.64	254.70	1.03	1.03	0.13	0.13	0.13	0.14	0.46	0.82	0.84	0.44	0.91	1.02	1.00	0.38	0.36
8	186.09	9.39	305.82	330.03	1.33	1.32	0.13	0.15	0.16	0.16	0.39	0.78	0.80	0.37	0.87	1.04	1.00	0.51	0.90
9	90.76	5.37	151.16	162.41	0.67	0.67	0.09	0.10	0.10	0.10	0.27	0.55	0.56	0.26	0.61	0.67	0.66	0.22	0.29
10	1.95	0.09	3.22	3.45	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.02	0.02	0.00	0.01
11	83.93	6.03	139.95	147.55	0.66	0.66	0.12	0.13	0.12	0.13	0.14	0.58	0.60	0.13	0.65	0.72	0.70	0.25	0.37
12	105.59	5.55	175.04	187.98	0.77	0.77	0.10	0.11	0.11	0.11	0.18	0.67	0.69	0.18	0.74	0.83	0.80	0.30	0.33
13	142.41	6.47	236.29	252.32	1.05	1.05	0.13	0.15	0.15	0.16	0.72	0.74	0.75	0.69	0.82	0.92	0.90	0.37	0.44
14	149.66	6.80	249.59	268.85	1.10	1.09	0.12	0.14	0.15	0.15	0.19	0.74	0.76	0.19	0.82	0.93	0.90	0.39	0.36
15	59.70	3.21	99.22	106.78	0.44	0.44	0.05	0.06	0.06	0.06	0.13	0.28	0.29	0.12	0.31	0.37	0.35	0.17	0.13
16	175.95	9.36	290.25	313.67	1.29	1.27	0.16	0.16	0.16	0.17	0.32	0.86	0.89	0.31	0.95	1.08	1.05	0.43	0.32
17	180.28	8.74	297.00	321.23	1.31	1.31	0.16	0.17	0.18	0.17	0.31	0.87	0.89	0.30	0.96	1.11	1.06	0.46	0.41
18	163.84	9.71	272.02	290.54	1.21	1.20	0.16	0.17	0.17	0.18	0.23	0.72	0.75	0.22	0.81	0.93	0.89	0.41	0.41
Fe Isotope % abundance	5.8		91.7	2.2	0.3														
Ni Isotope % abundance						68		26.3	1.19	3.66		1.08							
Cu Isotope % abundance											69.15			30.85					
Zn Isotope % abundance													49.2		27.7	4	18.5		0.6
Treatment %Abundance:	**54Fe**		**56Fe**	**57Fe**	**58Fe**	**58Ni**		**60Ni**	**61Ni**	**62Ni**	**63Cu**	**64Ni**	**64Zn**	**65Cu**	**66Zn**	**67Zn**	**68Zn**		**70Zn**
1	22.42		37.33	40.09	0.16	45.85		5.79	6.16	6.17	51.07	36.02	20.84	48.93	22.36	24.90	24.46		7.44
2	22.43		37.39	40.02	0.16	47.39		5.26	5.65	5.61	50.89	36.09	19.94	49.11	21.38	23.78	23.16		11.74
3	22.44		37.24	40.15	0.16	45.77		6.02	6.54	6.36	50.79	35.30	20.54	49.21	22.09	24.50	23.75		9.12
4	22.52		37.25	40.07	0.16	48.71		6.27	6.78	6.58	51.07	31.66	20.42	48.93	22.06	24.58	24.07		8.87
5	22.66		37.19	39.98	0.17	45.71		7.26	7.62	7.51	51.06	31.90	19.59	48.94	21.18	23.89	23.13		12.21
6	22.58		37.14	40.11	0.17	45.89		6.77	7.31	7.06	50.86	32.97	19.34	49.14	20.89	23.99	23.08		12.70
7	22.54		37.23	40.07	0.16	45.87		5.77	5.93	6.05	51.14	36.38	20.40	48.86	22.06	24.60	24.20		8.73
8	22.60		37.15	40.09	0.16	51.27		5.96	6.11	6.31	51.11	30.35	17.44	48.89	18.84	22.53	21.71		19.48
9	22.41		37.32	40.10	0.17	43.94		6.49	6.88	6.72	50.92	35.96	20.19	49.08	21.87	24.06	23.49		10.39
10	22.59		37.31	39.94	0.16	43.10		5.51	5.72	5.74	51.02	39.93	20.38	48.98	22.05	23.66	23.26		10.65
11	22.56		37.61	39.65	0.18	40.63		7.69	7.60	8.10	50.89	35.99	19.77	49.11	21.30	23.73	22.91		12.28
12	22.50		37.29	40.05	0.17	43.56		5.96	6.12	6.39	50.98	37.96	20.31	49.02	21.86	24.43	23.64		9.77
13	22.53		37.38	39.92	0.17	46.68		6.75	6.78	7.06	50.98	32.73	19.65	49.02	21.27	24.08	23.42		11.58
14	22.36		37.30	40.17	0.16	48.12		6.26	6.52	6.63	50.77	32.46	20.17	49.23	21.64	24.67	23.91		9.62
15	22.43		37.28	40.12	0.16	48.23		6.50	6.90	6.90	51.09	31.47	20.03	48.91	21.55	25.30	24.21		8.92
16	22.52		37.16	40.15	0.16	48.31		6.21	6.27	6.59	51.14	32.62	20.74	48.86	22.13	25.12	24.49		7.52
17	22.54		37.13	40.16	0.16	48.52		6.21	6.61	6.49	51.05	32.17	20.06	48.95	21.56	25.07	23.99		9.31
18	22.52		37.39	39.93	0.17	49.16		6.92	7.12	7.20	50.82	29.60	19.75	49.18	21.33	24.67	23.55		10.70

Table 4.1. ICP-MS analysis of transition metals in all 18 bottles. Treatments 1-6 contained [Zn^{2+}] amendments, treatments 7-12 contained [Cu^{2+}] amendments, and treatments 13-18 had no metal amendments. Concentrations are in ppm (mg/L) for each treatment containing 10g of sediment. Relative natural abundance of certain isotopes is given as well as the percent abundance recorded in each treatment.

4.2.3. Molecular Analyses

One of the plots generated from QIIME V2 was the Faith Phylogenic Diversity (PD) Significance which uses phylogenic distances to determine diversity among treatment groups. Faith PD is defined by taking a set of species equal to the sum of the lengths of all the branches on the tree that span the members of the treatment group (Faith, 1992). Faith PD measures the amount of the phylogenetic tree covered by the treatment group by summing the minimum number of branch lengths based on sequencing depth. Therefore, a higher number means more

branches, which means more diversity. Figure 4.19 shows the results for each of the metal and non-metal treatments.

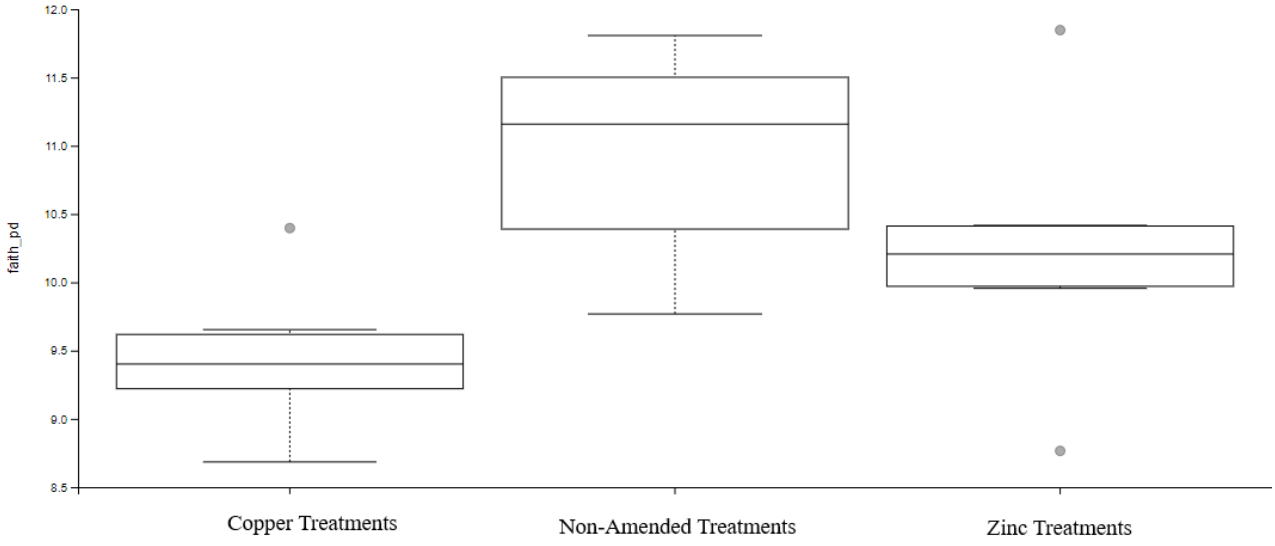

Figure 4.19. Faith Phylogenic Diversity Significance plot for the metal treatment groups. All replicates are shown for each treatment group (n=6).

A higher value on the y-axis of the plot indicates higher richness or more diversity. The box plot for the non-amended treatment group shows a wide interquartile range, indicating that the individual replicates differ from one another. However, the median for the non-amended treatment group is significantly higher than either metal group (copper and zinc). The Kruskal-Wallis p-value calculated across all groups is 0.02 signifying that the elevated essential metal concentrations lowered the microbial diversity. The Faith PD plot can suggest that a greater level of diversity indicates more activity, since at least two of the most active dechlorinating bottles were present in the non-amended treatments.

An alpha rarefaction curve (Figure 4.20) was created using amplicon sequence variants (ASV) and sequencing depth gathered from QIIME V2 after denoising the data. ASV functions by counting abundance and similarity in gene sequences (Callahan et al., 2017). ASV can detect

small biological variations and single nucleotide differences in DNA sequences, unlike operational taxonomic units (OTU) which can cluster taxonomically different sequences together (Callahan et al., 2017).

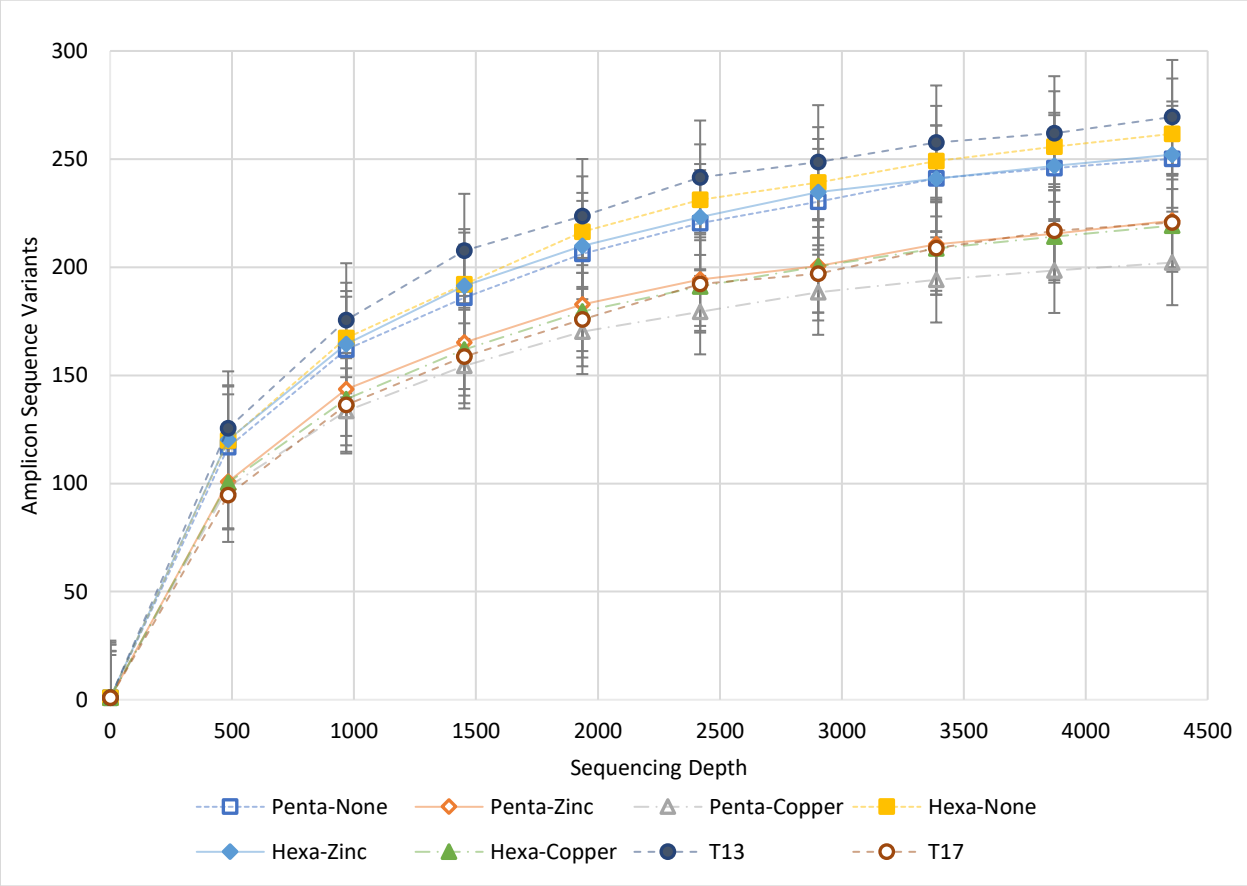

Figure 4.20. An alpha rarefaction curve was created using ASV and sequencing depth for all treatment groups based on condition, i.e. spiked PCBs and metals. Individual replicates that exhibited the most dechlorination, T13 and T17, are also included to compare their diversity with treatment groups. Error bars represent standard error across replicates.

All treatment groups spiked with hexa-chlorinated biphenyls (PCBs 149 and 136) exhibited higher microbial diversity except for the treatment group containing elevated copper concentrations. Additionally, the treatment groups spiked with penta-chlorinated biphenyls (PCBs 95 and 91) displayed lower biodiversity, but the penta-chlorinated microcosms with no

added metal concentrations followed a diversity path similar to the hexa-chlorinated treatments. T13, a single replicate spiked with hexa-chlorinated biphenyls and no metal amendments expressed the highest level of diversity of all treatments. T17, a single replicate spiked with penta-chlorinated biphenyls and no metal amendments displayed equal diversity to the penta-chlorinated zinc and hexa-chlorinated copper treatments after 4000 sequence depths. However, the diversity of T17 was lower than its replicates, shown as the penta-none treatment group (open blue squares in Figure 4.20). The alpha rarefaction curve indicated that microbial community diversity depended on both the PCB congener spiked and the metal concentrations.

Figure 4.21 shows the phylum abundances for each microcosm 1-18. Treatments 1-6 were amended with zinc(II), treatments 7-12 were amended with copper(II), and treatments 13-18 were not amended with any additional metal concentrations. The phylum abundances do not have any distinct patterns in relation to the metal amendments, but the higher percentage of *Chloroflexi*, the second most abundant phylum, indicates that dechlorinating bacteria were active and proliferating in each of the replicates.

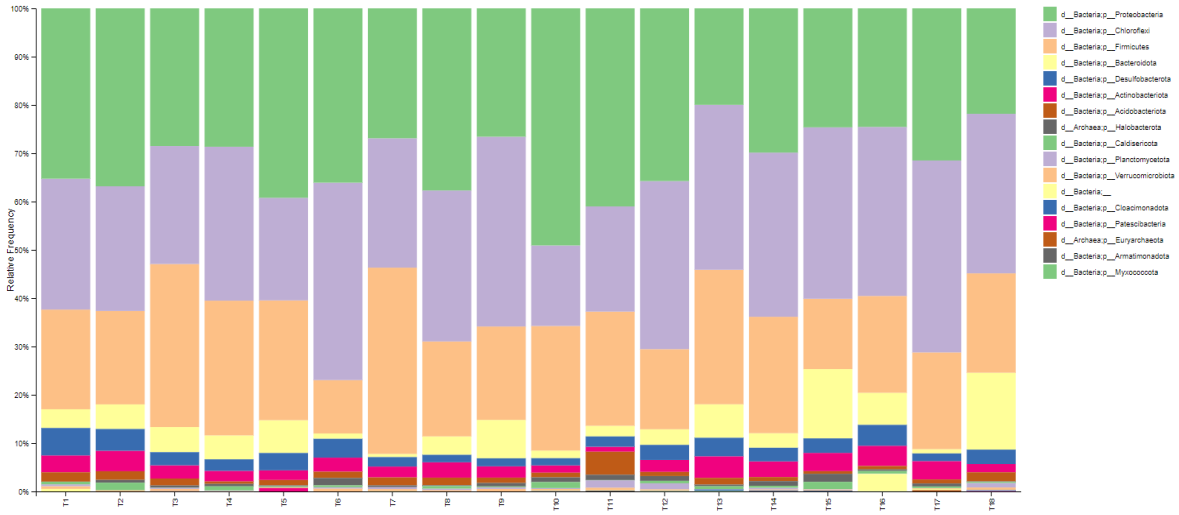

Figure 4.21. Taxonomic plot of each treatment generated using QIIME. The phylum *Chloroflexi* was at the second highest abundance for all sediment microcosms.

4.2.4. Discussion of Essential Metal Experiment Results

The use of TCE as a halo-primer reduced the lag time from around 100 days observed in a previous microcosm study conducted using sediment from the Twelve Mile Creek arm of Lake Hartwell (Pakdesukuk et al., 2013). PCB product congeners in my study appeared around day 50 for most microcosms suggesting that TCE upregulated the enzymes used for dechlorination and aided in growing the microbial community responsible for PCB dechlorination. The taxonomic plot generated from QIIME (Figure 4.21) indicated that the *Chloroflexi* phylum was around 30% for each treatment, making up a large portion of the microbial community present, which strongly suggested that the use of TCE aided in the phylum's growth. The *Chloroflexi* phylum encompasses the genera *Dehalococcoides*, *Dehalogenimonas*, and *Dehalobium* which are all known to be involved in dechlorination for either PCBs or TCE (Krzmarzick et al., 2012).

Treatments amended with zinc or copper exhibited a longer lag time in TCE dechlorination. The first day *cis*-DCE appeared in the metal amended treatments versus the non-amended metal treatments was found to be statistically significantly different implying that the use of elevated zinc and copper concentrations inhibited dechlorination. In the Lu et al. (2020) study of the effects of metals on PCB dechlorination, high concentrations of Cu^{2+} (50 mg/L) had an inhibitory effect on dechlorination compared to the treatments with no additional copper added. It is possible that these concentrations were high enough to suppress microbial growth leading to decreased PCB dechlorination rather than having a direct influence on enzymatic function. In my study, the concentration of copper per treatment was around 0.07 mg/L and likely did not have a toxic effect for the microbes but instead hindered the enzymatic function responsible for TCE and PCB dechlorination. In a study by Kuo and Genthner (1996), the concentration of copper and its impact on 2-chlorophenol anerobic microbial dechlorination

indicated a hormesis effect since Cu^{2+} at 0.01 mg/L increased the rate of dechlorination compared to the control with no copper amendments, while levels of Cu^{2+} above 5 mg/L showed no degradation of 3-chlorobenzoate (3CB). Additionally, the authors found that the lag time for 3CB microbial degradation increased when increasing copper concentrations from 1.0 mg/L to 2.0 mg/L and the dechlorination rate also decreased. A hormesis effect was not observed in my study, which could be due to the copper concentration being greater than 0.0 1mg/L (0.07 mg/L). However, Kuo and Genthner (1996) suggested that the elevated copper concentration used in my study likely does not have a toxic effect on the anerobic microbial community responsible for dechlorination but inhibits biodegradation via enzymatic pathways.

Copper and zinc toxicity to fermentative bacteria has been previously explored by Lin and Shei (2008) who showed an IC_{50} (half-maximal inhibitory concentration) value with concentrations much higher than my study, 4.5 mg/L Zn^{2+} and 6.5 mg/L Cu^{2+}. Interestingly in the Lin and Shei (2008) study, Cu^{2+} at 3 mg/L showed an increase in hydrogen production, implying a hormesis effect with copper concentrations. Alternatively, any concentration of Zn^{2+} inhibited hydrogen production compared to the control group with no metal amendments; likely zinc is not beneficial to fermenters (Lin and Shei, 2008). Fermentative bacteria are vital for TCE and PCB dechlorination since they produce the hydrogen needed for reductive dechlorination. Therefore, the metal amendments in my study could have affected fermentative bacterial growth which indirectly led to a decrease in dechlorination. However, it seems unlikely that the elevated copper concentrations, 0.07 mg/L or 1.06 µM per treatment, decreased hydrogen production based on the results of Lin and Shei (2008). On the other hand, the elevated zinc in my study, 0.09 mg/L or 1.32 µM, could have inhibited hydrogen production leading to the decrease in PCB dechlorination observed.

Sulfate-reducing bacteria may be more sensitive to copper amendments than the microbial community responsible for dechlorination. Sani et al. (2001) reported that at 6 µM Cu^{2+} the growth rate of sulfate-reducing bacteria decreased by around 25% compared to controls with no copper additions. Sani et al. (2001) also observed elevated zinc concentrations up to 16 µM had no permanent effect on cell growth as the proliferation was similar to the control group with no zinc amendments. Therefore, it is suggested that the concentration of zinc used in my study (1.3µM) was not toxic to the sulfate-reducing bacteria. Sulfate-reducers can be beneficial for the dechlorination of TCE or PCBs since the bacteria can supply a carbon source in the form of acetate to the dechlorinators (Ding et al., 2018).

Metal-reducing bacteria are imperative for dechlorinators since they provide vitamin B12 (co-facter for RDases), Fe^{2+} (needed for iron-sulfur co-factors) and acetate (carbon source). Iron reduction is dominant in Lake Hartwell since the sediments are rich in iron, indicating that metal reducers are critical and present in Lake Hartwell sediment; thus, there is likely an abundance of iron reducers in my Town Creek microcosms. Additionally, some metal-reducers, such as *Geobacter* and *Shewanella* (in the phylum *Proteobacteria*), are known to reduce TCE (Zhong et al., 2024). Reporting on the effects of iron reduction in media containing *Geobacter* cells, increasing concentrations of copper and zinc, (from 0 mg/L to 50 mg/L), decreased the cell percentage of *Geobactor* and iron reduction, indicating elevated concentrations of copper and zinc had a toxic effect (Wang et al., 2023). Therefore, it is possible the concentration of zinc and copper in my study inhibited iron reduction by decreasing metal-reducing bacterial cell percentages. Since *Geobacter* can reduce TCE, it is likely that the zinc and copper concentrations could have decreased *Geobacter* cells leading to a decrease in TCE reduction.

Microcosms with enriched cultures collected from contaminated urban river sediments were analyzed for their dechlorination of PCE in the presence of elevated zinc and copper concentrations (5, 10, 50 mg/L) (Wang et al., 2022). Complete dechlorination of PCE to ethene was observed after one month in the controls with no metal amendments and in the zinc and copper treatments up to 50 mg/L indicating that the fastidious organohalide respiring bacterial community was unaffected by the high concentrations of zinc or copper (Wang et al., 2022). However, at 50 mg/L it took around 200 days for complete dechlorination of PCE after subsequent enrichment transfers suggesting that 50 mg/L or higher concentrations of zinc or copper had a toxic effect on the microbial community responsible for dechlorination (Wang et al., 2022). Interestingly, *Dehalococcoides* abundance increased with increasing concentrations of both zinc and copper, but *Dehalobactor* abundance decreased with increased metal concentrations (Wang et al., 2022). However, the addition of zinc and copper increased the lag time observed for the dechlorination of PCE to ethene, implying that the metals affected enzymatic functions for dechlorination but did not have a toxic effect on organohalide respiring bacterial growth. Given the Wang et al. (2022) study, it is likely that the concentrations used in my study were not high enough to inhibit the growth of dechlorinating bacteria and instead affected the enzymes responsible for dechlorination.

In a study comparing increased concentrations of hazardous metals (Cu^{2+}, Cd^{2+}, Cr^{3+} and Pb^{2+}) on the dechlorination of PCBs in sediment communities and enriched cultures revealed that sediment microbial communities were more sensitive to the addition of the metals than an enriched culture of *Dehalococcodies mccartyi* strain CG1 (Lu et al., 2020). These results imply that the higher concentrations of metals affected other vital microbial species such as fermenters found in the sediment, rather than directly inhibiting dechlorinating bacteria. In fact, elevated

concentrations of Pb^{2+} and Cu^{2+} increased richness of *Dehalococcoides* by reducing the overall alpha diversity in the microcosms, suggesting that the genus has a higher tolerance to metal concentrations than other microbes present in the sediment (Lu et al., 2020). However, increased metal concentrations decreased overall dechlorination of PCB 180, indicating that elevated metal concentrations may affect the syntrophic metabolic network found in mixed microbial communities leading to inhibition of PCB dechlorination.

In my study, significant changes in EF were only recorded for treatments containing metal amendments, which were also shown to have lower microbial diversity. Prior studies conducted using sediment-free microbial *in vitro* assays or enriched cultures indicated mainly a preference for dechlorinating the (-) enantiomer (Yu et al., 2018; Qiu et al., 2020). Only a few RDase genes, *pcbA4* and *pcbA5* (determined previously from an enriched culture of *Dehalococcoides mccartyi* strains CG4 and CG5 respectively), were detected in the *in vitro* assays for the dechlorination of chiral PCBs 132, 174, and 180 (Qiu et al., 2020). However, 97 out of 135 *in vitro* assays contained no known PCB RDase encoding genes, indicating that there are likely many unidentified, novel RDases that are responsible for enantioselective dechlorination (Qiu et al., 2020). Not many strains of *Dehalococcoides* have been isolated, so perhaps there are specific strains of these fastidious bacteria present in sediment communities that contain various RDase genes responsible for specific PCB degradation and enantioselectivity. A pure culture and crude cell lysate study using *Dehalococcoides mccartyi* strain CG1 found that *pcbA1* preferentially attacked the (-) enantiomer in chiral PCBs 183 (2,2’3,4,4’,5’,6-heptachlorobiphenyl), 174 and 132, insinuating that the RDase gene *pcbA1* is stereoselective (Yu et al., 2018).

In a microcosm study conducted using sediment from the Twelve Mile Creek arm of Lake Hartwell spiked with Aroclor 1254, Pakdeesusuk et al. (2003) showed that PCB 149 and 132 did not degrade enantioselectivity, correlating with my study in microcosms containing no metal amendments. However, PCB 95, the product congener of PCB 149, did accumulate a significant EF value after incubation, though at the time the elution order of PCB 95 was unknown, so no conclusions were drawn on which enantiomer was favored (Pakdeesusuk et al., 2003). However, the methods used by Pakdeesusuk et al. (2003) to quantify enantiomer concentrations were the same as the ones used in my study, so it can be inferred that the elution order was the same and that (+)-95 accumulated in the microcosms since the EF was calculated using E1/(E1+E2) where E1 is the first eluent and the (-) enantiomer using the Chirasil-Dex column. However, in my study, all product congeners were racemic.

In a study by Wong et al. (2001), analysis showed a variety of non-racemic results from sediment cores collected in the Twelve Mile Creek arm of Lake Hartwell. As PCBs migrated from the point source, accumulation of the (+) enantiomer was shown for PCBs 149 and 136, indicating that the (-) was favored for dechlorination in the Hartwell sediment microbial community. The ERs for PCB 91 shifted to be more racemic as samples moved away from the point source, while PCB 95 became significantly less racemic (Wong et al., 2001). The elution order for PCB 91 and 95 enantiomers were unknown, so no conclusions were drawn about which enantiomer accumulated. However, the methods used in my study to quantify enantiomer concentrations were the same as used by Wong et al. (2001). Therefore, it can be implied that (+)-95 concentrations increased as samples were collected further from the point source. PCB 91 can be inferred to have a higher (-) enantiomer closer to the point source.

Contrastingly, in my study, the microcosms containing metal amendments accumulated more of the (-) enantiomer after incubation, suggesting that the (+) enantiomer was favored for dechlorination. Only the penta-chlorinated biphenyl microcosms with elevated copper concentrations displayed an increase in (+)-PCB 91 enantiomer, indicating that the (-)-91 was favored for dechlorination. Although, in one highly dechlorinating microcosm with no metal amendments (T17), PCB 91 accumulated more of the (-)-enantiomer, suggesting that (+)-91 was favored for dechlorination in this individual treatment. Additionally, PCB 95 in the T17 microcosm had a lower EF value, also indicating that the (+)-95 enantiomer was favored for dechlorination. The results suggest that the presence of elevated copper concentrations affected PCB 91 enantioselective degradation, but not PCB 95. Any chiral product congeners quantified in the hexa-chlorinated biphenyl microcosms accumulated as racemates, which suggests that the enzymes were enantioselective in degrading the parent congeners but did not influence product enantioselectivity. However, it was noted that the addition of metals decreased overall microbial diversity, so it is possible that specific strains of dehalogenating bacteria responsible for certain steps in the dechlorination process were not present in these microcosms.

In my study, the two replicates that exhibited the most dechlorination (T13 and T17) were non-amended but had different diversity indices, suggesting that a diverse microbial community may not be an imperative factor for PCB dechlorination. A prior study observing long-term dechlorination of PCBs in contaminated sediment indicated the addition of parent PCBs decreased the evenness and richness of microbial communities in the sediment (Xu et al., 2022). It is likely that PCBs propel the shift in the microbial species, leading to a less diverse community structure. However, in my study T13 was indicated as the most diverse replicate (Figure 4.20).

I hypothesized that the use of hexa-chlorinated biphenyls as the parent PCBs stimulated a more diverse dechlorinating community than treatments spiked with penta-chlorinated biphenyls. When combining multiple *Dehalococcoides* strains (CG1, CG4, CG5), researchers found that new, emerging dechlorination pathways further degraded PCB congeners (Chen et al., 2024). Therefore, it is likely that different strains of dechlorinating bacteria are responsible for each step of the dechlorination process. Spiking with hexa-chlorinated biphenyls increases the number of intermediate dechlorination products, which can lead to the enrichment of specific bacteria strains to further degrade the PCBs. Since T17, a high-dechlorinating replicate, was spiked with penta-chlorinated biphenyls, there are less intermediate dechlorination products which may explain a less diverse community found in the replicate.

My study suggested that elevated zinc(II) and copper(II) concentrations inhibited TCE and PCB dechlorination by increasing the lag time before cis-DCE appeared in the microcosms and lowering the concentration of PCB product congeners. Additionally, the presence of zinc and copper affected enantioselective degradation of all PCB parent congeners apart from PCB 91 in zinc treated microcosms. The use of zinc and copper decreased overall microbial diversity in the treatments, so it is possible that the lack of microbial diversity led to overall decrease in dechlorination. Perhaps some strains of bacteria that are responsible for enantioselective dechlorination were inhibited due to elevated metal concentrations. Though it is more likely that zinc and copper affect enzymatic reactions leading to enantioselectivity since *Dehalococcoides* is suggested to have a higher tolerance for metal concentrations. However, the elevated concentrations may affect the synergistic microbial population that aids in the growth of dechlorinating bacteria which could lead to an indirect inhibition of enantioselective dechlorination. In addition to the zinc and copper, the parent congeners spiked in the treatments

also affected microbial diversity. Microcosms spiked with hexa-chlorinated biphenyls exhibited a greater microbial diversity overall compared to treatments spiked with penta-chlorinated biphenyls likely due to more intermediate dechlorination products. It is reasonable to suspect that various strains of dechlorinating bacteria are involved in specific steps of the dechlorination process for PCBs. Therefore, having more intermediate dechlorination products could enrich the specific strains needed for further degradation.

Further research needs to be conducted on whether zinc and copper concentrations inhibit vital microbial populations that aid dechlorinating bacterial growth or if these metal concentrations inhibit imperative enzymatic functions responsible for dechlorination. Additionally, the use of elevated metal concentrations and their effect on enantioselective degradation needs to be further explored to determine if the metals are impacting specific enzymatic reactions, such as forming ligands with co-factors, or inhibiting strains of bacteria with enantioselective dechlorinating enzymes.

4.3 Enantiomer Experiment Results

4.3.1 PCB-95 Dechlorination

Treatments in the Enantiomer Experiment were created by enriching culture from bottle T17 from the Essential Metals Experiment, a highly dechlorinating microcosm. T17 was originally spiked with PCBs 91 and 95, penta-chlorinated biphenyls. For the Enantiomer Experiment, the enrichments were spiked with either PCB (+)-95, (-)-95, or racemic (r)-95. Each treatment included three replicates (see Table 3.4). Figures 4.22-4.24 display the decreasing concentration of each of the spiked PCB.

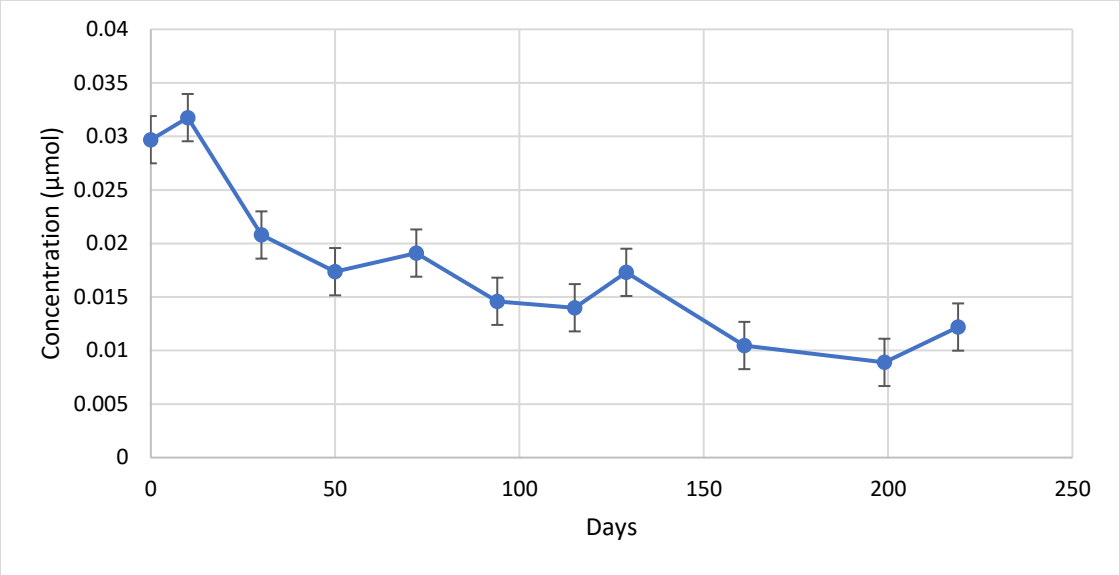

Figure 4.22. Decreasing concentration of (-)-95 in treatments spiked with the individual (-) enantiomer. Error bars represent standard error across replicates.

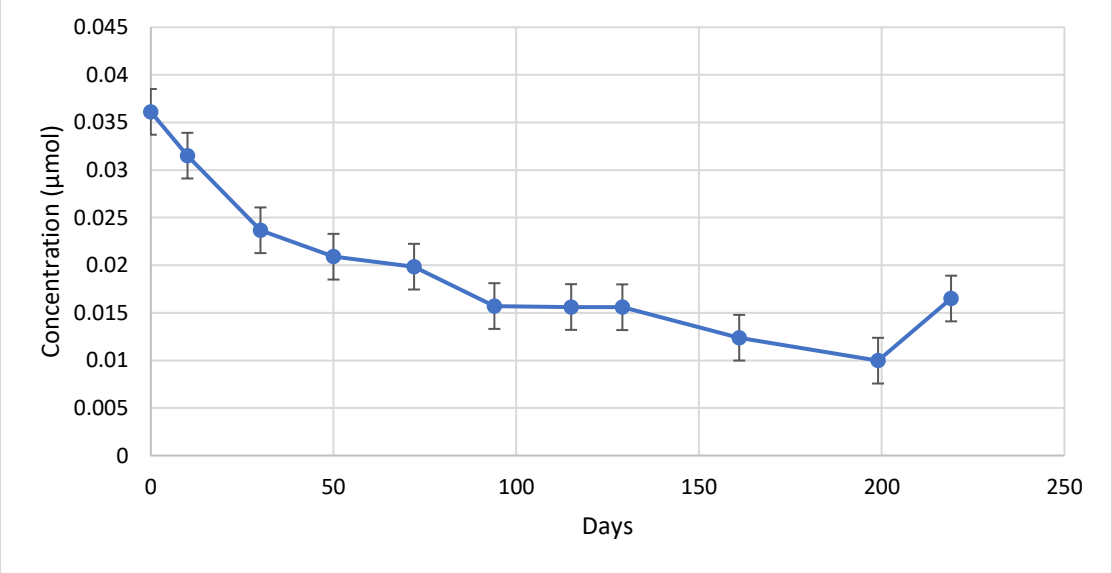

Figure 4.23. Decreasing concentration of (+)-95 in treatments spiked with the individual (+) enantiomer. Error bars represent standard error across replicates.

No dechlorination products were observed in treatments spiked with individual enantiomers of PCB 95; however, the concentration of PCB 95 did significantly decrease over time in these treatments (regression t-test reported p-values < 0.005). It is possible that the

product congeners were lower than the limit of detection for the GC (0.1 µmol for PCBs 51 and 53, 0.08 µmol for PCB 19). Concentrations of the parent congeners that were spiked into the treatments were much lower than my previous experiments (0.49-0.52mg/L). Separating racemic commercial products into individual enantiomers using HPLC was tedious and not well practiced; thus, the concentrations were lower than my previous experiments.

Figure 4.24 illustrates an even dechlorination of both enantiomers of PCB 95 in the racemic treatments. However, there were miniscule differences in the dechlorination of each enantiomer that were dramatically different and are displayed in Figures 4.26 and 4.27. Overall, each treatment in this experiment, racemic or individual enantiomers, showed the dechlorination of PCB 95 over time.

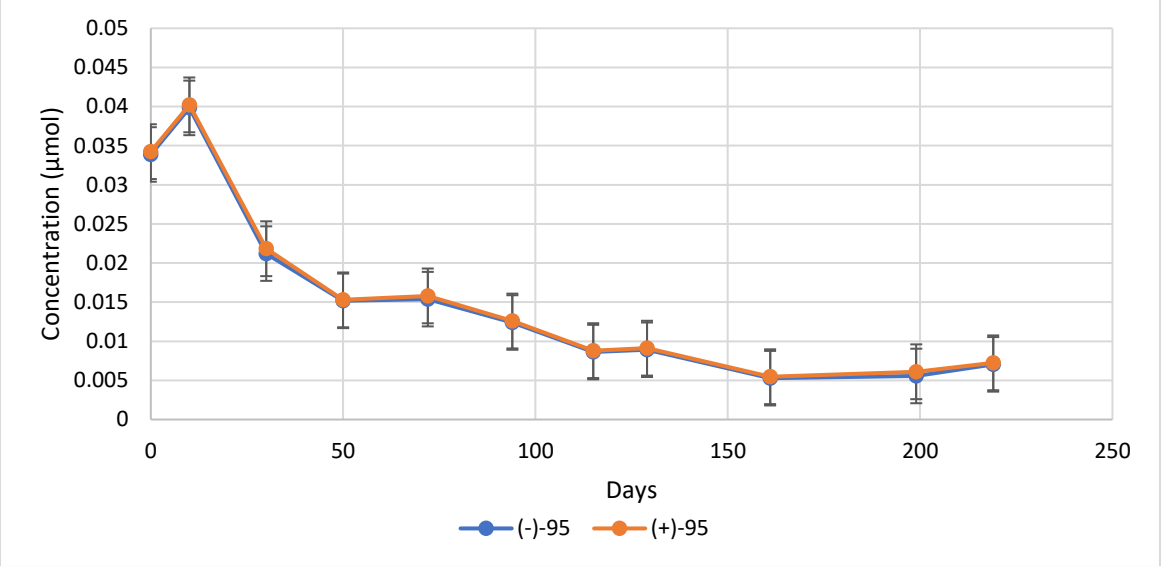

Figure 4.24. Decreasing concentrations of (+)-95 and (-)-95 in treatments spiked with the racemic congener. Error bars represent standard error across replicates.

Figure 4.25 shows the dechlorination products in two out of the three racemic replicates. No dechlorination products were detected in any of the individual enantiomer treatments.

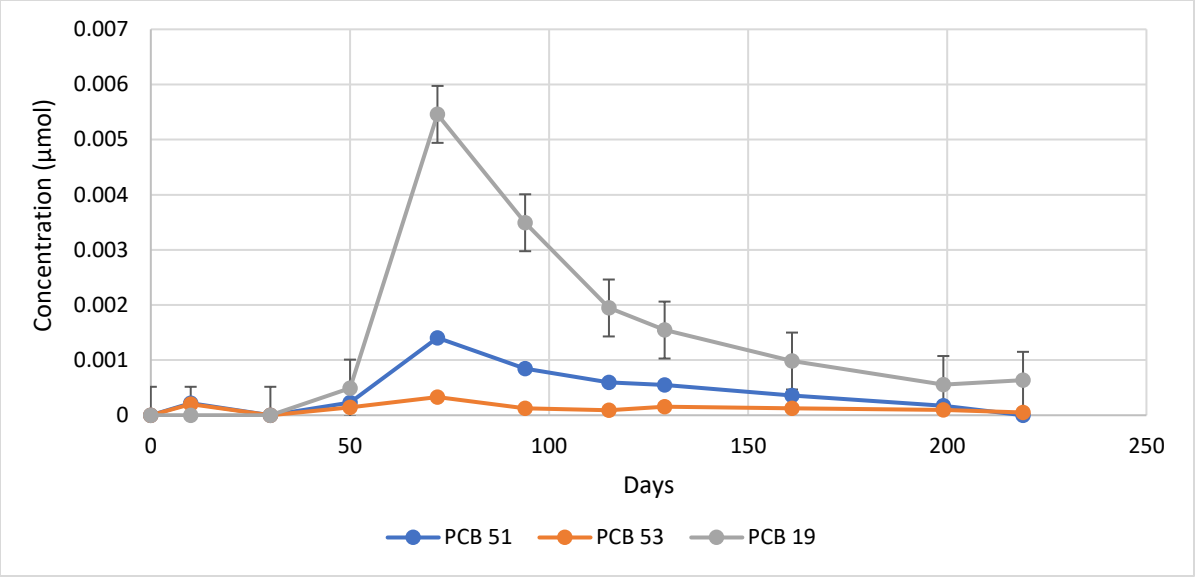

Figure 4.25. Accumulation of dechlorination products for PCBs 95 and 91 in two out of the three racemic treatments. Error bars represent standard error across replicates. Only one replicate, bottle 8, displayed PCB 51 so no error bars were generated for PCB 51.

PCB 51 is a dechlorination product of PCB 91, and likely appeared in these microcosms due to residual concentrations of PCB 91 from the parent culture that was spiked originally with both PCB 95 and PCB 91. Both PCB 51 and 53 dechlorinate to PCB 19, so for the one replicate that has PCB 51 accumulating, the congener that is favored for dechlorinating into PCB 19 is unknown in that treatment since both PCB 53 and 51 were present in the microcosm (Figure 4.11).

Figures 4.26 and 4.27 show the EF values over time for both PCB 95 and PCB 91 in two racemic replicates. I decided to show the changing EF values over time instead of comparing day 0 to day 279 since the data suggested that the EF values changed almost weekly. There is an overall trend for both PCB 91 and PCB 95, but it is imperative to show how the changes in EF values may not be constant.

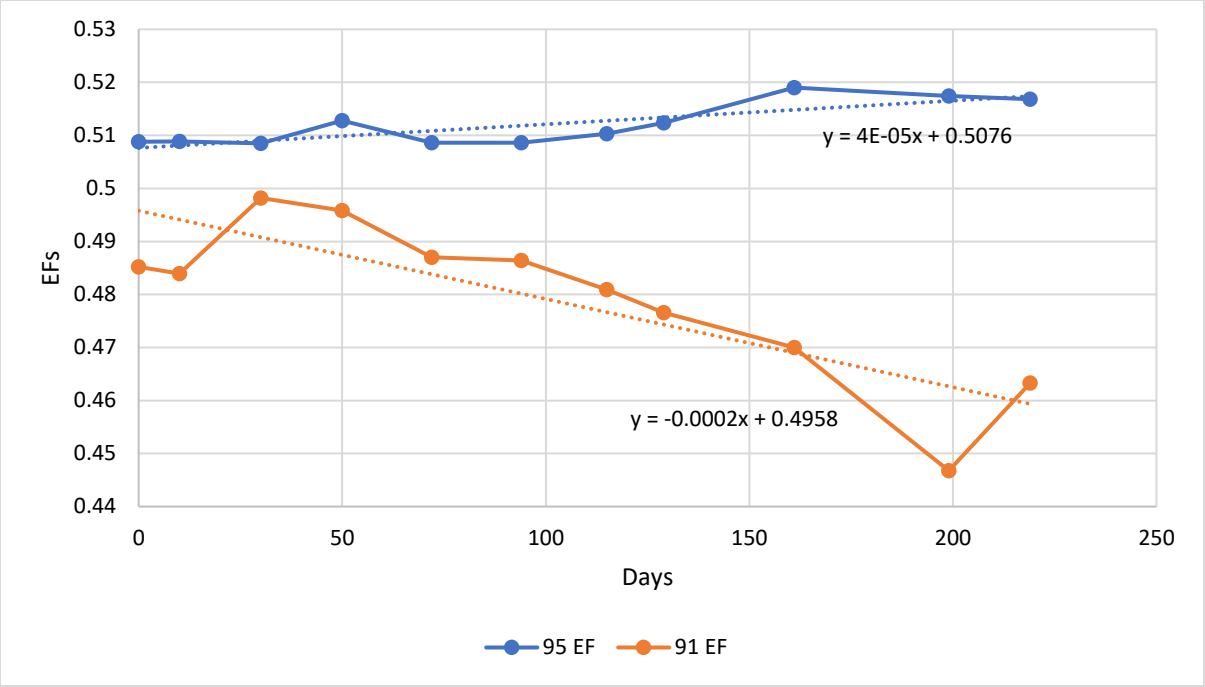

Figure 4.26. Changing EF values for PCB 95 and 91 over time in racemic replicate 7. Regression line was generated for a t-test comparing day 0 to day 279 EFs.

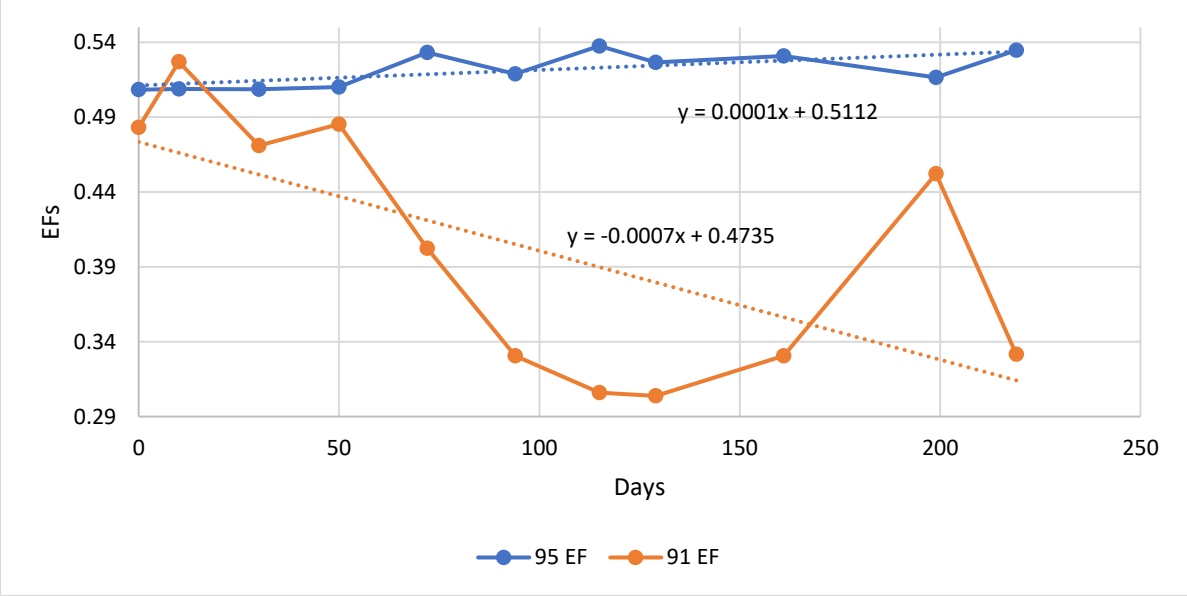

Figure 4.27. Changing EF values for PCB 91 and 95 over time in racemic replicate 8. Regression line was generated for a t-test comparing day 0 to day 279 EFs.

Racemic replicate 7 reported a significant p-value for both PCB 95 and PCB 91 EF values from day 0 to the final day (279 d) using a regression t-test (0.004 and 0.001, respectively) indicating that the EF changed significantly over time. Figure 4.27 indicates that racemic replicate 8 had changing EF values for both PCB 95 and 91; however, the regression t-test reported that the changes were not statistically significant (p-values were 0.07 for both PCBs).

The two racemic replicates resulted in products of PCB 95 while only one replicate (8) showed dechlorination products of PCB 91. Residual concentrations of PCB 91 from the enrichment of the sediment cultures were recorded in all the microcosms, and while the concentrations were low, one replicate did show further dechlorination of PCB 91 to PCB 51 (Figure 4.25). The EF values for PCB 91 did not steadily increase or decrease indicating that likely both enantiomers were favored for dechlorination. Overall, the EF value for PCB 95 increased slightly, indicating that the (+) enantiomer accumulated in the microcosms and the EF value for PCB 91 decreased, meaning the (-) enantiomer accumulated in the treatments.

Since the microcosms spiked with individual enantiomers did not produce any quantifiable dechlorination products, perhaps the presence of both enantiomers is necessary to stimulate microbial activity. Likely the interaction of both enantiomers can upregulate and induce the enzymes responsible for dechlorination.

4.3.2 Discussion of Enantiomer Experiment Results

As discussed earlier in the Essential Metals Experiment, prior studies using sediment-free *in vitro* assays or pure cultures of *Dehalcoccoides mccartyi* indicated mainly a preference for dechlorinating the (-) enantiomer (Yu et al., 2018; Qiu et al., 2020). Qiu et al. (2020) quantified 38 of 164 sediment cultures as containing RDases *pcbA4* or *pcbA5* and only one culture

contained *pcbA1*. However, 135 of the cultures dechlorinated PCBs in an enantioselective manner, indicating that *pcbA4*, *pcbA5*, and *pcbA1*, may not be the only RDases responsible for PCB dechlorination (Qiu et al., 2020). Additionally, the identified RDases are bifunctional, meaning they dechlorinate PCE and PCBs. It is likely that non-identified RDases exist for dechlorinating only PCBs and could be enantioselective.

Only a few strains of *Dehalococcoides* that can dechlorinate PCBs have been isolated, so it is possible that there are other specific strains of these fastidious bacteria present in sediment communities that contain various RDase genes responsible for specific PCB degradation and enantioselectivity. An enriched culture and crude cell lysate study using *Dehalococcoides mccartyi* strain CG1 observed that *pcbA1* preferentially attacked the (-) enantiomer in chiral PCBs 183, 174 and 132, suggesting that the RDase gene *pcbA1* (identified by isolating strain CG1) was stereoselective (Yu et al. 2018; Wang et al., 2014). Additionally, reinforcement of this hypothesis is a study using strain CG1 that reported the accumulation of (+)-91 as a product from the dechlorination of PCB (-)-132 at an EF of greater than 0.9, meaning that the (-)-91 was favored for dechlorination (Huang et al., 2024).

In a microcosm study conducted using sediment from the Twelve Mile Creek arm of the Lake Hartwell watershed spiked with Aroclor 1254, Pakdeesusuk et al. (2003) found that the product congener of 149, PCB 95, accumulated a significant EF value after incubation. My retrospective analysis showed that (-)-95 was favored for dechlorination in the microcosms. Contrastingly Huang et al. (2024) showed that the EF value for PCB 91 decreased, indicating that the (+)-91 was favored for dechlorination from the racemic parent congener, PCB 132 using an enriched culture of *Dehalococcoides mccaryti* strain CG1. Neither the parent congeners, PCB 149 nor PCB 132 showed any significant enantiomeric fractions after incubation (Pakdeesukuk

et al., 2003). Additionally, my results are supported by the findings of Huang et al. (2024), since the (+)-91 enantiomer in the racemic replicate was favored for dechlorination, leading to a lower EF value.

In another study, sediment cores were collected throughout the Twelve Mile Creek arm of Lake Hartwell that displayed changing enantiomeric ratios (ERs) calculated by (+)/(-) for PCBs 149, 136, 95, and 91 as reported by Wong et al. (2001). My retrospective analysis found that as PCBs migrated from the point source, accumulation of the (+) enantiomer was shown for PCBs 149 and 136, indicating that the (-) was favored for dechlorination in the sediment microbial community at several different locations. The ERs for PCB 91 shifted to be more racemic as samples moved away from the point source, while PCB 95 became significantly less racemic. The (+)-95 enantiomer concentrations increased as samples were collected further from the point source, and the (-) enantiomer for PCB was at a higher concentration closer to the point source, which is Town Creek. Sediment for my study was collected at Town Creek, which aligns with my results indicating that (-)-91 accumulated in the microcosms. The (-)-95 enantiomer was favored for dechlorination in my study with a statistically significant EF value indicating non-racemic levels of PCB 95, which was in contrast to the findings of Wong et al. (2001). The difference could be due to my use of halo-priming and enriching my treatments prior to analyzing the EF values. It is likely that those techniques proliferated specific bacterial strains responsible for enantioselective dechlorination.

A study using contaminated surface sediments from various urban rivers in China quantified EF values for nine PCB congeners (Lu et al., 2021). Of 11 samples containing PCB 91, nine of the samples resulted in an EF value greater than 0.52, and three with an EF value lower than 0.48 indicating that a majority of the samples favored (+)-91 dechlorination. Only one

sample detected PCB 95 and recorded an EF value less than 0.48, implying that the (+)-95 enantiomer was favored for dechlorination. In my treatments, the (+)-91 enantiomer was also favored for dechlorination, but the (-)-95 was favored for dechlorination. However, one sample for PCB 95 does not provide much statistical power for comparison to my study.

Another study collected EF values for various chiral PCBs in Hudson River (NY, USA) sediment, air, water, and phytoplankton (Asher et al., 2007). PCB 95 was found to be non-racemic in all phases aside from the air samples. At the time, researchers did not know the elution order of PCB 95 using the Chiralsil-Dex column, but I can infer the results since my methodology was the same. The (+)-95 enantiomer accumulated in each phase, expect air. The microbial community present in the Hudson River Estuary favored the (-)-95 enantiomer for dechlorination, which supports the findings of my study. Samples from the Hudson River Estuary had inconclusive results for PCB 91 since it was infrequently detected in the samples.

The variation of EF values across pure cultures or sediment cultures is likely due to the difference in the microbial community. In the enriched *Dehalococcoides mccaryti* strain CG1 cultures, the (-) enantiomer appeared to be favored regardless of the parent congener spiked (Yu et al., 2018; Qiu et al., 2020). Since my study partially contradicted these findings, it was possible that the microbial community in Town Creek sediment had a diverse set of *Dehalococcoides* strains that were responsible for the difference in EF values. Additionally, only the presence of both enantiomers resulted in dechlorination products, even though the dechlorination of PCB 95 was statistically enantioselective in one racemic replicate.

For future studies, individual enantiomers of PCB 91 should be spiked into sediment microcosms since 91 appeared to be more favorable for dechlorination over PCB 95. Dang et al. (2013) reported non-racemic values for PCB 91, while PCB 95 was racemic in the water column,

suggesting that PCB 91 may be more favored for enantioselective dechlorination. Even at low concentrations, the microbes present in the sediment continued to dechlorinate PCB 91 in an enantioselective manner, favoring the (+).

If my study were to be repeated, I suggest spiking with higher concentrations of (-)-95 and (+)-95 in the microcosms to decrease the possibility of the product congeners being lower than the detection limit in the GC (0.03 mg/L for PCBs 51 and 53, 0.02 mg/L for PCB 19). I did not spike with higher concentrations of the product congeners since the HPLC methods are tedious and time-consuming. Spiking with higher concentrations of parent congeners can also help induce enzymes for dechlorination or proliferate dechlorinating bacteria (Bedard et al., 2006).

5. CONCLUSIONS, IMPLICATIONS, AND RECOMMENDATIONS

My studies provide novel information regarding the dechlorination of TCE and PCBs in microcosms conducted using contaminated sediment from Town Creek, SC, USA. In this chapter, I provide the main takeaways of my research in the conclusion section. In the implications section, I provide reasoning for continuing research based on my findings. The recommendations section provides information on future directions to further scientific knowledge on the dechlorination of PCBs and TCE in natural systems.

5.1 Conclusions

In my first study, the use of TCE as a halo-primer reduced the lag time previously observed from microcosms using sediment from the Lake Hartwell watershed. In comparison to an enriched *Dehalococcodies* culture, the SRNL culture, the lag time before PCB dechlorination was greater in my sediment treatments. Likely there are bifunctional RDases that were present in the SRNL culture that were responsible for dechlorinating TCE and PCBs which led to a faster accumulation rate for the product PCB congener. However, the sediment microcosms dechlorinated the parent congeners to downstream products to a greater extent than the SRNL culture, indicating that the sediment microbial community likely has more strains of bacteria that are specialist in dechlorination of PCBs.

My results suggest that there are specific RDases for only PCB dechlorination that were likely present in the sediment microcosms and absent in the SRNL culture. The SRNL culture had only been exposed to PCE for generations, so it is unlikely that there were any bacterial strains or RDases responsible for only PCB dechlorination. Although, when compared to other studies reporting PCB dechlorination using sediment from natural systems, the rates for my sediment microcosms and the SRNL culture were slower. The literature provides evidence that

likely the microbial community and sediment composition influenced the rate and which product congeners were produced. The presence of more intermediate products may stimulate further bacterial growth and enzymatic functions leading to faster dechlorination rates.

My second study using elevated zinc(II) and copper(II) concentrations inhibited TCE and PCB dechlorination by increasing the lag time. Metal amendment increased the lag time of TCE dechlorination to *cis*-DCE by around 35 days in sediment microcosms. The presence of zinc and copper lowered the concentration and diversity of PCB products. Sediment microcosms containing metal amendments displayed only one dechlorination product, PCB 91, from the parent congener, PCB 149. Additionally, no terminal products, PCB 19 (from dechlorination of PCB 95 and 91 in the penta-chlorinated biphenyls and PCB 149 in the hexa-chlorinated biphenyls) or PCB 54 (from dechlorination of PCB 136), were reported in the metal amended treatments.

The presence of zinc and copper affected enantioselective degradation of all PCB parent congeners except for PCB 91 in the zinc amended microcosms. Sediment microcosms with no metal amendments and PCB 91 in the zinc treatments did not display significant EF values from day 0 to day 389. Metal amendments accumulated more of the (-) enantiomer after incubation, determining that the (+) enantiomer was favored for dechlorination. Only the penta-chlorinated biphenyl microcosms with elevated copper concentrations displayed an increase in (+)-PCB 91 enantiomer, indicating that the (-)-91 was favored for dechlorination.

The use of zinc and copper decreased overall microbial diversity in the treatments, so it is possible that the lack of microbial diversity led to an overall decrease in dechlorination. The Faith PD plot may suggest that a greater level of diversity indicates more activity, since at least two of the active dechlorinating bottles were present in the non-amended treatments (Figure

4.19). The Kruskal-Wallis p-value calculated across all groups was 0.02 signifying that the elevated essential metal concentrations lowered the microbial diversity. The alpha rarefaction curve showed that the treatments containing metal amendments exhibited lower microbial diversity compared to the non-amended treatments (Figure 4.20).

The presence of zinc and copper affected both the lag time for TCE and PCB dechlorination, PCB product concentrations and diversity, EF values and sediment microbial diversity. Metal amendments increased the lag time for both TCE and PCB dechlorination. Copper and zinc influenced the dechlorination products of PCB 149 and PCB 95, leading to a decrease in diversity of the product congeners and their concentrations. All metal amended microcosms except for PCB 91 in zinc treatments displayed significant EF values. The elevated concentrations of zinc and copper led to an overall decrease in the diversity of the sediment microbial community.

In addition to the zinc and copper, the parent congeners spiked in the treatments also affected microbial diversity. Microcosms spiked with hexa-chlorinated biphenyls exhibited a greater microbial diversity overall compared to treatments spiked with penta-chlorinated biphenyls (Figure 4.20). In my study, T13 was the most diverse replicate (Figure 4.20). Based on these data, spiking with hexa-chlorinated biphenyls as the parent PCBs stimulated a more diverse microbial community than treatments spiked with penta-chlorinated biphenyls.

In the third study, sediment microcosms were spiked with racemic and individual enantiomers of PCB 95 with residual concentrations of PCB 91. Treatments with individual enantiomers of PCB 95 reported no dechlorination products. Only the microcosms spiked with racemates of PCB 95 resulted in dechlorination products. Two out of the three racemic replicates showed further dechlorination of PCB 95. Since these microcosms were created from

enrichments, there was residual concentrations of the parent congener PCB 91, which was further dechlorinated in one treatment containing racemic PCB 95. Interestingly, the dechlorination of both PCB 91 and PCB 95 were enantioselective in these treatments. The EF values for both PCB 91 and 95 did not steadily either increase or decrease indicating that likely both enantiomers were favored for dechlorination. Overall, the EF value for PCB 95 increased slightly, indicating that the (+) enantiomer accumulated in the microcosms and the EF value for PCB 91 decreased, meaning the (-) enantiomer accumulated in the treatments. In the racemate treatments, (-)-95 and (+)-91 were favored for dechlorination.

5.2 Implications

Many natural systems are co-polluted with TCE, PCBs, and hazardous metals making my study important to understanding how elevated metal concentrations can affect dechlorination. Copper and zinc are naturally occurring metals in natural systems that had not been previously explored for their effect on PCB dechlorination. Town Creek was a unique environmental location to observe how these metals affect PCB dechlorination since the only other pollutants were chlorinated solvents.

Perhaps some strains of bacteria that are responsible for enantioselective dechlorination were inhibited due to elevated metal concentrations. Though it is more likely that zinc and copper affect enzymatic reactions leading to enantioselectivity since *Dehalococcoides* is suggested to have a higher tolerance for metal concentrations. However, the elevated concentrations may affect the synergistic microbial population that aids in the growth of dechlorinating bacteria which could lead to an indirect inhibition of enantioselective dechlorination.

Microcosms spiked with hexa-chlorinated biphenyls exhibited a greater microbial diversity overall compared to treatments spiked with penta-chlorinated biphenyls likely due to more intermediate dechlorination products. It is reasonable to suspect that various strains of dechlorinating bacteria are involved in specific steps of the dechlorination process for PCBs. Therefore, having more intermediate dechlorination products could enrich the specific strains needed for further degradation.

Speculating based on my results and the literature; it may be possible that the interaction of both enantiomers with the enzymes induced further dechlorination in my Enantiomeric Experiment. It is also likely that dechlorination products of the individual enantiomers were below the limit of detection for the GC (0.1 µmol for PCBs 51 and 53, 0.08 µmol for PCB 19) since there was a consistent decrease in the parent congeners in the non-racemic treatments.

Remediation is the final goal of my research, but further areas need to be explored before bioremediation techniques for PCBs can be employed. Given my research, I can infer that systems with higher concentration of metals are going to exhibit longer lag times for dechlorination of PCBs due to the toxicity on the synergistic microbial community. To remediate, perhaps higher concentrations of imperative bacteria such as fermentators, metal-reducers, sulfate-reducers, etc. should be present in the media administered to PCB and metal co-contaminated aquifers. Or, cultures of these vital bacteria could be exposed to higher metal concentrations over time in an attempt to increase their resistance to the metal toxicity.

5.3 Recommendations for Future Research

To expand on my study observing the effects of TCE as a halo-primer for PCB dechlorination in sediments and pure cultures, future research should focus on identifying more RDases that may be responsible for only PCB dechlorination. Likely the lack of further PCB

dechlorination products in the SRNL culture was due to the culture not containing bacterial strains that can dechlorinate specific PCB congeners. Additionally, this study could be replicated with more PCB congeners to determine if the SRNL culture can further dechlorinate PCBs. A limitation of the study was that it included only one congener (PCB 136) that had a dechlorination pathway with only one intermediate before reaching a terminal product. For example, PCB 149 could be used as a parent congener in the SRNL culture since it has more diverse dechlorination pathways than PCB 136 (Figures 4.3 and 4.11). If the SRNL culture shows diversity in its dechlorination, and can further dechlorinate PCB 149 products, then perhaps the *Dehalococcoides* strains present in the enriched culture have more bifunctional RDases for TCE and PCB dechlorination than observed in my study. Identifying novel RDases that can only dechlorinate PCBs and the bacterial strains that employ them could help with creating more effective enriched cultures designed to dechlorinate PCBs. These enriched cultures could be used at hazardous waste landfills containing PCBs to remediate the tons of contaminated sediment and soil stored in them.

My study using elevated zinc and copper concentrations indicated that these metals inhibited microbial diversity. However, further research needs to be conducted on whether zinc and copper concentrations inhibit vital microbial populations that aid dechlorinating bacterial growth or if these metal concentrations inhibit the enzymatic functions responsible for dechlorination. Additionally, the use of elevated metal concentrations (either essential or hazardous) and their effect on enantioselective degradation needs to be further explored to determine if the metals are impacting specific enzymatic reactions, such as forming ligands with co-factors, or inhibiting strains of bacteria with enantioselective dechlorinating enzymes. I suggest focusing on using enriched cultures with elevated zinc and copper concentrations, since

it may be easier to run further DNA analysis on these cultures instead of sediment microbial communities. By using enriched cultures, it may be simpler to obtain analysis on the RDase concentrations to determine if zinc and copper are affecting the induction of particular RDases or bacterial strains. Additionally, further studies could focus on changing concentrations of known cofactors for *Dehalococcoides* along with manipulating zinc and copper concentrations to determine if zinc and copper are forming ligands with certain cofactors which leads to the inhibition of PCB dechlorination.

In my study with sediment microcosms spiked with enantiomers of PCB 95, I noticed that PCB 91, in residual concentrations, was more favored for dechlorination. For future studies, individual enantiomers of PCB 91 should be spiked into Town Creek sediment microcosms. Even at low concentrations, the microbes present in the sediment continued to dechlorinate PCB 91 in an enantioselective manner, favoring the (+). If my study were to be repeated, I suggest spiking with higher concentrations of (-)-95 and (+)-95 in the microcosms to decrease the possibility of the product congeners being lower than the detection limit in the GC. Since separating the enantiomers using HPLC methods is tedious, I was not able to achieve higher concentrations (-)-95 and (+)-95 in my microcosms. Spiking with higher concentrations of parent congeners can also help induce enzymes for dechlorination or proliferate dechlorinating bacteria.

Additionally, it would be important to determine the RDases responsible for enantioselective dechlorination. I suggest using enriched cultures spiked with higher concentrations of individual enantiomers and conducting DNA and enzyme analysis. DNA analysis will identify which strains of bacteria are enriched in the cultures with specific enantiomers to determine if any of those strains are enantioselective. Furthermore, enzyme analysis can determine which RDases are responsible for enantioselective dechlorination. Since

my results suggest that both enantiomers need to be present to induce PCB dechlorination, it may be interesting to identify if there are more bacterial strains or RDases present in racemic treatments.

APPENDIX

Supplemental Methodology

There was leftover PCB 19 (ordered by a previous student from AccuStandard), so I used the remainder for my stock solutions. To determine how much in mass of PCB 19 was left, I weighed the vial with the solids on a microscale before dissolving all the solids in isooctane and weighing the dried, empty vial. The different milligram amounts were due to stochiometric synthesizing by AccuStandard and were not requested to be larger than 5 mg, or to already be dissolved in a solvent.

I created my calibration curve solutions of all PCB congeners from the prior stock solutions of each PCB. PCBs 54 and 53 were in 50 mg/L concentrations, so 27 mL were removed and dried down to 2 mL dissolved in isooctane. Both of the 2 mL were added to a 50 mL volumetric flask. PCBs 51, 102, 96 were in 100 mg/L concentrations, so 13.5 mL were removed and dried to 2 mL. All three of the aliquots of 2 mL were added to the same 50 mL volumetric flask mentioned above. PCBs 136 and 149 were in 360. 9mg/L concentrations, so 3.74 mL were removed and added to that flask. PCBs 95 and 91 were in 326.4 mg/L concentrations, so 4.14 mL were removed and added to the same 50 mL volumetric flask. PCB 19 is in 257.5 mg/L concentration, so 5.24 mL were removed and added to the volumetric flask. All of them added made a volume of 31 mL. Using isooctane, the flask was diluted up to the 50 mL mark. Once at the 50 mL mark, 25 mL was removed by decanting into a 25 mL volumetric flask. Then the contents were transferred to a clean 50 mL volumetric to be diluted with 25 mL of isooctane to get a concentration of 13.5 mg/L. This process was repeated four more times to get total PCB standard (congeners 19, 53, 51, 54, 96, 102, 95, 91, 149, and 136) concentrations of 27 mg/L, 13.5 mg/L, 6.75 mg/L, 3.375 mg/L, 1.6875 mg/L, and 0.84375 mg/L. Lastly, 16.6

µL of 60.2 mg/L Aldrin was added to each of the standards using a microliter syringe. This brought the final concentration of Aldrin to 0.04 mg/L in each of the standards, except the 0.844 mg/L had a total volume of 50 mL. Thus, 33.2 µL of Aldrin was added to that standard.

Early on, the calibration process encountered an issue due to contamination of the GC. GC-ECD peaks began to flatline at the 13.5 mg/L PCB standard solution. This caused a permanent contamination of PCB 136 on the gold seal that rests below the inlet liner. Thus, all consumables (inlet liner, septum, o-ring and gold seal with washer) were replaced to remove the contamination. The first 10 inches of the column was also cut and re-installed in case PCBs were trapped in the beginning of the column. All these repairs solved the contamination problem. To avoid future contamination issues, the 13.5 mg/L and 27 mg/L stock solutions were never run on the GC again.

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