BIOREMEDIATION OF MARINE SEDIMENTS CONTAMINATED BY A MIXTURE OF PERSISTENT ORGANIC POLLUTANTS

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

in Biotechnology

by
Miray KURTOĞLU

January 2025
İZMİR

We approve the thesis of **Miray KURTOĞLU**

Examining Committee Members:

Asst. Prof. Dr. Hale DEMİRTEPE
Department of Environmental Engineering, İzmir Institute of Technology

Assoc. Prof. Dr. Altan ÖZKAN
Department of Environmental Engineering, İzmir Institute of Technology

Assoc. Prof. Dr. Burcu KAPLAN TÜRKÖZ
Department of Food Engineering, Ege University

24 January 2025

Asst. Prof. Dr. Hale DEMİRTEPE
Supervisor, Department of
Environmental Engineering,
İzmir Institute of Technology

Assoc. Prof. Dr. Hatice Eser ÖKTEN
Co-Supervisor, Department of
Environmental Engineering,
İzmir Institute of Technology

Asst. Prof. Dr. Arzu UYAR
Head of the Department of
Biotechnology

Prof. Dr. Mehtap EANES
Dean of the Graduate School of
Engineering and Sciences

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to my advisor, Asst. Prof. Dr. Hale DEMİRTEPE, for her invaluable guidance, encouragement, and support throughout my thesis study. Her expertise and dedication have been instrumental in the successful completion of this work. I would also like to extend my heartfelt appreciation to my co-advisor, Assoc. Prof. Dr. Hatice Eser ÖKTEN, whose insightful feedback and unwavering support have greatly enriched this study. It has always been my aspiration to work with remarkable female scientists, and I am deeply honored to have learned from and collaborated with such exceptional mentors.

I also sincerely thank my jury members, Assoc. Prof. Dr. Altan ÖZKAN and Assoc. Prof. Dr. Burcu KAPLAN TÜRKÖZ, from the Department of Food Engineering at Ege University, for their valuable time and efforts as part of the jury. I also am deeply grateful to the respected scholars, Dr. Afife GÜLEÇ, Assoc. Prof. Dr. Ali Oğuz BÜYÜKKİLECİ, Assoc. Prof. Dr. Alper BAYRAKDAR, Asst. Prof. Dr. Mertol GÖKELMA, and Prof. Dr. Ferda SOYER, for their valuable contributions in terms of materials, equipment, knowledge, and support throughout my academic journey.

Through this journey, I have learned that no great accomplishment can ever be achieved alone but together with those who stood by me every step of the way. To my dearest friends —Anastasiia 'Nastya' KORZHOVA, Berkutay ZAİM, Berna Bahar ÖREN, Deniz TURAN, Filiz BAŞER, İlker ERÜNSAL, Melike CİHAN, Naz KÖK, Sinan SANCAK, Şebnem GENİŞOĞLU, and Yalın Çağdaş EŞSİZ—your belief in me carried me further than I ever thought possible.

I owe everything to my incredible parents, who have been my rock and my greatest source of strength. Your endless support, patience, and love have been the secret ingredient. To my beloved cats —Eko, Irma, Kiki, and Tekir— and the countless stray animals I care for, thank you for being the chaotic yet comforting force in my life. You've been my furry therapists and reminders that small acts of love matter.

Last but not least, thank you, Miray.

This study was funded by the Scientific and Technological Research Council of Türkiye (TÜBİTAK, Grant No: 122Y005), and Izmir Institute of Technology Coordination of Scientific Research Projects (Grant No: 2024IYTE-1-0038).

ABSTRACT

BIOREMEDIATION OF MARINE SEDIMENTS CONTAMINATED BY A MIXTURE OF PERSISTENT ORGANIC POLLUTANTS

Persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), existing as mixtures in the environment, pose significant risks to ecosystems and human health. Removing these pollutants from contaminated environments, i.e. remediation, offers a solution to this global issue. This study investigated the effectiveness of bioremediation strategies, specifically bioaugmentation (BA) and biostimulation (BS), in sediment microcosms using historically contaminated sediments from shipbreaking yards in the heavily industrialized area of Aliağa, İzmir. Microbial analyses revealed 37.6% abundance of *Chloroflexi*-related microorganisms in the sediments, which were subsequently enriched for BA treatment. BS treatment involved adding micronutrients to stimulate native microbial activity. BA and BS achieved higher removal efficiencies for total PCBs (14% and 19%, respectively) and PBDEs (33% and 24%) compared to natural attenuation (12% for PCBs and 8% for PBDEs) set, proving the importance of external amendments for better removal. Degradation rates for PCBs were higher in BA (0.00124 chlorine per biphenyl per day-Cl/bp/day) than in BS (0.00085 Cl/bp/day) whereas PBDE degradation was faster in BS (0.00945 bromine per diphenyl ether per day-Br/dp/day) compared to BA (0.00741 Br/dp/day). Although BA and BS strategies reduced total PCB and PBDE concentrations, the formation of lower halogenated congeners indicated an ecotoxicological risk. PCB-52 exhibited consistently high risks while BDE-28 escalated to high risk in BS, and BDE-153 fluctuated between high and moderate risks across all treatments. These findings emphasized the importance and necessity of conducting laboratory-scale studies prior to in situ bioremediation applications to evaluate removal efficiencies and potential risks.

ÖZET

KARIŞIM HALİNDEKİ KALICI ORGANİK KİRLETİCİLERLE KİRLENMİŞ DENİZ SEDİMANLARININ BİYOİYİLEŞTİRİLMESİ

Poliklorlu bifeniller (PCBs) ve polibromlu difenil eterler (PBDEs) gibi kalıcı organik kirleticiler, çevrede karışım halinde bulunarak ekosistem ve insan sağlığı için önemli riskler oluşturur. Bu küresel sorunun çözümü bu kirleticilerin çevresel ortamlardan uzaklaştırılması, yani iyileştirilmesidir. Bu çalışma, endüstriyel faaliyetlerin yoğun olduğu Aliağa, İzmir, Türkiye'deki gemi söküm sahalarından alınan kirlenmiş sedimanlar kullanılarak, biyoogmentasyon (BO) ve biyostimulasyon (BS) gibi biyoiyileştirme stratejilerinin etkinliğini sediman mikrokozmları ile incelemiştir. Mikrobiyal analizler, sedimanlarda *Chloroflexi* filumu ile ilişkili mikroorganizmaların çokluğunu (%37,6) ortaya koymuştur ve bu mikroorganizmalar zenginleştirilerek BO uygulaması için kullanılmıştır, BS uygulamasında ise doğal mikrobiyal aktiviteyi teşvik etmek amacıyla ortama mikrobesinler sağlanmıştır. BO ve BS, toplam PCB'ler için sırasıyla %14 ve %19, PBDE'ler için ise %33 ve %24 olarak giderim verimlilikleri sağlamış; bu değerler, doğal giderim (PCB'ler için %12 ve PBDE'ler için %8) setine kıyasla daha iyi performans gösterdiği için harici katkıların önemini kanıtlamıştır. PCB'ler için bozunma hızları BO'da (0,00124 klor/bifenil/gün-Cl/bp/gün) BS'den (0,00085 Cl/bp/gün) daha yüksek iken, PBDE'lerin bozunma hızı BS'de (0,00945 brom/difenil eter/gün-Br/dp/gün) BO'ya (0,00741 Br/dp/gün) kıyasla daha hızlı olmuştur. BO ve BS stratejileri toplam PCB ve PBDE konsantrasyonlarını azaltmış olsa da, zamanla daha düşük halojenli bileşiklerin oluşumu ekotoksikolojik riski işaret etmiştir. PCB-52 sürekli olarak yüksek risk sergilerken, BDE-28 BS'de yüksek risk kategorisine çıkmış ve BDE-153 tüm uygulamalarda yüksek ve orta risk seviyeleri arasında değişiklik göstermiştir. Bu bulgular, in situ biyoiyileştirme uygulamalarından önce laboratuvar ölçekli çalışmaların yapılmasının, giderim verimliliği ve potansiyel riskleri değerlendirme açısından önemini ve gerekliliğini vurgulamaktadır.

TABLE OF CONTENTS

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

ABBREVIATIONS

AB	Aliağa Bay
AGE	Agarose gel electrophoresis
AOPs	Advanced oxidation processes
ASM-1	Artificial seawater medium-1
ASM-2	Artificial seawater medium-2
ATSDR	Agency for Toxic Substances and Disease Registry
BA	Bioaugmentation
Br/dp	Bromine per diphenyl ether
BS	Biostimulation
C/C_0	The ratio of the current concentration to the initial concentration
Cl/bp	Chlorine per biphenyl
ddPCR	Droplet digital PCR
DDT	Dichlorodiphenyltrichloroethane
deca-BDE	Decabromodiphenyl ether/Decabrominated diphenyl ether
di-CBs	Dichlorinated biphenyls
DI	Distilled water
dw	Dry weight
EDDS	Ethylenediamine-S,S-disuccinic acid
EI	Electron ionization
FRTR	Federal Remediation Technologies Roundtable
GC-MS	Gas chromatography-mass spectrometry
hepta-BDEs	Heptabrominated diphenyl ethers
hepta-CBs	Heptachlorinated biphenyls
hexa-BDEs	Hexabrominated diphenyl ethers
hexa-CBs	Hexachlorinated biphenyls
IS	Internal standard
IUPAC	International Union of Pure and Applied Chemistry
K_{oc}	Organic carbon-water partitioning coefficient
K_{ow}	Octanol-water partitioning coefficient
LC50	Lethal concentration 50%

mono-CBs	Monochlorinated biphenyls
NA	Natural attenuation
NB	Nemrut Bay
NC	Negative control
NCBI	National Center for Biotechnology Information
NOEC	No observed effect concentration
nona-BDEs	Nonabrominated diphenyl ethers
nona-CBs	Nonachlorinated biphenyls
nZVI	Nanoscale zero-valent iron
octa-BDEs	Octabrominated diphenyl ethers
octa-CBs	Octachlorinated biphenyls
PAHs	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
penta-BDEs	Pentabrominated diphenyl ethers
penta-CBs	Pentachlorinated biphenyls
PCBs	Polychlorinated biphenyls
PCR	Polymerase chain reaction
POPs	Persistent organic pollutants
RQ	Risk quotient
SBY	Ship breaking yards
SS	Surrogate standard
TBBPA	Tetrabromobisphenol A
tetra-BDEs	Tetrabrominated diphenyl ethers
tetra-CBs	Tetrachlorinated biphenyls
tri-BDEs	Tribrominated diphenyl ethers
tri-CBs	Trichlorinated biphenyls
UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency

CHAPTER 1

INTRODUCTION

The marine environment is essential as it is the home of diverse ecosystems, provides natural resources, and keeps the Earth's climate stable. However, it is also the ultimate sink for all types of discharges, such as wastewater, atmospheric deposition, and surface runoff. Therefore, marine environmental contamination is a global challenge that needs to be addressed to support healthier ecosystems. Particularly, persistent organic pollutants (POPs) are of great concern since they persist in the environment for extended periods of time and pose a significant risk to the environment and human health due to their toxic and bioaccumulative character. When POPs enter an aquatic environment, they accumulate in the sediments with high organic content. Hence, the sediments become both sinks and sources of POPs contamination in the marine environment.

The strategies to reduce the contamination and, thereby, the risk posed by POPs are called remediation. The remediation strategies involve physical, chemical, and biological techniques to isolate the contamination, reduce the concentration, and/or transform the toxic compounds into less harmful products. Among the techniques, bioremediation has been considered the most sustainable one. However, the application of bioremediation techniques does not guarantee the mitigation of risks as they might result in the formation of more toxic products. Hence, before applying the bioremediation techniques to a contaminated environment, small-scale laboratory tests should be conducted to elaborate on the possible degradation mechanisms, the end-products, and the risks involved.

Aliağa region in İzmir, Türkiye is a heavily industrialized area, where land-based activities involving oil refineries, petrochemical facilities, and iron-steel production plants, and coastal activities, such as ports and shipbreaking yards exist. Previous studies have shown that the atmosphere, soil, seawater, and sediments in the region are contaminated by a mixture of POPs and POP-like chemicals (Odabasi et al. 2017a). These studies identified the shipbreaking yard area as a prominent POPs contaminated zone.

The shipbreaking operation involves the dismantling of deck cranes, transformers, pumps, painted boards, cables, furniture, and flooring material. During the operation, leakage occurs from these materials, which involve the additive chemicals. These chemicals, which are known for their stability, insulating properties, and flame retardancy are mainly polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). In short, shipbreaking operations may result in the release and accumulation of these POPs into the marine environment.

Previously, the presence of dehalorespiring bacteria was linked to the POP contamination in the heavily industrial areas. In these regions, natural biodegradation processes were monitored by enhancing the environment with those bacteria along with nutrient supplementation to observe improvements in POP degradation efficiency (Hendrickson et al. 2002). This demonstrated that bioremediation is an effective method for removing POPs from the contaminated sediments, and can contribute to solving this global challenge. However, no research has been conducted on the remediation of sediments in the Aliağa region, which is a hot-spot for POPs in Türkiye.

The main aim of this study is to investigate the effectiveness of bioremediation strategies to remove POPs that appear as a mixture in ship breaking yard sediments. Small-scale laboratory reactors (microcosms) have been established to represent three bioremediation strategies and to compare their outcomes. The specific objectives are:

1. To determine the abundance of dehalorespiring bacteria in Aliağa region sediments.
2. To test the effectiveness of bioaugmentation using the enriched indigenous microorganisms, biostimulation using micronutrients, and natural attenuation reflecting the natural biodegradation capacity of the ship breaking yard sediments.
3. To examine the differences between bioremediation strategies in reducing the POPs concentrations, enhancing the rate of biodegradation, achieving biodegradation pathways, and altering the ecotoxicological risk posed by these POPs.

The study has three novel aspects. The first aspect is related to the use of historically contaminated sediments. The ship breaking operations have been ongoing since 1977, hence the POPs in the sediments might be strongly sorbed, which is a challenge for their removal. Since most of the literature studies observe biodegradation in artificially contaminated sediments, the degradation rates proposed by such studies would not represent the natural environmental conditions. Therefore, the results of this

study are expected to provide insights into the environmental biodegradation rates. Secondly, organohalide respiring bacteria, which can utilize PCBs and PBDEs as electron acceptors, are investigated in the study area for the first time. Here, the hypothesis is that they might be abundant in the ship breaking yard sediments, compared to the sediments in the close vicinity. Lastly, the biodegradation of POPs as a mixture has been the focus of a limited number of studies. Therefore, this study would help in understanding the concurrent removal of PCBs and PBDEs in the sediments.

In Chapter 2, detailed information on the general characteristics of PCBs and PBDEs, degradation mechanisms in sediments, remediation of contaminated sites, and Aliağa region as the contaminated area of interest was given.

In Chapter 3, the molecular, chemical, and instrumental techniques used in this study were explained in addition to the relevant data analyses.

In Chapter 4, the results of environmental DNA analysis relating to the enrichment studies were shared. Then, the anaerobic degradation trends and rates of bioremediation strategies tested in sediment microcosm studies were presented and discussed. Lastly, ecotoxicological risks of PCBs and PBDEs were evaluated.

In Chapter 5, the conclusion was made upon the results of the bioremediation strategies based on their comparative effectiveness in reducing PCB and PBDE levels. Recommendations for future research were shared.

CHAPTER 2

LITERATURE REVIEW

2.1. Persistent Organic Pollutants

Persistent organic pollutants (POPs) are a group of chemicals known for their persistence in the environment, tendency to bioaccumulate in organisms, and detrimental effects on human health and ecosystems (Jones and de Voogt 1999). These substances are highly resistant to degradation, build up in biological systems, and can be transported over long distances through air, resulting in widespread contamination (Wania and Mackay 1996). The Stockholm Convention on Persistent Organic Pollutants, an international treaty, pursues to safeguard human health and the environment by regulating or phasing out the production and use of POPs (United Nations Environment Programme (UNEP) 2001).

These substances have a propensity to accumulate in the fatty tissues of living organisms, including humans. This accumulation increases with each trophic level in the food chain, leading to high concentrations in top predators (Kelly et al. 2007). El-Shahawi et al. (2010) stated that POPs exhibit various toxic effects, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and endocrine disruption.

POPs originate from a variety of sources, both intentional and unintentional. Intentional production and use, particularly in the past, include pesticides like dichlorodiphenyltrichloroethane (DDT) and industrial chemicals like polychlorinated biphenyls (PCBs) (Jones and de Voogt 1999). Unintentional production of POPs arises as byproducts of industrial activities, such as waste incineration or the combustion of organic materials (Lohmann et al. 2007). Natural sources, like volcanic eruptions and forest fires, contribute to background levels, but their impact is generally less significant than

anthropogenic sources. Additionally, contaminated sites act as reservoirs, continuously releasing POPs into the environment (Meijer et al. 2003).

2.1.1. Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are human-made organic chemicals, listed as one of the POPs by the Stockholm Convention in 2004. Structurally, PCBs consist of two benzene rings connected by a single bond, with one to ten chlorine atoms attached to their biphenyl structure, resulting in 209 possible congeners (Figure 1). Chlorines attached to the 2 or 6 positions are referred to as *ortho* chlorines, those attached to the 3 or 5 positions are called *meta* chlorines, and those at the 4 position are known as *para* chlorine. In addition, chlorine substitutions on a biphenyl structure are categorized as singly flanked, doubly flanked, or unflanked based on the presence of neighboring chlorines. A singly flanked chlorine has one adjacent chlorine on the same benzene ring. For instance, if chlorines are attached at positions 2 and 3, the chlorine at position 3 is singly flanked because it is adjacent to a chlorine at position 2. A doubly flanked chlorine is surrounded by chlorines on both adjacent positions. For example, a chlorine at position 4 is doubly flanked when chlorines are also present at positions 3 and 5. In contrast, an unflanked chlorine has no neighboring chlorines on either adjacent positions. For instance, a chlorine at position 4 is unflanked if there are not any chlorines at positions 3 or 5 (Wiegel and Wu 2000).

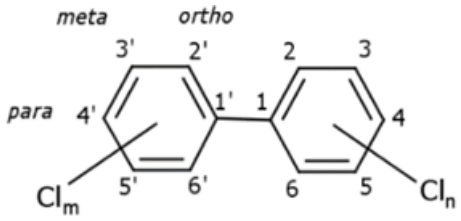

Figure 1. The molecular structure of PCBs.

Two main nomenclature systems are commonly used to identify PCB congeners. The first describes the specific carbon positions on the biphenyl rings where chlorine atoms are attached. In this system, the chlorine positions are listed sequentially. For example, 2,3,3',6-tetrachlorobiphenyl indicates chlorines attached to carbons 2, 3, 6 and 3'. A variation of this system groups the positions of each ring separately and may omit the prime symbols for simplicity, resulting in notations like 236-3. The second widely used system, developed by (Ballschmiter and Zell 1980) and by the International Union of Pure and Applied Chemistry (IUPAC) rules, assigns a unique number to each PCB congener to simplify identification. This system orders congeners based on the number of chlorine atoms and their arrangement, prioritizing unprimed positions over primed ones. Congeners are sequentially numbered from PCB-1 (monochlorobiphenyl) to PCB-209 (decachlorobiphenyl), covering all possible configurations. PCB congeners with an equal number of chlorine atoms are referred to as homologs. Hence, ten homolog groups are present: mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and deca-PCBs.

PCBs were extensively utilized in various applications, including as dielectric fluids in capacitors and transformers, heat transfer and hydraulic fluids, flame retardants, lubricants, cutting oils, and as additives in products such as paints, carbonless copy paper, adhesives, pesticides, sealants, and plastics (Erickson 2001). Their widespread industrial use was mainly due to their chemical stability, low flammability, and favorable physical characteristics, including excellent electrical insulating properties (Bedard and Quensen III 1995). This combination of desirable traits led to the production of PCBs under various trade names across multiple countries. Each of these names refers to commercial mixtures of PCBs produced in specific regions and shows differences in manufacturing practices and usage preferences. Some of the most well-known PCB trade names include Aroclor (USA), Kanechlor (Japan), Delor (Czechoslovakia), and Clophen (Germany/Europe). Aroclor was a trade name for PCB mixtures produced by the Monsanto Company in the United States. It became one of the most widely recognized PCB brands globally. Aroclor products were used in transformers, capacitors, hydraulic systems, paint additives, and plasticizers due to their insulating and fire-resistant properties. Each Aroclor mixture is designated by a number that reflects its chlorine content; for example, Aroclor 1254 contains around 54% chlorine by weight. Commonly used formulations, such as Aroclor 1242, 1254, and 1260, were extensively employed in industrial applications throughout the U.S. (Kodavanti and Loganathan 2017). In Japan, PCBs were produced by Kanegafuchi Chemical Company under the brand name Kanechlor. These PCB mixtures

were commonly used in electrical equipment and various industrial applications. Like Aroclor, Kanechlor products are numbered to indicate their chlorine content, such as Kanechlor 300, 400, and 500, with increasing numbers signifying higher levels of chlorination. Kanechlor products share similar properties with Aroclor but were primarily distributed in Japan (Bursian, Newsted, and Zwiernik 2011). Delor was a PCB brand produced in Czechoslovakia by Chemko Strážske. These PCBs were commonly used in electrical equipment like capacitors and transformers. Delor mixtures, comparable in composition and application to Aroclor and Kanechlor, were specifically designed for the Central and Eastern European market (Bursian, Newsted, and Zwiernik 2011). Clophen was a well-known PCB trade name produced by Bayer AG in Germany and widely distributed throughout Europe. Clophen was used extensively in transformers, capacitors, paints, plasticizers, and sealants. Clophen products, such as Clophen A30, A40, and A50, are differentiated by their chlorine content, with higher numbers indicating greater chlorination. These products were manufactured and applied primarily in Western Europe (Kodavanti and Loganathan 2017). As a result of these extensive applications and desirable properties, an estimated 1.3 million tons of PCBs were produced worldwide between 1930 and 1993 (Breivik et al. 2007).

PCBs are hydrophobic and exhibit low water solubility and vapor pressure, particularly as chlorine content increases (Mackay et al. 2006). Their octanol-water partition coefficients ($\log K_{ow}$) range from 3.90 to 8.26, indicating a strong affinity for organic matter and lipid-rich tissues with an increased level of chlorination. However, the Henry's Law constants are not influenced by the chlorination level of the congeners. The physicochemical properties of common PCB compounds are given in Table 1, which includes the compound name and formula, average molecular weight, Henry's Law constant, melting and boiling points, $\text{Log}K_{oa}$, $\text{Log}K_{ow}$, and vapor pressure. These properties lead to significant environmental contamination and persistence, especially in sediment particles in aquatic ecosystems (Abramowicz et al. 1993; Safe 1994). PCBs preferentially adhere to sediments and bioaccumulate in the fatty tissues of organisms, such as zooplankton, shellfish, and fish (Iseki et al. 1981; Galceran et al. 1993; Monosson 1999). This accumulation magnifies through the food web, eventually reaching higher trophic levels, including birds, terrestrial animals, and humans (Agency for Toxic Substances and Disease Registry (ATSDR) 2000; Hermanson and Johnson 2007).

Table 1. The physicochemical properties of PCBs (Source: US EPA CompTox Chemicals Dashboard, 2024a).

CAS Number	PCB Congener	Chemical Formula	Average Molecular Weight (g/mole)	Experimental Average					
				Henry's Law ($atm.m^3/mole$)	Melting Point (°C)	Boiling Point (°C)	$LogK_{oa}$	$LogK_{ow}$	Vapor Pressure (mmHg)
2051-60-7	PCB-1	$C_{12}H_9Cl$	188.65	7.36e-4	33.7	274	-	4.47	1.38e-3
2051-61-8	PCB-2			6.13e-4	16.7	285	6.82	4.64	7.35e-3
2051-62-9	PCB-3			5.73e-4	77.8	193	6.80	4.61	6.03e-3
13029-08-8	PCB-4	$C_{12}H_8Cl_2$	223.1	2.30e-4	61.5	-	7.18	4.95	2.75e-3
34883-39-1	PCB-9			2.80e-4	-	-	-	5.13	1.38e-3
25569-80-6	PCB-6			2.50e-4	-	-	-	5.02	-
34883-43-7	PCB-8			2.30e-4	44.0	-	7.40	5.09	1.35e-3
38444-73-4	PCB-19	$C_{12}H_7Cl_3$	257.54	2.30e-4	-	-	-	5.48	-
37680-65-2	PCB-18			2.50e-4	-	-	7.60	5.52	1.05e-3
38444-78-9	PCB-16			2.00e-4	-	-	-	5.31	-
55712-37-3	PCB-25			-	-	-	-	-	-
7012-37-5	PCB-28			2.00e-4	58.0	-	-	5.62	1.40e-4
38444-85-8	PCB-22			1.40e-4	-	-	-	5.42	-

(cont. on next page)

Table 1 (cont.)

35693-99-3	PCB-52	$C_{12}H_6Cl_4$	291.98	2.00e-4	87.0	-	8.47	6.17	2.54e-5
41464-39-5	PCB-44			1.40e-4	-	-	8.36	5.90	-
41464-46-4	PCB-71			-	-	-	-	-	-
73575-53-8	PCB-67			1.00e-4	-	-	-	-	-
32690-93-0	PCB-74			1.00e-4	-	-	-	6.67	-
32598-10-0	PCB-66			1.20e-4	-	-	9.02	6.11	4.62e-5
41464-43-1	PCB-56			-	-	-	-	-	-
38379-99-6	PCB-95	$C_{12}H_5Cl_5$	326.42	1.20e-4	-	-	8.79	6.55	-
37680-73-2	PCB-101			9.00e-5	77.0	-	9.06	6.07	1.55e-5
38380-01-7	PCB-99			7.80e-5	-	-	-	7.21	2.20e-5
38380-02-8	PCB-87			7.40e-5	-	-	-	6.85	1.70e-5
38380-03-9	PCB-110			-	-	-	9.06	6.22	-
31508-00-6	PCB-118			-	110	-	9.82	7.12	4.99e-6
52663-62-4	PCB-82			-	10.0	378	-	-	-
52663-63-5	PCB-151	$C_{12}H_4Cl_6$	360.86	5.90e-5	-	-	-	6.85	2.29e-6

(cont. on next page)

Table 1 (cont.)

68194-13-8	PCB-147	$C_{12}H_4Cl_6$	360.86	5.10e-5	-	-	-	-	-
38380-04-0	PCB-149			-	-	-	9.27	7.28	8.43e-6
51908-16-8	PCB-146			2.50e-5	-	-	-	7.12	-
35065-27-1	PCB-153			2.30e-5	103	-	9.73	6.53	1.99e-6
35065-28-2	PCB-138			2.10e-5	79.0	-	9.80	7.35	2.47e-6
52663-64-6	PCB-179	$C_{12}H_3Cl_7$	395.31	2.40e-5	-	-	-	-	-
52663-68-0	PCB-187			-	-		9.87	7.00	-
52663-69-1	PCB-183			-	83.0	-	-	7.30	-
38411-25-5	PCB-174			1.40e-5	-	-	-	-	-
52663-70-4	PCB-177			-	-	-	-	-	-
68194-16-1	PCB-173			1.40e-5	-	-	-	-	-
35065-29-3	PCB-180			1.00e-5	112	-	9.88	7.72	5.79e-7
35065-30-6	PCB-170			9.00e-6	-	-	-	-	6.28e-7
52663-75-9	PCB-199	$C_{12}H_2Cl_8$	429.75	1.00e-5	-	-	-	-	-
52663-76-0	PCB-203			-	-	-	-	-	-

(cont. on next page)

Table 1 (cont.)

52663-78-2	PCB-195	$C_{12}H_2Cl_8$	429.75	1.10e-5	-	-	-	-	-
35694-08-7	PCB-194			1.00e-5	-	-	-	8.04	7.94e-9
40186-72-9	PCB-206	$C_{12}HCl_9$	464.19	-	-	-	-	8.92	-

PCBs are chemically a complex mix of isomers or congeners that vary widely in structure and mechanisms of action (Pessah et al. 2019). These congeners are typically categorized into two groups based on their structure: those with biphenyl rings that lack chlorines in the ortho positions, adopting a coplanar orientation in solution, and those with one to four ortho-substituted chlorines, resulting in progressively noncoplanar orientations (Figure 2). The former group is known as dioxin-like PCBs (DL PCBs), while the latter is referred to as non-dioxin-like PCBs (NDL PCBs). Exposure to low levels of dioxin and dioxin-like PCBs has been connected to numerous negative effects on several organ systems, with notable impacts on the liver, skin, and immune system, and are potentially carcinogenic (Bock 2017; Mellor, Steinmetz, and Cronin 2016; Wheeler, Rothhammer, and Quintana 2017; Lauby-Secretan et al. 2013).

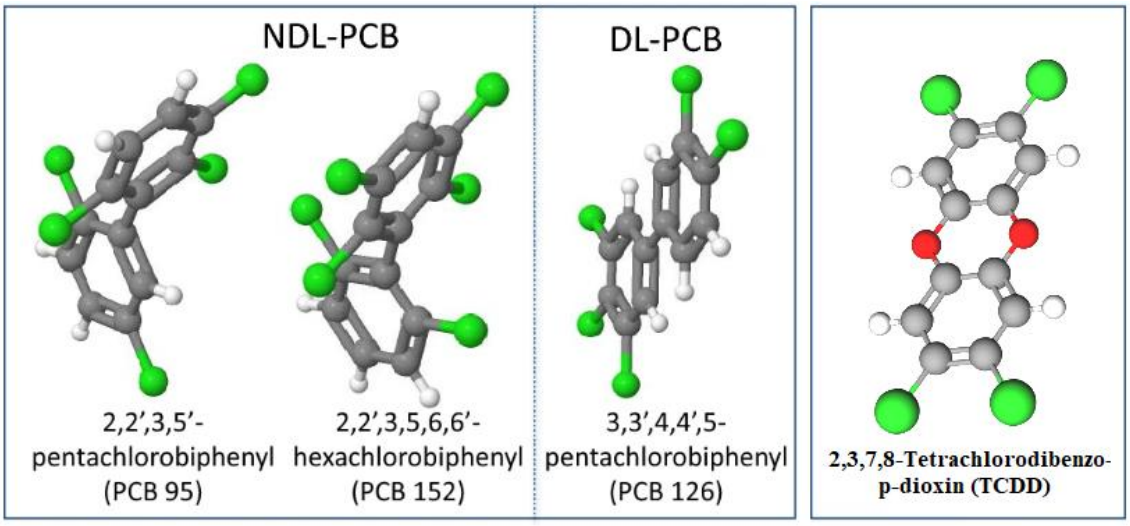

Figure 2. Examples of noncoplanar and coplanar PCBs, along with the structure of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (Source: Pessah et al. 2019).

The toxicity of PCBs can also be linked to NDL congeners. Toxicity assessments for these congeners are typically conducted on model organisms such as *Daphnia magna* and *Pimephales promelas* (fathead minnow), with endpoints such as no observed effect concentration (NOEC) and median lethal concentration (lethal concentration 50%, LC50) values (Hinzman 1993; Suedel, Dillon, and Benson 1997). Table 2 summarizes the data collected for the ecotoxicological risk evaluation including the NOEC and LC50 values

available in the literature for the given PCB congeners and also the corresponding test organisms. These values were derived from the ECOTOX database, EPI Suite software and relevant literature (US EPA ECOTOX Knowledgebase 2024b).

Table 2. Data used for ecotoxicological risk evaluation of the selected PCB congeners.

Chemicals	Species	Class	Endpoint	Concentration (ng/L)	K_{oc} (L/kg)
PCB-28	*Daphnia magna*	Invertebrates	LC50	160000	1.51e+04
PCB-52	*Daphnia magna*	Invertebrates	LC50	30000	2.74e+04
PCB-101	*Daphnia magna*	Invertebrates	LC50	10000	6.77e+04
PCB-153	*Pimephales promelas*	Fish	NOEC	25000	2.27e+05
PCB-138	*Pimephales promelas*	Fish	NOEC	25000	1.53e+05
PCB-180	*Pimephales promelas*	Fish	NOEC	25000	4.40e+05

2.1.2. Polybrominated Diphenyl Ethers

Polybrominated diphenyl ethers (PBDEs) are another group of POPs, regulated by the Stockholm Convention. They are synthetic aromatic organic compounds with a diphenyl ether backbone, capable of being brominated into 209 distinct configurations (Fromme et al. 2009). These configurations are known as PBDE congeners (Figure 3).

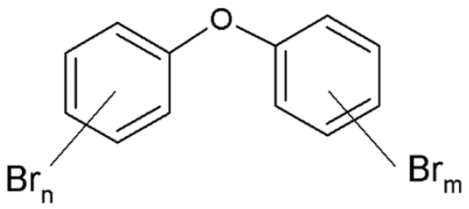

Figure 3. The molecular structure of PBDEs.

The positions of bromine in the structure, and hence the numbering of congeners, are used similarly to those of PCBs, i.e., the same IUPAC numbering for PCBs can be

used for PBDEs. According to the number of bromines in the structure, PBDEs can be grouped into homologs, where ten groups exist.

PBDEs were extensively used as flame retardants in consumer products such as electronics, furniture, textiles, and building materials. They were commonly integrated into materials as additives to plastics, foams, and fabrics. PBDEs were primarily produced as three major commercial formulations: Penta-BDE, Octa-BDE, and Deca-BDE, each varying in bromination levels and specific applications. Penta-BDE mixtures primarily contained pentabromodiphenyl ethers, along with lower brominated congeners (e.g., tetra- and tribromodiphenyl ethers). Penta-BDE was widely used in flexible polyurethane foam, commonly found in furniture, mattresses, automotive seating, and certain textiles. It provided effective fire resistance, particularly in products that require cushioning, like sofas and upholstered furniture (Alaee 2003). Octa-BDE mixtures contained octabromodiphenyl ethers as the primary component, with hexabromodiphenyl ethers and heptabromodiphenyl ethers as secondary components. Octa-BDE was primarily used in high-impact plastics, such as those found in electronics casings, including computers, televisions, and other household appliances. Its application in hard plastics made it effective for fire resistance in materials prone to electrical sparking or overheating (La Guardia, Hale, and Harvey 2006). Deca-BDE primarily consisted of decabromodiphenyl ether, the most heavily brominated PBDE congener. Deca-BDE was used extensively in electronics, textiles, and automotive components (La Guardia, Hale, and Harvey 2006).

PBDEs are highly hydrophobic, exhibit low water solubility, and have low vapor pressures. These properties are primarily influenced by the number and position of bromine atoms on the molecule. PBDEs with higher bromination levels are more lipophilic, less soluble, and less volatile (Table 3). As a result, highly brominated PBDEs readily adhere to soils and sediments, where they persist and serve as long-term reservoirs, gradually leaching into other environmental compartments over time (O'Driscoll et al. 2016).

Toxicity assessments for PBDEs are typically conducted on model organisms such as *Daphnia magna* and *Fundulus heteroclitus* (mummichog), with endpoints such as NOEC and LC50 values (Davies and Zou 2012; Key et al. 2009; Maskoameng 2006; Nakari and Huhtala 2008). Table 4 summarizes the data collected for the ecotoxicological risk evaluation including the NOEC and LC50 values available in the literature for the given PBDE congeners and also the corresponding test organisms. These values were

derived from the ECOTOX database, EPI Suite software and relevant literature (US EPA ECOTOX Knowledgebase 2024b).

Table 3. The physical and chemical properties of PBDE commercial mixtures.

Property	Penta-BDE	Octa-BDE	Deca-BDE
Molecular weight (g/mole)	564.75	801.47	959.22
Melting point (°C)	-7 to -3 [a]	85 to 89 [a]	290 to 306 [a]
Boiling point (°C)	>300 [b]	>330 [a]	>320 [b]
Aqueous solubility (mg/L at 25 °C)	0.0133 [a] 0.0024 (penta-BDE component) [a] 0.0109 (tetra-BDE component) [a]	<0.001 [a] 0.00198 (hepta-BDE component) [a]	<0.0001 [c]
Log K_{ow}	6.64 – 6.97 [a] 6.57 [a]	6.29 [a]	6.27 [a] 6.265 [a]
Henry's Law constant ($atm.m^3$/mole at 25 °C)	3.5×10^{-6} – 1.2×10^{-5} [a]	7.5×10^{-8} – 2.6×10^{-7} [a]	1.62×10^{-6} – 4.40×10^{-8} [b]
Solid state vapor pressure P_s (Pa at 25°C)	8.60×10^{-7} [d]	1.26×10^{-7} [d]	9.28×10^{-9} [d]
Log K_{oc}	4.89 – 5.10 [e]	5.92 – 6.22 [e]	6.80 [e]

[a] ATSDR, 2004,
[b] WHO, 1994,
[c] Hardy, 2002,
[d] US EPA, 2010,
[e] US EPA, 2014.

Table 4. Data used for ecotoxicological risk evaluation of the selected PBDE congeners.

Chemicals	Species	Class	Endpoint	Concentration (ng/L)	K_{oc} (L/kg)
BDE-28	*Daphnia magna*	Invertebrates	LC50	110710	1.71e+04
BDE-47	*Fundulus heteroclitus*	Fish	NOEC	50000	5.32e+04
BDE-99	*Daphnia magna*	Invertebrates	LC50	54000	5.81e+04
BDE-100	*Daphnia magna*	Invertebrates	LC50	11120	5.75e+05
BDE-153	*Daphnia magna*	Invertebrates	NOEC	14600	1.74e+03
BDE-209	*Daphnia magna*	Invertebrates	NOEC	500000	4.78e+04

2.2. Occurrence of PCBs and PBDEs in Environmental Matrices

PCBs and PBDEs have been detected in various environmental media and ecological beings. Because of their hydrophobic property, they can bind easily to the organic fraction of particulate matter. They have been found in the air, soil, sediments, and sewage sludge (Darnerud et al. 2001; Karickhoff, Brown, and Scott 1979; Jaward et al. 2004; Pan et al. 2007).

PCBs and PBDEs are found across diverse environmental matrices globally, reflecting the influence of urbanization, industrial activities, and waste management practices. Examples of these pollutants and their measured concentrations in different regions and matrices are summarized in Table 5. Electronic waste recycling sites in Tianjin and various land use types in Shanghai, China, including industrial, residential, and agricultural areas, showed high PBDE concentrations in soil (Wu et al. 2015; Wu et al. 2019). Similarly, the Pangani River basin featured urbanized highlands and cultivated lowlands near the river, reflecting diverse anthropogenic influences (Hellar-Kihampa et al. 2013). Heavily industrialized areas like Masan Bay in Korea and rapidly urbanizing regions such as East Lake in Wuhan, China, showed significant contamination (Hong et al. 2010; Yun et al. 2015). Even natural habitats, like the coastal sediment of British Columbia, Canada, had PCB and PBDE accumulation which threatens wildlife (Kim et al. 2022). The Aegean Sea coastlines of Greece and Turkey, situated between eastern and western industrialization, serve as an intersection of urban and industrial influences (Lammel et al. 2015). Similarly, the Red River Delta in Vietnam, a region shaped by extensive electronic waste recycling practices, presented a distinct example of pollutant presence in indoor dust and air (Tue et al. 2013). These locations illustrated the diverse environmental contexts where these persistent organic pollutants are found, emphasizing the widespread nature of their distribution.

PCBs and PBDEs have also been detected in vegetation, wildlife, fish and other marine species, terrestrial species, and human body (Zhang et al. 2023; Khairy et al. 2021; Yu et al. 2012; Holma-Suutari et al. 2016; Wen et al. 2008). Bioconcentration refers to the absorption and retention of substances, such as PCBs and PBDEs, by an organism directly through respiration, either from water in aquatic environments or from air in terrestrial ecosystems. Bioaccumulation, on the other hand, involves the uptake and

concentration of these chemicals within an organism through multiple pathways, including respiration, ingestion, and physical contact. Biomagnification occurs when PCBs and PBDEs are transferred up the food chain, resulting in their concentrations being higher in predators than would be expected under equilibrium conditions between the organism and its environment (Neely 1980). These POPs tend to accumulate in the fatty tissues of living organisms, where they can persist over time. As they pass through the food chain, e.g., via fish, shellfish, or birds, their levels increase and potentially reach harmful concentrations in top predators such as eagles, polar bears, and humans (Alexander 1999). Therefore, the occurrence and monitoring of their concentrations in various environmental matrices worldwide are crucial. This allows for a better understanding of their distribution and accumulation patterns, which can lead to efforts aimed at mitigating their impacts.

Table 5. Concentrations of PCBs and PBDEs from several locations (ND: Not detected).

Place	Sampling Year	Details on Sampling Site	Source	Type of POP	Concentration Range	Reference
Tianjin, China	2015	Electronic waste recycling sites	Soil	PBDE	5.9–2699 ng/g	Wu et al. (2019)
Shanghai, China	2012	Automobile manufacturing	Soil	PBDE	12.2–142 ng/g	Wu et al. (2015)
Shanghai, China	2012	Commercial and residential areas	Soil	PBDE	7.0–131.5 ng/g	Wu et al. (2015)
Shanghai, China	2012	Agricultural area	Soil	PBDE	4.3–49.3 ng/g	Wu et al. (2015)
Pangani, Tanzania	2009-2010	River basin	Sediment	PCB, PBDE	0.4–11.0 ng/g, 0.04–2.18 ng/g	Hellar-Kihampa et al. (2013)
Masan Bay, Korea	2004	Industrial area	Sediment	PCB, PBDE	0.07 – 24 ng/g, 1.25 – 19 ng/g	Hong et al. (2010)
East Lake, China	2013	Close to rapid urbanization and industrialization	Sediment	PCB, PBDE	ND – 107.1 ng/g, 9.7 – 151.3 ng/g	Yun et al. (2015)
Coast of British Columbia, Canada	2018-2020	Resident killer whale habitat	Surface sediment	PCB, PBDE	0.012–223 ng/g, 0.019–166 ng/g	Kim et al. (2022)
Aegean Sea coastlines, Greece and Turkey	2012	Urban and industrial center	Air	PCB, PBDE	5.8–85.7 pg/m^3, 0.40–13.85 pg/m^3	Lammel et al. (2015)
			Water	PCB, PBDE	7.48–33.4 pg/m^3, 3.11–6.24 pg/m^3	
Red River Delta, Vietnam	2008	Electronic waste recycling sites	Indoor Dust	PCB, PBDE	4.8–320 ng/g, 130–12,000 ng/g	Tue et al. (2013)
			Air	PCB, PBDE	1000–1800 pg/m^3, 620–720 pg/m^3	

2.3. Degradation Mechanisms in Sediments

When PCBs and PBDEs enter into an aquatic environment, they tend to accumulate in organic matter-rich sediments due to their high hydrophobicity and low water solubility (Jones and de Voogt 1999; Eljarrat and Barceló 2004). Hence, sediments act as reservoirs for these POPs. They also serve as active zones where biotic and abiotic degradation processes take place (Lohmann et al. 2007). The accumulation of PCBs and PBDEs in sediments increases the risk of bioaccumulation in aquatic food webs, posing ecological and toxicological threats. Consequently, understanding the degradation mechanisms of these contaminants in sediments is significantly important for effective pollution management and remediation strategies.

2.3.1. Abiotic Mechanisms

Abiotic degradation mechanisms significantly influence the transport and transformation of PCBs in sediments through processes such as volatilization, solubilization, sorption, and sedimentation. Lower-chlorinated congeners are more prone to volatilization and solubilization, leading to their loss during sediment resuspension, while highly chlorinated congeners bind to sediment particles, accumulating in deeper layers due to their lower mobility (Chiarenzelli, Scrudato, and Wunderlich 1997; Sanders, Hamilton-Taylor, and Jones 1996; Li et al. 2009). Apart from these natural processes, chemical and electrokinetic (EK) methods have shown promise in enhancing PCB degradation. Advanced oxidation processes (AOPs), such as persulfate oxidation activated by $FeSO_4$, achieved up to 80.5% degradation, with conditions like low pH favoring dechlorination and hydroxylation (Tang et al. 2015). EK methods, including persulfate activation under alkaline conditions and nanoscale zero-valent iron (nZVI) application, achieved PCB removal rates of 40.5% and 20.4%, respectively, despite challenges like poor reagent transport and high organic content (Fan et al. 2016). Electrodialytic cells (ED) further improved PCB removal, achieving up to 83% efficiency by enhancing desorption and reagent contact (Gomes et al. 2015). These mechanisms and

strategies emphasize the potential of combining natural processes with engineered solutions to address PCB contamination in sediments.

Abiotic pathways for PBDE degradation offer effective solutions to reduce their environmental persistence and toxicity. AOPs, which generate strong oxidants such as hydroxyl and sulfate radicals, are particularly effective when combined with reductive debromination, enabling the complete mineralization of PBDEs (Luo et al. 2011). Photodegradation and photocatalytic methods also play a critical role, especially for highly brominated PBDEs like BDE-209, by accelerating debromination and structural breakdown (Sun et al. 2012). Photocatalysts such as TiO_2 enhanced with copper have demonstrated improved efficiency in debromination processes (Lv et al. 2016). Additionally, iron-based materials, including zero-valent iron (Fe^0) and nano bimetallic materials (e.g., Ni/Fe), facilitate the stepwise debromination of PBDEs, often producing less toxic aliphatic and aromatic acids as degradation products (Wu et al. 2016). Microwave-assisted degradation further enables the complete mineralization of PBDEs adsorbed on porous minerals, demonstrating its potential for treating compounds like BDE-209 (Sun, Hu, and Cheng 2020). Collectively, these physicochemical approaches highlight innovative strategies for mitigating the environmental and health risks posed by PBDEs.

2.3.2. Biotic Mechanisms

Microorganisms show great diversity and adaptability, enabling them to metabolize a wide range of substrates, including halogenated organic compounds. These compounds, which originate from both natural and industrial sources, are degraded by microorganisms across various environments through diverse metabolic strategies (Gribble 2023). Despite their diversity, the fundamental metabolic processes in microorganisms share two common principles. First, **electron transfer** occurs upon organohalides, where energy is produced via oxidation-reduction reactions. In these reactions, electron donors are oxidized, and acceptors are reduced, eventually generating ATP (Figure 4). Second, **carbon metabolism** allows halogenated compounds to serve as

carbon sources, supporting microbial growth (Bossert, Häggblom, and Young 2004). Both PCBs and PBDEs can serve in these functions.

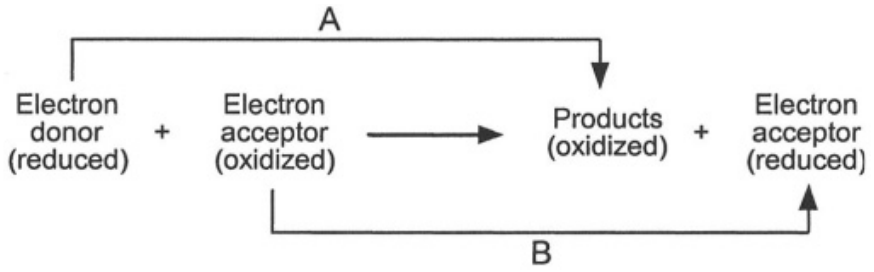

Figure 4. Schematic representation of a redox reaction cycle. While path A represents the primary electron transfer, path B indicates the recycling of the reduced electron acceptor back into the cycle.

Environmental factors that shape microbial community structure and activity are highly dynamic and vary across space. In multiphasic systems like soils and sediments, the redox environment forms a continuum—from highly oxidized conditions near the surface, dominated by aerobic microorganisms, to increasingly reduced conditions at greater depths. This gradient is driven by microbial activity and limited atmospheric gas exchange and supports a variety of electron acceptors which include inorganic species like nitrate (NO_3^-), sulfate (SO_4^{2-}), and iron (Fe^{3+}), as well as less common species like selenate (SeO_4^{2-}) and arsenate (AsO_4^{3-}) (Ghiorse and Wilson 1988; Stolz and Oremland 1999; Oremland et al. 2001). These alternative electron acceptors support the respiration of facultative and strict anaerobic microorganisms and enable them to thrive in anoxic environments (Bossert, Häggblom, and Young 2004).

2.3.2.1. Anaerobic Degradation

In oxygen-depleted settings, the absence of molecular oxygen limits the activity of aerobic microorganisms, but mixed microbial communities adapt by relying on

alternative electron acceptors. One critical process in these conditions is **reductive dehalogenation**, where organohalides act as terminal electron acceptors. This process is mediated by anaerobic microorganisms and involves the enzymatic cleavage of carbon-halogen bonds, replacing halogens with hydrogen (Bossert, Häggblom, and Young 2004). Reductive dehalogenation is driven by electron donors such as hydrogen, acetate, and formate, which fuel microbial respiration. These donors are essential for anaerobic degradation processes and are often shared among competing microbial populations, influencing the efficiency of dehalogenation (Löffler et al. 2004).

Specifically, microbial reductive dechlorination is a biological process where microorganisms gain energy by replacing chlorine atoms on PCBs with hydrogen atoms under anaerobic conditions (Wiegel and Wu 2000). In this reaction, molecular hydrogen typically serves as the electron donor while PCBs serve as the terminal electron acceptor themselves (Borja et al. 2005). Through the anaerobic reductive dechlorination of highly chlorinated PCB congeners, the chlorines at the meta and para positions are preferentially removed while the biphenyl framework remains unaffected (Abramowicz 1995). Figure 5 illustrates an example of a potential pathway for anaerobic degradation.

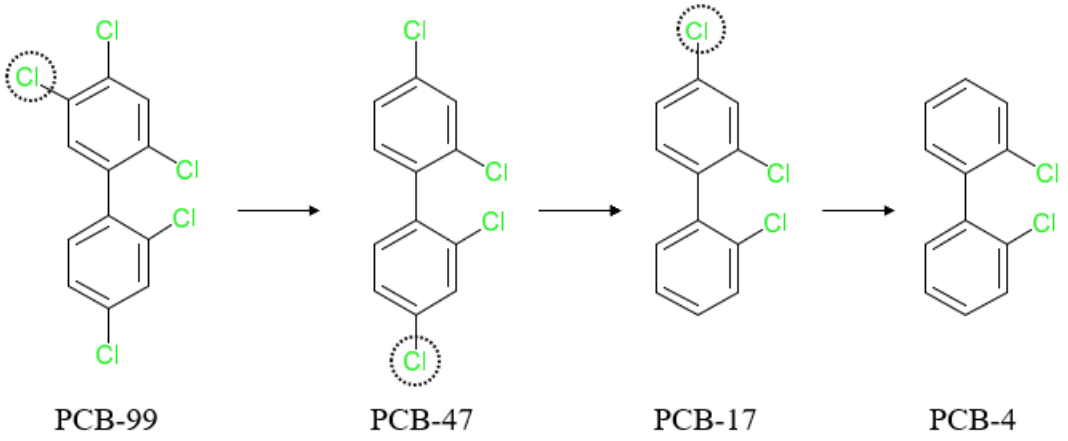

Figure 5. Anaerobic degradation involving the replacements of chlorines with hydrogen atoms on the biphenyl ring.

The preferential removal of meta- and para-substituted chlorine atoms reduces the abundance of highly chlorinated PCB congeners while increasing that of lower-

chlorinated congeners with ortho-substitutions. This removal process results in a change in the coplanar structure of the congeners and reduces their dioxin-like toxicity through anaerobic dechlorination (Abramowicz 1995). Furthermore, the lower chlorinated daughter molecules can be completely broken down into carbon dioxide and water by aerobic microorganisms through a sequential process of anaerobic and aerobic microbial degradation (Bedard and Quensen III 1995). Additionally, the depletion of highly chlorinated congeners lowers PCB exposure levels, potentially reducing their carcinogenicity and bioaccumulation risks (Abramowicz 1995; Wiegel and Wu 2000; Bedard and Quensen III 1995).

The first evidence of microbial reductive dechlorination was shown by Brown and collegues with the Hudson River sediments contaminated by Aroclor 1242 (Brown et al. 1984). The study has shown that the PCB profiles in the sediment differed from the original Aroclor 1242 mixture, with an increase in ortho-substituted mono- and dichlorobiphenyls, and a decrease in tri-, tetra-, and pentachlorobiphenyls. In addition to this research on in situ anaerobic dechlorination, a laboratory study was carried out to validate the environmental dechlorination of Aroclor 1242 in Hudson River sediments. Over a 16-week incubation period, microorganisms from the sediments removed 53% of the total chlorine and increased the proportion of mono- and dichlorobiphenyls from 9% to 88%. Dechlorination primarily occurred at the meta and para positions while ortho-substituted congeners accumulated (Quensen III, Tiedje, and Boyd 1988). PCB dechlorination activity was observed even in sediments that were not previously contaminated with PCBs after their amendment. The PCB-free sediment obtained from Sandy Creek Nature Center (SCNC) in Georgia, USA, and the PCB-contaminated sediment from Woods Pond (WP) in Massachusetts, USA were amended with PCB-62. While dechlorination was detected in both cases, the rate and extent of dechlorination were significantly higher in the WP sediments. This suggests that microbial communities in PCB-contaminated sediments have adapted over time to utilize PCBs more effectively possibly due to environmental selection pressures (Wu, Bedard, and Wiegel 1997).

PCB-dechlorinating bacteria, which utilize PCBs as substrates for reductive dechlorination, are primarily members of the *Dehalococcoides mccartyi* species within the phylum *Chloroflexi*. In addition, other genera such as *Dehalobacter* and *Dehalogenimonas*, as well as members of the *Geobacteraceae* family within the phylum *δ-Proteobacteria*, also play significant roles in the anaerobic degradation of PCBs (Xiang 2020). In addition, phylogenetically related but distinct *Chloroflexi* bacteria such as

Dehalobium chlorocoercia DF-1 and bacterium *ortho*-17 (*o*-17) link their growth to the reductive dechlorination (Wu et al. 2002; Cutter et al. 2001). *Dehalococcoides mccartyi* are remarkable microorganisms that rely exclusively on halorespiration of chlorinated organic compounds for growth. These strictly anaerobic, small, disk-shaped cells lack cysts, spores, and cell walls. They have a doubling time of approximately two days when they are provided with acetate, hydrogen, and an appropriate chlorinated organic compound (Bedard 2008).

Similar to the microbial mechanisms involved in the anaerobic degradation of PCBs, PBDEs can also be reductively debrominated by anaerobic microorganisms in sediments. Gerecke et al. 2005 provided the first direct evidence of BDE-209 reductive debromination in anaerobic conditions, with a 30% reduction over 238 days in mesophilic digester sludge. This transformation yielded nona- and octa-BDE congeners. The addition of primers, i.e. chemically related compounds, doubled the degradation rate which emphasized the potential for amendments to enhance this process. Tokarz III et al. (2008) demonstrated that BDE-209 underwent reductive debromination in anaerobic sediments, producing nona-, octa-, hepta-, and hexa-BDEs. Additionally, BDE-99, a penta-BDE, was further debrominated into tetra- and tribromodiphenyl ether congeners. Lee and He (2010) investigated the reductive debromination of an octa-BDE mixture (hexa- to nona-BDEs) in anaerobic microcosms established with soils and sediments from 28 locations. Within 2 months, products ranging from hexa- to mono-BDEs were generated and tetra-BDEs constituted 50% of the total products. Some studies also have explored the influence of different electron donors and buffer solutions enriched with mineral salts and vitamins (Qiu et al. 2012; Huang, Chang, and Lee 2014; Lee and He 2010; Tokarz III et al. 2008).

Various microorganisms capable of reductive debromination of PBDEs have been identified. *Dehalococcoides mccartyi* strains, such as GY50, TZ50, and MB, were fundamental PBDE-respiring bacteria. Strain GY50 debrominated tetra- and penta-BDEs to diphenyl ether (Ding et al. 2017). Strain TZ50 utilized reductive dehalogenases to transform penta- and tetra-BDEs into diphenyl ether (Zhao et al. 2021). Strain MB has been shown to debrominate penta-BDEs to di- to tetra-BDEs while also dechlorinating PCBs (Xu et al. 2022). *Pseudomonas* sp. SCSWA09 also contributed to PBDE debromination (Qiu et al. 2012), as well as *Clostridium* sp. in anaerobic sludge (Shih, Chou, and Peng 2012). Additionally, *Dehalobacter restrictus* PER-K23 and *Desulfitobacterium hafniense* PCP-1 have been identified as anaerobic microorganisms

capable of transforming PBDEs (Robrock, Korytár, and Alvarez-Cohen 2008). *Sulfurospirillum multivorans* demonstrated the ability to debrominate deca-BDE, producing hepta- and octa-BDE congeners, although it did not show any activity against an octa-BDE mixture (He, Robrock, and Alvarez-Cohen 2006).

2.3.2.2. Aerobic Degradation

Aerobic degradation of PCBs is a process carried out by microorganisms, initiated by the enzyme biphenyl 2,3-dioxygenase. This enzyme catalyzes the first step, where PCBs react with oxygen to form intermediates like chlorobenzoates and chlorocatechols. However, PCBs are rarely fully mineralized by a single organism due to metabolic bottlenecks and the formation of toxic intermediates (Pieper 2005).

Various microorganisms play a role in this process, including both Gram-negative and Gram-positive bacteria such as *Pseudomonas*, *Paraburkholderia*, *Achromobacter*, *Comamonas*, *Rhodococcus*, and *Bacillus* (Erickson and Mondello 1992; Hofer, Backhaus, and Timmis 1994; Péloquin and Greer 1993; Labbé, Garnon, and Lau 1997; Masai et al. 1995; Kimbara 2005; Ohtsubo et al. 2004). For example, *Pseudomonas pseudoalcaligenes* KF707 and *Paraburkholderia xenovorans* LB400 were well-characterized species with biphenyl 2,3-dioxygenase enzymes capable of transforming a broad range of PCB congeners.

The efficiency of PCB degradation varies among microorganisms and is highly dependent on enzymatic diversity and specificity. Lower-chlorinated PCB congeners are generally more easily degraded, while highly chlorinated congeners present greater challenges. *Paraburkholderia xenovorans* LB400, for instance, exhibited broad substrate specificity, while other strains might have more limited degradation capabilities (Mondello 1989).

Aerobic biotransformation of PBDEs involves microbial processes where specific bacterial strains metabolize PBDEs and release bromide ions or forms hydroxylated intermediates. In particular, *Rhodococcus jostii* RHA1 and *Paraburkholderia xenovorans* LB400, well-known bacteria for PCB degradation, have shown the ability to transform mono- through penta-BDEs, with LB400 also acting on certain hexa-BDE congeners

(Robrock et al. 2009). The efficiency of PBDE transformation decreases with higher bromination levels due to increased hydrophobicity and steric hindrance. RHA1 exhibited nearly complete debromination for some PBDEs, releasing stoichiometric quantities of bromide, while LB400 tended to produce hydroxylated PBDE intermediates rather than fully debrominating the compounds. This indicated differences in enzymatic pathways, with RHA1 more effectively cleaving C-Br bonds. In another study, over 11 months of aerobic incubation, PBDE levels in sewage sludge were reduced by 62–78%, with degradation enhanced by yeast extract and 4-bromobiphenyl. The most abundant congener, BDE 209, followed first-order kinetics with half-lives of 6.0 to 8.2 months. The study suggested that both lower brominated congeners and hydroxylated derivatives were formed during the process (Stiborova et al. 2015).

Other bacterial genera capable of aerobic degradation of PBDEs include *Gemmatimonas*, *Pseudomonas*, *Staphylococcus*, *Cupriavidus*, *Ensifer*, and *Hydrogenophaga*, which represent core degraders commonly found in PBDE-adapted sediments (Yang et al. 2015).

2.4. Remediation of Contaminated Sites

Contaminated sites refer to the areas confirmed to contain hazardous pollutants resulting from human activities, posing significant risks to human and environmental health, and have been determined to require cleanup according to Regulation on Control of Soil Contamination and Contaminated Sites due to Point Sources (CSCCS) of Türkiye (Ministry of Environment Urbanization and Climate Change 2010). Various activities and processes such as industrial activities, agricultural practices, waste disposal, urban development, accidents and spills contribute to environmental pollution, leading to contaminated sites. While preventative measures can be implemented at identified discharge points to mitigate future pollution, existing contamination often requires extensive remediation. As defined by CSCCS, remediation encompasses systematic actions taken to control or reduce the risks posed by contaminated sites, including strategies to remove, reduce, or transform pollutants.

In the USA, awareness of the dangers posed by contaminated sites to human health and the environment grew significantly in the late 1970s, capturing national attention. In response, Congress enacted the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly known as Superfund, in 1980. This legislation facilitated the identification and management of sites contaminated by hazardous substance releases. It also included a list of sites, namely National Priorities List, that has priority in cleanup. The U.S. government also established the Federal Remediation Technologies Roundtable (FRTR) in 1990 to foster collaboration among agencies in addressing the cleanup of these contaminated sites (FRTR 1990). In the 2000s, the European Parliament acknowledged the pressing need for regulations to manage soil use and mitigate the impact of external factors. While strategies for soil resource conservation were subsequently developed, no direct legislation addressed the identification and treatment of soil contamination. Despite the absence of a dedicated directive, 13 existing EU directives indirectly relate to soil contamination prevention and management, providing guidance on remediation practices without explicitly prioritizing the issue. In Türkiye, based on the implementation of the CSCCS in 2010, "Contaminated Sites Information System" now serves as a central database for recording and managing information related to contaminated sites.

2.4.1. Remediation Techniques

Contaminated site remediation methods are broadly divided into two: in situ (on-site) and ex situ (off-site). These approaches often involve a combination of techniques, which are classified into physical, chemical, or biological treatments, aimed at removing, isolating, or separating pollutants from soil, sediment, or water. The selection of remediation strategies depends on factors such as site conditions, the nature and properties of the contaminants, their distribution and concentration, and the intended future use of the site (Mulligan, Fukue, and Sato 2009). Therefore, the treatments applied may differ. In situ remediation methods are more advantageous and sustainable compared to ex situ methods, as they avoid disrupting the natural structure of the site and incurring excavation and transportation costs (FRTR, 2021). However, in situ applications can

present challenges in certain site conditions, such as the effective distribution of chemical and biological agents within the contaminated area (Khan, Husain, and Hejazi 2004). Therefore, when selecting an in situ remediation method, it is crucial to identify potential difficulties that may arise during field implementation and develop sustainable solutions to address them.

2.4.2. Bioremediation and Its Advantages

Bioremediation, an emerging field within environmental remediation, utilizes microorganisms' natural capacity to break down or transform pollutants into less toxic forms. This method provides a sustainable and often more economical alternative to conventional physical and chemical approaches, with minimal impact on the surrounding environment (Atlas and Philp 2005). It relies on the metabolic activities of various microorganisms, including bacteria and fungi, which produce enzymes capable of degrading complex organic pollutants such as petroleum hydrocarbons, pesticides, and industrial solvents (Das and Chandran 2010). By adjusting environmental conditions or introducing specific microbial strains, bioremediation can be optimized and directed to address particular contaminants (Tyagi, da Fonseca, and de Carvalho 2011).

Bioremediation offers a compelling alternative for contaminated site remediation due to its numerous advantages over conventional techniques. First and foremost, bioremediation is often more cost-effective than physical or chemical methods, particularly for large-scale or in-situ applications due to its lower operational and maintenance costs (Atlas and Philp 2005). This economic advantage stems from the elimination of excavation and transportation costs associated with ex-situ methods. Additionally, bioremediation typically employs less invasive techniques, minimizing disturbances to ecosystems and avoiding the generation of substantial hazardous waste streams (Adams et al. 2015). This approach aligns with the growing emphasis on sustainable remediation practices and reducing the environmental footprint of remediation projects. By utilizing natural processes, bioremediation decreases reliance on energy-intensive technologies, minimizes the use of harsh chemicals, and fosters environmental sustainability (Strong and Burgess 2008). This aligns with the global push

towards greener remediation solutions. Furthermore, bioremediation has demonstrated efficacy in addressing a wide array of contaminants, encompassing petroleum hydrocarbons, chlorinated solvents, and pesticides (Das and Chandran 2010). This versatility positions it as a valuable tool for remediating complex contaminated sites with multiple contaminants.

2.4.3. Bioremediation Strategies

2.4.3.1. Natural Attenuation

Natural attenuation, also referred to as monitored natural attenuation (MNA), depends on the natural capacity of native microorganisms to break down pollutants without the need for external assistance (Wilson 2011). This passive approach is often employed when the rate of natural degradation is sufficient to achieve remediation goals within an acceptable timeframe and regulatory framework.

Natural attenuation is a cost-effective and environmentally friendly option, as it avoids the need for extensive engineering interventions and the addition of exogenous materials. However, it requires comprehensive site characterization and long-term monitoring to ensure that the natural processes are effectively degrading the contaminants and that no adverse impacts are occurring (Terzaghi et al. 2018). The success of natural attenuation depends on various factors, including the type and concentration of pollutants, the presence of suitable electron acceptors, and the hydrogeological conditions of the site.

Between 1987 and 1998, Lake Hartwell experienced natural capping of PCB-contaminated sediments through clean sediment deposition at several locations. This was in correlation with the expectation that ongoing deposition of clean sediment would cap the PCB-contaminated material and thereby isolate it from the food chain based on the WASP4 modeling simulations reported in the Final Record of Decision for Lake Hartwell (US EPA 1994). Therefore, MNA was chosen as a remedy for the contaminated sediments. Pakdeesusuk et al. (2003) conducted microcosm studies to investigate microbial activity in Lake Hartwell sediments with samples collected in 1998. The results

confirmed that indigenous microbial communities were capable of reductive dechlorination, preferentially at the meta and para positions. These processes mirrored field observations, showing reductions in total chlorine content from 4.9 to 3.0 per biphenyl after 260 days of incubation. However, dechlorination plateaued with the accumulation of ortho-substituted congeners which suggested limitations in natural microbial degradation. The study emphasized that MNA's success depended heavily on the continuous deposition of clean sediment to isolate recalcitrant PCBs (Pakdeesusuk et al. 2003). Then, Pakdeesusuk et al. (2005) expanded on earlier findings by comparing sediment core data collected from multiple locations across the lake in 1987 and 1998. Their analysis revealed that while natural capping had occurred at most locations of the lake, some areas showed limited sedimentation and slower recovery. These cores allowed an analysis of site-specific differences in natural attenuation processes. Congener-specific analyses revealed that in situ reductive dechlorination was progressing albeit at a very slow rate. There were decreases in the higher chlorinated PCB congeners and increases in lower chlorinated congeners. The similarity between microcosm results and field data validated the ongoing microbial activity in Lake Hartwell sediments but also highlighted significant site-specific variations in the effectiveness of MNA (Pakdeesusuk et al. 2005). Then, Sivey and Lee (2007) focused on the spatial variability of MNA in Lake Hartwell. Using sediment cores collected in 2004, they evaluated changes in PCB concentrations over nearly two decades by comparing these data to historical samples from 1987 and 1998. Their findings showed that while some areas exhibited reductions in PCB concentrations near the sediment-water interface, other locations demonstrated limited improvement or even increased chlorination degrees. These results indicated that MNA's effectiveness was uneven across the lake, influenced by sedimentation rates, geochemical conditions, and microbial activity. They emphasized the need for supplemental strategies in areas where natural processes were insufficient (Sivey and Lee 2007).

2.4.3.2. Biostimulation

Biostimulation aims to enhance the activity and growth of native pollutant-degrading microorganisms by optimizing environmental conditions. This process may

include adding nutrients such as nitrogen and phosphorus, electron donors or acceptors, or adjusting pH and temperature to establish conditions that enhance microbial activity (Tyagi, da Fonseca, and de Carvalho 2011). This strategy capitalizes on the metabolic potential of the indigenous microbial community, which often possesses the genetic capability to degrade contaminants but may be limited by environmental constraints.

Biostimulation is a flexible method that can be utilized across diverse contaminants and site conditions. However, its effectiveness requires a deep understanding of the site's microbial ecology and the specific needs of the target microorganisms. Over-stimulation can result in adverse effects, such as excessive microbial proliferation, nutrient depletion, and the production of unwanted byproducts. As a result, careful monitoring and fine-tuning of biostimulation parameters are essential for achieving efficient and sustainable remediation.

Biostimulation of native PCB-dechlorinating bacteria has been successfully achieved through halopriming with halogenated aromatic compounds. Bedard and May (1996) were the first to report the stimulation of weathered Aroclor 1260 dechlorination in sediments following the addition of 2,5,3′,4′-tetrachlorobiphenyl (PCB-70). Later, it was demonstrated that brominated biphenyls (PBBs) could similarly prime PCB dechlorination, with 2,6-dibromobiphenyl reducing ~75% of highly chlorinated PCBs to less toxic forms within 100 days (Bedard, Van Dort, and Deweerd 1998). However, Sowers and May 2013 suggested that the intentional introduction of relatively high concentrations of halogenated biphenyls into the environment could face regulatory challenges. Several halogenated benzoates, phenols, benzenes and other halogenated compounds were also tested for priming PCB dechlorination, but they all failed to be as effective as PBBs (Deweerd and Bedard 1999; Cho et al. 2002).

Lactate, acetate, and pyruvate were shown to enhance PCB dechlorination in Taiwan's Keelung River sediment microcosms under methanogenic and sulfate-reducing conditions, with lactate promoting more extensive dechlorination than the other compounds (Chang, Liu, and Yuan 2001). Building on prior research, a mixture of lactate, propionate, acetate, and butyrate was utilized as electron donors with the fungicide pentachloronitrobenzene (PCNB) as a halogenated electron acceptor to stimulate native PCB-dechlorinating bacteria (Krumins et al. 2009). The researchers demonstrated that this combination significantly enhanced the dechlorination of weathered PCBs in Anacostia River sediment microcosms. The mole fraction of less-chlorinated congeners

(2–4 chlorines per biphenyl) increased by 20 ± 1.9% over a 415-day incubation period (Krumins et al. 2009).

Biostimulation has also been observed using slow-release electron donors. For instance, the addition of Fe^0 as a source of cathodic hydrogen significantly stimulated microbial dechlorination of specific PCB congeners in microcosms containing PCB-contaminated Baltimore Harbor sediment (Rysavy, Yan, and Novak 2005). It was demonstrated that introducing 0.001 atm H_2 directly or 100 mg Fe^0 per gram of dry sediment reduced lag time and enhanced PCB dechlorination activity in these microcosms (Rysavy, Yan, and Novak 2005). Later, nanoscale zero-valent iron (nZVI) was tested for its ability to stimulate PCB dechlorination under in situ-like biogeochemical conditions in marine sediments from the Venice Lagoon, Italy (Zanaroli et al. 2012). The results revealed that nZVI effectively promoted PCB dechlorination while minimally affecting the indigenous microbial community (Zanaroli et al. 2012). On the other hand, the periodic addition of elemental iron effectively stimulated PCB dechlorination by promoting the growth of native *Dehalococcoides* populations in sediments from Lake Hartwell, New Bedford Harbor, and Roxana Marsh. Lower iron dosages (0.01 g iron per g dry sediment) added every six months were particularly effective in sustaining *Dehalococcoides* activity by maintaining low hydrogen levels and minimizing competition with methanogens and sulfate reducers (Varadhan, Khodadoust, and Brenner 2011). Fe^0 alone failed to promote reductive dechlorination of PCBs in microcosms with sediments from Raisin River and Duluth Harbor in Michigan, except when bioaugmented with an active dechlorinating culture. This suggested that biostimulation is unlikely to be effective at sites lacking a viable native population of PCB-dechlorinating microorganisms (Winchell and Novak 2008).

A study by Lee and He (2010) tested the debromination potential of soils and sediments from 28 locations using an octa-BDE mixture. Debromination occurred in 20 locations with octa-BDE dissolved in trichloroethene (TCE) and in 11 locations with octa-BDE dissolved in nonane. Within two months, debromination products ranging from hexa- to mono-BDEs were formed, with toxic tetra-BDEs making up 50% of the products (Lee and He 2010). Nitrogen addition in the form of NH_4Cl accelerated microbial degradation of BDE-47 through an increase in the abundances of *Dehalobacter* spp. and *Dehalococcoides* spp. in PBDE-contaminated mangrove sediments of Sai Keng in Hong Kong (Chen et al. 2015). Biochar derived from spent mushroom substrate has also been shown to enhance the microbial debromination of BDE-47 in mangrove sediments of Mai

Po in Hong Kong by stimulating the growth of organohalide-respiring bacteria, according to (Chen et al. 2018). In another study, biostimulation of BDE-209-contaminated sediments was achieved by adding a defined mineral medium containing sodium formate as carbon source and ethanol as electron donor. This approach led to a 55.3% reduction in BDE-209 over 180 days, and the accumulated products were identified as tetra-BDEs (Demirtepe and Imamoglu 2019). Pan et al. (2020) investigated the degradation of BDE-47 in mangrove sediments by adding various carbon sources, including formate, acetate, pyruvate, lactate, succinate, methanol, and ethanol. After two months, all carbon sources significantly enhanced degradation percentages. By the end of five months, methanol, acetate, and succinate showed higher degradation rates and shorter half-lives compared to sediments without added carbon, though overall degradation percentages were similar. As a result, additional carbon sources could enhance BDE-47 degradation in sediments in a carbon- and time-dependent manner (Pan et al. 2020). The effect of adding nZVI was tested also for the removal efficiency of BDEs in mangrove sediments. The nZVI promoted the degradation of BDE-209 and produced debrominated products within 12 months (Pan et al. 2021).

2.4.3.3. Bioaugmentation

Bioaugmentation involves the introduction of specific microbial strains, either isolated from the same contaminated site or obtained from another environment, to a polluted site to enhance the degradation of target contaminants. These exogenous microorganisms augment the existing microbial community and provide the necessary enzymes and metabolic pathways to break down contaminants that may be recalcitrant to degradation by indigenous populations (Thompson et al. 2005). This strategy is particularly effective when the native microbial community lacks the genetic capacity or diversity to efficiently degrade the pollutants present.

The effectiveness of bioaugmentation relies on the careful selection of microbial strains that are specifically suited to the site's environmental conditions and possess the necessary degradative abilities. Factors such as pH, temperature, nutrient levels, and the presence of co-contaminants play a crucial role in determining the success of this

approach (Hussein et al. 2022). Furthermore, the introduced microorganisms must be able to compete with and establish themselves within the existing microbial community to achieve sustained pollutant degradation.

Bioaugmentation in PCB-contaminated sediments was first studied in microcosms, where various electron donors and halogenated compounds promoting dechlorination were used alongside the addition of the *Dehalococcoides mccartyi* strain 195 (Krumins et al. 2009). This approach achieved a 7–8% reduction in chlorine within 415 days, demonstrating its effectiveness in enhancing PCB breakdown (Krumins et al. 2009). May et al. (2008) reported the isolation of *Dehalobium chlorocoercia* DF-1 from sediments in Charleston Harbor and demonstrated its ability to dechlorinate PCBs with double-flanked chlorines. In subsequent soil microcosm experiments, bioaugmentation with DF-1 stimulated the reductive dechlorination of weathered Aroclor 1260 (4.6 ppm) and achieved an 8.9 mol% reduction of specific PCB congeners within 145 days (May et al. 2008). Payne, May, and Sowers (2011) demonstrated that bioaugmentation with DF-1 in sediment mesocosms contaminated with weathered Aroclor 1260 was highly effective. Within 120 days, this approach achieved a 56% mass reduction in penta- through nona-chlorobiphenyls. These congeners were primarily converted into tetrachlorobiphenyls, which are more susceptible to aerobic degradation. The study highlighted a significant improvement in dechlorination rates compared to earlier research (Payne, May, and Sowers 2011). Previous studies reported rates of 0.2 Cl/biphenyl within 415 days and 0.35 Cl/biphenyl within 145 days (Krumins et al. 2009; May et al. 2008). In contrast, Payne et al. (2011) achieved a rate of 0.7 Cl/biphenyl in just 120 days.

In another microcosm study, sediments contaminated with PCBs from Baltimore Harbor were bioaugmented with a mixture of indigenous PCB dehalorespiring microorganisms and a culture containing a rare ortho-dechlorinating strain alongside a non-indigenous strain targeting double-flanked chlorines (Fagervold et al. 2011). The microcosms were amended with PCB-151 and Aroclor 1260. After 300 days, bioaugmentation resulted in reduced lag times for dechlorination activity, increased microbial populations (100- to 1000-fold), and modified dechlorination pathways. The findings demonstrated that the inoculated microorganisms effectively competed with indigenous populations, cooperatively enhanced dechlorination, and altered the mechanisms of PCB degradation (Fagervold et al. 2011). In another study with microcosms with marine sediments chronically contaminated by PCBs, the addition of pure *Dehalococcoides mccartyi* culture was compared with natural attenuation under

anaerobic conditions (Matturro et al. 2016). Both approaches led to reductions in highly chlorinated PCBs and increases in less chlorinated congeners, with similar degradation patterns observed in bioaugmented and non-bioaugmented microcosms. For example, highly chlorinated congeners such as PCB-153 (2,2',4,4',5,5'-hexachlorobiphenyl) and PCB-180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) decreased by 32.5% and 23.8%, respectively, after 70 days without bioaugmentation. The results highlighted the efficiency of the native autochthonous microbial community, including *D. mccartyi*, in PCB biodegradation, with limited additional impact from bioaugmentation (Matturro et al. 2016).

Bioaugmentation was employed as a bioremediation strategy for BDE-209-amended sediments from a pond in Ankara, Turkey using *Dehalobium chlorocoercia* strain DF-1 (Demirtepe and Imamoglu 2019). Sediment microcosms were incubated anaerobically for 180 days, and the degradation produced tri-, tetra-, and penta-BDEs as intermediates. Bioaugmentation resulted in a 40.2% reduction in BDE-209 and showed moderate effectiveness when compared to 55.3% with biostimulation and 30.9% with natural attenuation (Demirtepe and Imamoglu, 2019). A recent study by Xu et al. (2022) evaluated the effectiveness of bioaugmentation using *Dehalococcoides mccartyi* strains (CG1 and TZ50) to enhance the debromination of PBDEs, focusing specifically on a penta-BDE mixture (BDE-47, -99, and -100). Sediments collected from a wetland in Singapore were used in microcosm experiments in which they were inoculated with CG1 and TZ50 at varying cell densities and spiked with penta-BDEs. Low, moderate, and high cell densities for each strain were defined as 0.1×10^6 and 0.5×10^6 cells/mL, 2.6×10^6 cells/mL, and 6.8×10^6 and 13.9×10^6 cells/mL, respectively. The highest removal efficiency was observed in microcosms inoculated with 6.8×10^6 cells/mL of CG1, achieving 89.9 ± 7.3% removal of penta-BDEs within 60 days. The penta-BDEs were completely detoxified into diphenyl ether. Bioaugmentation significantly enhanced debromination compared to control groups, where no substantial activity was observed (Xu et al. 2022).

2.4.4. Bioremediation of Mixed Contaminants

Environmental contamination rarely involves a single pollutant. Instead, it often possesses complex mixtures of hazardous substances. This is particularly true for industrial sites, electronic waste processing areas, and marine environments near these facilities, where PCBs and PBDEs frequently co-exist. Therefore, effective remediation strategies should be considered to address these mixtures and restore the relevant contaminated sites. However, limited studies have been carried out to observe the degradation of pollutants simultaneously.

Song et al. (2015) investigated the usefulness of in situ bioremediation of soils contaminated with electronic waste and focused on the anaerobic degradation of PCBs and PBDEs. The study utilized lactate as an electron donor to stimulate microbial activity. It was observed that higher halogenated PCBs and PBDEs underwent degradation over 90 days. This process resulted in the accumulation of intermediates with four to seven chlorine or bromine atoms. Meanwhile, the concentrations of lower halogenated compounds stayed stable. This transformation was facilitated by ions and the microbial activity achieved by dissimilatory iron-reducing bacteria (DIRB) and arylhalorespiring bacteria. The functional microbial species played a dual role, first oxidizing ferrous ions to ferric ions and then reducing ferric ions back to ferrous ions to drive dehalogenation. This synergistic role of soil bacteria and ion cycling highlighted their critical contribution to the breakdown of PCBs and PBDEs together (Song et al. 2015).

Wang et al. (2015) explored the potential of phytoremediation using corn (*Zea mays L.*) to remove copper, PCBs, and PBDEs from electronic waste-contaminated soil. The contaminated soil was treated with varying concentrations of ethylenediamine-S,S-disuccinic acid (EDDS), a biodegradable chelating agent, to enhance pollutant solubility and plant uptake. Corn was cultivated for 42 days, and pollutant accumulation in plant tissues like shoots and roots was analyzed. In shoots, PCB accumulation increased by an average of 58.7% across all EDDS treatments compared to the control, with lower chlorinated congeners like tri- and tetra-PCBs dominating. In roots, PCB accumulation was higher at elevated EDDS concentrations, with hexa- and hepta-PCBs appearing in small amounts. Similarly, PBDE concentrations increased in both shoots and roots following EDDS application. BDE-28, BDE-47, and BDE-209 emerged as the dominant

PBDE congeners, with roots showing higher overall PBDE accumulation than shoots. Changes in soil contaminant levels were not reported. The findings suggested that EDDS could improve the bioavailability of PCBs and PBDEs which facilitated their transfer from soil to plants (Wang et al. 2015).

In another study, Ye et al. (2015) investigated a combined approach to remediate soil contaminated with PCBs, PBDEs, polycyclic aromatic hydrocarbons (PAHs), lead, and nickel. The remediation process involved an initial soil washing step using a peanut oil-water solvent system enhanced with tea saponin, followed by phytoremediation through the planting of vetiver grass (*Vetiveria zizanioides*). These substances are safe, eco-friendly, and efficient solvents utilized to remove organic contaminants from polluted soils during remediation efforts. This innovative combination of washing and planting achieved the removal of 36% of residual PCBs and 45% of residual PBDEs from the soil (Ye et al. 2015).

Another study explored the bioremediation of sediments collected from a wetland in Singapore, contaminated with individual and co-occurring aromatic organohalides, including PCBs, PBDEs, and tetrabromobisphenol A (TBBPA), using bioaugmentation with the pure isolate of *Dehalococcoides mccartyi* CG1 (in low and high cell densities) and the *Dehalococcoides*-containing enrichment culture F derived from the marine sediment (Xu et al. 2024). The sediment microcosms were spiked with individual organohalide pollutants, including Aroclor1260, PCB-180 (the dominant congener in Aroclor1260), PBDEs (a mixture of BDE-100, -99, and -47, or TBBPA. Additionally, two co-contaminated setups were prepared: the first included Aroclor1260, PBDEs, and TBBPA (Co-contamination scenario 1), while the second combined PCB-180, PBDEs, and TBBPA (Co-contamination scenario 2). The concentrations of the pollutants were arranged as the levels typically found at contaminated sites, and this enabled a realistic evaluation of bioremediation performance. Lactate and pyruvate were also added as both electron donors and potential carbon sources for microbial growth. Biotic (sediments with indigenous microbiota) and abiotic controls (sterilized sediments) were established to isolate the effects of bioaugmentation with *Dehalococcoides* populations. For individual contaminant dehalogenation, the study demonstrated that bioaugmentation with *Dehalococcoides mccartyi* CG1 at a high cell density (CG1-H) significantly enhanced the dehalogenation of individual contaminants like Aroclor1260, PCB-180, PBDEs, and TBBPA compared to controls. For Aroclor1260, CG1-H achieved a 3.3-fold higher dechlorination rate than the control, and for PCB-180, CG1-H preferentially removed

meta-chlorines, generating PCB-153 as a key product. Conversely, enrichment culture F demonstrated superior performance in dechlorinating PCB-180, achieving an 18.7-fold higher dechlorination rate compared to the control and producing PCB-47 as the predominant product. For PBDEs, CG1-H achieved a 27.4-fold enhancement in debromination rate over the control, with diphenyl ether as the dominant product. However, enrichment culture F showed limited efficacy in PBDE debromination. For TBBPA, bioaugmentation with CG1 (both low and high densities) accelerated the debromination rate by up to 2.1 times compared to controls, converting over 99% of TBBPA into BPA. For co-contaminant dehalogenation, under co-contamination scenario 1, CG1-H led to significant improvements exhibiting dehalogenation rates of Aroclor1260, PBDEs, and TBBPA being 2.7, 43.7, and 3.1 times higher than in the controls, respectively. PBDEs and TBBPA were nearly completely debrominated, while hexa- and hepta-CBs in Aroclor1260 were converted to tetra- and penta-CBs. Notably, bioaugmentation with *Dehalococcoides mccartyi* CG1 at a low cell density (CG1-L) demonstrated a synergistic effect in co-contaminated sediments, with approximately double the dehalogenation rates for Aroclor1260 and PBDEs compared to mono-contaminated setups. In co-contamination scenario 2, enrichment culture F outperformed CG1-H in PCB-180 dechlorination, achieving a rate constant of 3.70×10^{-3} d^{-1} compared to 3.21×10^{-3} d^{-1} for CG1-H. However, CG1-H exhibited superior PBDE debromination (44.3-fold enhancement) and comparable performance for TBBPA. These findings suggested that co-contamination may enhance the activity of CG1, possibly due to a halopriming effect from TBBPA.

2.5. Contaminated Area of Interest: Aliağa Region

The Aliağa region, located on the western coast of Turkey, is an industrial hub characterized by its ship breaking yards, petrochemical facilities, steel production plants, and oil refineries. While these industries contribute significantly to the local and national economy, they are also major sources of environmental pollution, particularly in marine ecosystems.

Studies conducted in the area have identified various pollutants, including PAHs, polychlorinated naphthalenes (PCNs), PCBs and PBDEs in the air (Aydin et al. 2014; Odabasi et al. 2017b; 2015). Similarly, PCBs, PAHs, PBDEs, and PCNs have been detected in soil, while metals, PAHs, PCBs, and organochlorine pesticides (OCPs) have been analyzed in sediments of the bay (Kara et al. 2015; Neşer et al. 2012; Yılmaz et al. 2016). In seawater, PCBs and PAHs have been identified as prevalent contaminants (Odabasi et al. 2017a). Moreover, biological matrices such as fish, pine trees, and honeybees have revealed the presence of metals, PCBs, PAHs, PBDEs, and PCNs (Pazi et al. 2017; Muzyed, Kucuksezgin, and Tuzmen 2017; Kargar et al. 2017; Odabasi et al. 2015).

Sediments in Aliağa act as reservoirs for PCBs and PBDEs, with reported concentrations varying widely depending on location and sampling year. For instance, Pazi et al. (2012) reported the total concentrations of both Aroclors 1254 and 1260 in Aliağa sediments ranging from 2.8 to 205 µg/kg (dry weight) from samples collected in 2009. Odabasi et al. (2017a) documented the total concentration of 41 PCB congeners between 2.7 and 2450 µg/kg in sediments sampled in 2009. Yılmaz et al. (2016) reported the total concentration of 41 PCB congeners ranging from 17 to 847 µg/kg (dry weight) in sediment samples from Aliağa ship breaking yards collected in 2013. Muzyed, Kucuksezgin and Tuzmen et al. (2017) identified the total concentrations of both Aroclors 1254 and 1260 in Aliağa sediments between 8.61 and 562 µg/kg (dry weight) in 2014, with the highest levels found in Nemrut Bay due to intense industrial activities. On the other hand, no previous studies investigating PBDE levels in the sediments of Aliağa region have been conducted. Nevertheless, studies showed the atmospheric PBDE levels in the region, which may indicate the possible contamination of sediments in the area (Odabasi et al. 2017b).

As a result, the presence of these pollutants, especially PCBs and PBDEs, highlights a critical concern in the Aliağa region. Since these pollutants are found as mixtures in the environment, humans and other organisms can be exposed to multiple pollutants simultaneously. It is crucial to evaluate PCBs and PBDEs in their mixed state to ensure comprehensive and effective remediation. Developing effective remediation techniques and addressing the challenges are essential for mitigating and controlling the risks associated with PCBs and PBDEs.

CHAPTER 3

MATERIALS AND METHODS

3.1. Reagents and Standards

The solvents n-hexane and acetone for chromatography, anhydrous sodium sulfate (granulated for organic trace analysis), copper fine powder (particle size <63 μm), and aluminum oxide 90 were purchased from Merck KGaA (Darmstadt, Germany). The individual surrogate standards of PCB-30 (2,4,6-CB) and PCB-185 (2,2',3,4,5,5',6-CB), internal standard Mirex and PCB-Mix 37 as a standard mixture of 18 PCB congeners were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The individual standards of BDE-77 (3,3',4,4'-BDE) and BDE-47 (2,2',4,4'-BDE), PCB Mix C-CS-01 as a standard mixture of 39 PCB congeners and BDE-COC as a standard mixture of 14 PBDE congeners were purchased from AccuStandard, Inc. (New Haven, USA). The individual standard of PCB-61 (2,3,4,5-CB) was supplied from CPAchem Ltd. (Stara Zagora, Bulgaria). BDE-OND as a standard mixture of 16 PBDE congeners were purchased from Wellington Laboratories Inc. (Ontario, Canada). All chemicals used in this study were of high-purity and analytical-grade quality.

3.2. Study Site and Sample Collection

The study area was located in the Aliağa industrial region, approximately 45 km north of the city of İzmir, Turkey, within a 5 km radius centered on Aliağa city center. The area also encompassed iron-steel plants, refinery and petrochemical facilities, ports, a natural gas-fired power plant, residential areas, and beaches. The primary aim of this

research was to investigate the bioremediation strategies for the sediments, which were deemed to be heavily contaminated by POPs, i.e. the region of the ship breaking yards. Additionally, we hypothesized that that specific region in Aliağa generally exhibited a high density of microorganisms belonging to *Chloroflexi* phylum. To test this hypothesis, sediment samples were also collected from adjacent areas to evaluate the presence and distribution of the *Chloroflexi* phylum. Therefore, the sampling points covered Aliağa Bay, Nemrut Bay, and the coastal area hosting the ship breaking yards.

The sediment sampling locations are shown on the map in Figure 6. Sediment samples were obtained using a Hydro-Bios Van Veen grab sampler, which retrieved sediments from the surface to a depth of 10 cm. The collected samples were transferred into glass jars, securely sealed, and stored in darkness. Upon reaching the laboratory, the samples were promptly refrigerated at 4°C.

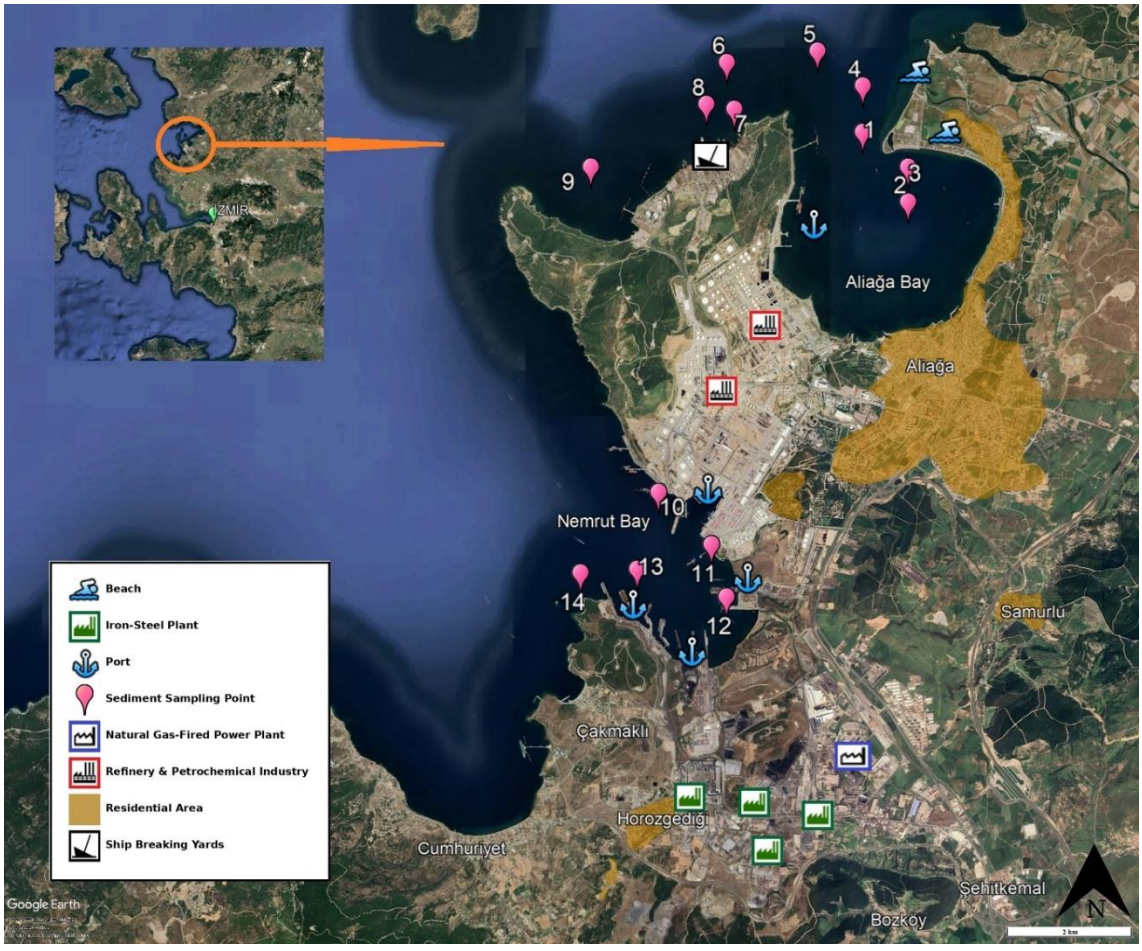

Figure 6. Sediment sampling and industrial activity map of Aliağa Region.

3.3. Detection of Microbiological Diversity

3.3.1. Preparation of Composite Sediments

Sediment samples collected from three regions, including Aliağa Bay (#1-5), the ship dismantling area (#6-9), and Nemrut Bay (#10-14) were grouped accordingly (Figure 6). Equal amounts of sediment were taken from each point and mixed in glass beakers that had been pre-cleaned with hexane. To achieve homogeneity in sediment samples, each was passed through an industrial analytical sieve system with mesh sizes of 2000 microns, 500 microns, and 63 microns. This process removed foreign materials such as stones, seashells, seaweed, sand, and clay, and increased cell density. As a result, three composite sediments were obtained.

3.3.2. Pre-Treatment for DNA Isolation

15 mL of wet sediment samples taken from the composite sediment of each region were divided into six 50 mL Falcon tubes. Then, 14 mL of distilled water (DI) was added, and the tubes were vortexed thoroughly. Following this, 1 mL of 10% sodium dodecyl sulfate (SDS) solution was added, and the tubes were placed in an ultrasonic bath for 20 minutes. After sonication, the tubes were centrifuged at 5000 rpm for 3 minutes, and the supernatant was transferred to new 50 mL Falcon tubes. The process of adding 15 mL of distilled water, vortexing, and centrifugation was repeated two more times, with the supernatants combined into the same Falcon tube each time. As sediment precipitation was still visible at the bottom of the combined supernatant tubes, the upper phase was carefully transferred to a new Falcon tube. These tubes were centrifuged at 9000 rpm for 10 minutes, after which the supernatant was discarded, and 1 mL of distilled water was added. The samples were vortexed thoroughly, combined into a single tube, and centrifuged again at 9000 rpm for 10 minutes. The pellet was resuspended in 1 mL of distilled water, vortexed intensively, and transferred into a 2 mL microcentrifuge tube.

The tube was then centrifuged at 15000 g for 5 minutes. Finally, 500 µL of distilled water was added to the resulting pellet, vortexed thoroughly, and the sample was submitted to the DNA isolation kit.

3.3.3. DNA Isolation from Composite Sediments

The EURx GeneMATRIX Soil DNA Purification Kit was used to isolate total microbial genomic DNA, following the manufacturer's protocol. The pre-treated mixture was directly submitted to the bead tubes. In the end, total DNA isolates were stored in the manufacturer's elution buffer at -20°C until further analyses.

3.3.4. Quantification of DNA, Polymerase Chain Reaction, and Agarose Gel Electrophoresis

DNA concentration and quality were assessed using the Thermo Scientific NanoDrop 8000 at the Biotechnology and Bioengineering Application and Research Center (CFB), within the Integrated Research Centers at Izmir Institute of Technology. Absorbance measurements for DNA samples were taken at wavelengths of 230 nm, 260 nm, and 280 nm. The purity of DNA was evaluated based on A_{260}/A_{280} and A_{260}/A_{230} ratios, which should exceed 1.6 and 2.0, respectively. First, the storage buffer was used to set the background and then concentrations were determined. All measurements were performed with a volume of 2 µL.

To confirm the presence of target microorganisms from the phylum *Chloroflexi*, DNA isolates were amplified using polymerase chain reaction (PCR) targeting the 16S rRNA gene. For this purpose, a specific primer set targeting the 16S rRNA gene of *Chloroflexi* phylum was employed. The primer sequences utilized in this study are listed in Table 6. This primer pair, 348F and 884R, was selected based on its specificity for the *Chloroflexi* phylum, particularly targeting groups known to possess organohalide respiration capabilities, as reported by (Fagervold, May, and Sowers 2007).

Table 6. Specific primer set for 16S rRNA of the *Chloroflexi* phylum.

Primer Set	Sequence (5'-3')
Forward primer (348F)	GAGGCAGCAGCAAGGAA
Reverse primer (884R)	GGCGGGACACTTAAAGCG

Amplification was performed using a Bio-Rad C1000 Touch thermal cycler, with each PCR reaction prepared to a final volume of 50 µL. The reaction mixture included 5 µL of 10X PCR buffer with $(NH_4)_2SO_4$, dNTP mix at a final concentration of 200 µM for each nucleotide, $MgCl_2$ at a final concentration of 1.5 mM, forward and reverse primers at 200 µM each, 2.5 units of Taq DNA polymerase per reaction, 100 ng/µL of template DNA, and sterile ultrapure water to complete the final volume.

The PCR amplification protocol included an initial denaturation at 94°C for 3 minutes, followed by 29 cycles of denaturation at 94°C for 30 seconds, annealing at 51°C for 30 seconds, and extension at 72°C for 60 seconds, with a final extension at 72°C for 10 minutes. Primer specificity was verified using a true negative control with *E. coli* ATCC 25922 DNA as the template, along with a separate negative control without template DNA to ensure the absence of contamination.

To analyze PCR products, 1% agarose gel electrophoresis (AGE) was conducted by dissolving 1 g of agarose in 100 mL of 1X Tris-acetate-EDTA (TAE) buffer through microwave heating. After cooling for approximately 10 minutes, 2.5 µL of ethidium bromide (EtBr) was added, and the solution was poured into a gel tray equipped with a comb to form wells. Once the gel was solidified, it was placed into an electrophoresis chamber filled with TAE buffer. A DNA ladder (10 µL) was loaded into the first well, and 10 µL of PCR product mixed with 2 µL of 6X loading dye was loaded into subsequent wells. The electrophoresis was run at 90 V for 1 hour until the DNA fragments migrated adequately through the gel. The expected fragment size was approximately 500 bp. After the run, the gel was removed, and DNA bands were visualized under UV light using the Bio-Rad VersaDoc 4000 MP imaging system. Band sizes were compared to the DNA ladder to confirm the size of the PCR products.

3.3.5. Sanger Sequencing

To purify PCR products prior to sequencing, a spin column-based DNA cleanup method was employed. First, 1 g of Sephadex G50 was mixed with 15 mL of autoclaved ultrapure water into a 50 mL Falcon tube. The tube was vigorously shaken for 2 minutes and then allowed to rest at room temperature for 10 minutes to ensure the Sephadex was properly hydrated and ready for use. This preparation ensured a clean and effective matrix for purifying the DNA.

Since each reaction was prepared with either forward or reverse primer in Sanger sequencing procedure, it was repeated twice for each geographical region sample. For the three geographical regions included in the study, a total of six reactions were prepared. 650 µL of the hydrated Sephadex slurry was loaded into spin columns with collection tubes and allowed to settle for 5 minutes, ensuring the separation of phases. The spin columns were centrifuged at 5400 rpm for 2 minutes, and the water collected in the collection tubes was discarded. The spin column tops were carefully transferred into clean Eppendorf tubes. 10 µL of PCR product was pipetted onto the Sephadex column. The columns were centrifuged again at 5400 rpm for 2 minutes, and the purified DNA was collected in the Eppendorf tubes. This step efficiently removed primers, unincorporated nucleotides, and other impurities, and left the DNA ready for further analysis. Once the PCR products were purified, their concentration and quality were measured using the Thermo Scientific NanoDrop 8000 spectrophotometer. For PCR products of 200–500 bp, the required DNA concentration is 3–10 ng per reaction according to the User Guide of Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit. Since our target DNA region was approximately 500 bp, 10 ng of DNA was used per reaction with a total volume of 10 µL. The rest of the reaction mix contained the following components: 1 µL of BigDye Terminator v3.1 Ready Reaction Mix, 1 µL of 3.2 µM primer, 1 µL of 5X Sequencing Buffer, and sterile ultrapure water to reach the final reaction volume. The tubes were placed in the thermal cycler. The cycle sequencing PCR protocol consisted of initial incubation at 96°C for 1 minute, 30 cycles of denaturation at 96°C for 10 seconds, annealing at 50°C for 5 seconds, extension at 60°C for 4 minutes, and an infinite hold at 4°C.

After the cycle sequencing reaction, the samples underwent a second round of purification using Sephadex G50 spin columns. The spin columns were prepared as described earlier, and 10 µL of the cycle sequencing product was loaded onto each column. The samples were centrifuged at 5400 rpm for 2 minutes, and the purified products were collected in clean Eppendorf tubes. All of the purified products were transferred to the Applied Biosystems MicroAmp Optical 96-Well Reaction Plate with Barcode which was then placed into Applied Biosystems Hitachi 3500 Genetic Analyzer. The resulting chromatogram files, formatted as ABI files, were visualized using FinchTV software. In addition, the sequence results were screened directly in 16S rRNA sequence database for Bacteria and Archaea optimized for highly similar sequences (megablast) via National Center for Biotechnology Information (NCBI) BLAST tool. Match/Mismatch scores were adjusted as 1,-2. Then, sequences obtained from three regions were aligned with the complete genome reference sequence of *Dehalococcoides mccartyi* 195 (accession number NC_002936.3) obtained from the Nucleotide database on the NCBI website, using CodonCode Aligner v.11.0.2 software.

3.3.6. Droplet Digital PCR

Droplet digital PCR (ddPCR) method, suitable for microbial abundance studies even at very low DNA concentrations, was also applied to the samples. The purposes of this analysis were to determine the ratio of microorganisms belonging to the *Chloroflexi* phylum within the total microbial consortium for three regions, and to start the enrichment procedures with the composite sediment sample where the *Chloroflexi* phylum was most represented. The protocol was performed for the absolute quantification of 16S rRNA gene copy numbers. The 348F/884R specific primers were designed specifically for the *Chloroflexi* phylum. Additionally, a universal primer set selected from conserved regions of the 16S rRNA gene was used for all bacteria (Table 7). Therefore, it was possible to determine the ratio of *Chloroflexi* phylum to total bacteria.

Table 7. Universal primer set for 16S rRNA of the bacterial kingdom.

Primer set	Sequence (5'-3')
Forward primer (16S-F)	AGGGAATCTTCSGCAATGGG
Reverse primer (16S-R)	ACGCCCAATAAATCCGGACA

Throughout this procedure, Bio-Rad QX200 Droplet Digital PCR System was used with all proper and compatible equipment included. Each ddPCR reaction was prepared with a 20 µL PCR mix containing 8 µL of 2X ddPCR EvaGreen Supermix, variable volumes of nuclease-free water, 0.8 µL each of forward and reverse primers, and 20 ng of DNA from each sample. The prepared ddPCR assay mixture was loaded into a disposable DG8 cartridge placed in a cartridge holder. Next, 70 µL of droplet generation oil for EvaGreen was added to each of the eight oil wells. The cartridge was then sealed with a DG8 gasket and inserted into the QX200 Droplet Generator. After droplet generation, the droplets were carefully transferred to a new ddPCR 96-well PCR plate. The plate was heat-sealed with pierceable aluminum foil using the PX1 PCR plate sealer and placed in the T100 thermal cycler. Thermal cycling conditions included an initial denaturation at 95°C for 5 minutes, followed by 39 cycles of 95°C for 30 seconds and 55°C for 1 minute, and two final steps at 98°C for 5 minutes and an infinite hold at 4°C. Once PCR was completed, the sealed plate was transferred to the QX200 Droplet Reader. The droplets were then analyzed using Bio-Rad QuantaSoft software. Analysis was set up and initiated to detect droplets with an optical detector. At the end of the plate reading, QuantaSoft software v.1.7.4.0917 was used to analyze the data. Positive droplets were selected from the 2D amplitude plot for each well. Finally, the concentration of the 16S rRNA gene, expressed as copies/µL, was calculated for the three samples.

3.4. Preparation and Subculturing of Enrichment Culture

The enrichment medium (Table 8) was prepared as artificial seawater medium-1 (ASM-1) based on the specifications described by previous studies (Berges, Franklin, and

Harrison 2001; Bedard, Ritalahti, and Löffler 2007; Wolin, Wolin, and Wolfe 1963; Lee and He 2010; Yan and Novak 2004). Yeast extract was added due to its rich amino acid, vitamin, and mineral content, sodium acetate (a chlorine-free organic substrate) was added as the carbon source, and PCB-61 (2,3,4,5-tetrachlorobiphenyl) and BDE-47 (2,2',4,4'-tetrabromodiphenyl ether) were added as electron acceptors. These compounds were considered more suitable for the enrichment study as they were moderately halogenated tetra congeners rather than heavily halogenated compounds. This would allow the microorganisms to readily utilize them for their growth.

Table 8. Components of enrichment medium for a total volume of 100 mL.

Component	**Amount**
Yeast extract	0.01 g
Sodium acetate (10 mM)	0.082 g
Nitrilotriacetic acid	0.015 g
$MgSO_4 \cdot 6H_2O$	0.03 g
Resazurin (1000X)	0.1 mL
$AlCl_3$	1 mg
Artificial seawater medium-1	See Appendix A.

To begin with, yeast extract, sodium acetate, $AlCl_3$, nitrilotriacetic acid and $MgSO_4 \cdot 6H_2O$ were mixed in the artificial seawater medium excluding Stock Solution 6 (S6, Appendix A) due to the heat sensitivity of the vitamins it contains, and the pH was adjusted to 7. The resulting medium was purged with high-purity nitrogen for 3 minutes and autoclaved at 120 °C and 1.1 atm for 20 minutes. Then, 1 g of wet composite sediment belonging to the ship dismantling area, S6 and resazurin was mixed in 100 mL of the autoclaved medium in a laboratory glass bottle within the anaerobic chamber, where the O_2 concentration was 0.1%. Lastly, 140 ppb of PCB-61 and 15 ppb of BDE-47 were added

to the initial culture. The concentrations of the electron acceptors were adjusted to be 10 times the solubility of the compounds in water. Each culture was prepared in duplicates from the initial setup throughout the entire process. The culture was incubated without agitation in the dark at 25°C.

To maintain the viability of active cultures, periodic transfers were performed into freshly prepared and sterilized ASM-1, increasing the volume by 50 mL each time until reaching a total of 250 mL over four subcultures. Subculturing involved transferring 1:10 from the last subculture into fresh ASM-1 under N_2:CO_2:H_2 (78%:20%:2%) atmosphere in the anaerobic chamber and it was considered a potential mechanism that the 2% hydrogen in the chamber would dissolve in the medium and act as an/the electron donor. Additionally, the concentration of electron acceptors was also increased by a factor of 0.2 with each transfer, starting at 1x and resulting in concentrations of 1.2x, 1.4x, and 1.6x. The initial cultures were completed after 30 days, and the first subcultures were prepared. Subsequent subcultures were prepared approximately every 90 days, monitoring the removal performance of PCB-61 and BDE-47 at certain time intervals. The optimal appearance of the enrichment cultures approaches transparency, exhibiting a yellowish hue. In the presence of oxygen, the culture transitions to a pink color due to resazurin. Throughout the monitoring of the enrichment cultures, when the medium was observed to turn into pink, 280 mM L-cysteine solution was added to achieve a final concentration of 1.4 mM. The growth of microorganisms of interest from the phylum *Chloroflexi* was monitored by dehalogenation of PCB-61 and BDE-47.

Starting from Subculture 2, PCB-61 and BDE-47 were added at increasing concentrations with each transfer, but at different levels for each compound. To ensure consistency in the analysis, three key factors were addressed: comparability across subcultures, overcoming the distinction between the varying concentrations of PCB-61 and BDE-47, and minimizing discrepancies between duplicate subcultures. To achieve this, measured concentrations at any time were normalized to the initially added concentrations for each compound. The ratio was expressed as C/C_0 ratios in Section 4.2. Additionally, measurements from duplicate bottles were averaged.

3.5. Microcosm Setup

This study aimed to evaluate the effectiveness of the bioaugmentation method in transforming PCBs and PBDEs into less harmful products. Therefore, the microcosm reactor sets were designed to comparatively investigate this effect and include the necessary control sets (Table 9). A composite sediment sample was prepared from sediments collected along the coastline where the ship dismantling facilities were located. To achieve a homogeneous mixture, equal quantities of sediment samples #7 and #8 were passed through a 2 mm industrial sieve. The composite sediment was stored in hexane-washed glass jars and kept in darkness until the microcosm setups were prepared inside the anaerobic chamber.

Enriched Subculture #4, as explained in Section 3.4, was added to the bioaugmentation reactors along with the composite sediment. In the biostimulation reactors, composite sediment was combined with a growth medium devoid of microorganisms. The purpose of this set was to examine the effects of the microbial culture independently from those of the micronutrients. For this reason, only the growth medium ASM-1 was added to the sediments. The natural attenuation reactors contained only composite sediment and filtered artificial seawater medium-2 (ASM-2). The artificial seawater medium prepared for this set did not contain the micronutrients, e.g. yeast extract, vitamins, sodium acetate, etc., that were included in ASM-1. In the negative control reactors, composite sediments were topped with the enriched Subculture #4, after filtering it through a 0.22 µm PTFE filter. The purpose of the negative control set was to assess the effects of the consumed and depleted micronutrients and carbon sources in the enriched subculture, excluding the influence of microorganisms in it. In the sterile control reactors, composite sediment, filtered ASM-2, and 43 mg of mercury (II) chloride (0.5 mg $HgCl_2$/g wet sediment) were added to inhibit microbial activity (Demirtepe and Imamoglu 2019). To ensure continued sterility during prolonged reactor operation, the sterile control reactors were autoclaved for 20 minutes at 1.1 atm pressure and 120°C for three consecutive days. The pH of ASM-2 used in the natural attenuation and sterile control sets was adjusted to 8.21, matching the actual seawater pH at sampling points #7 and 8. Instead of adding natural seawater collected from the field to the natural attenuation

and sterile control sets, it was decided to use artificial seawater across all sets to ensure consistency, avoiding differences in composition from the other sets.

Table 9. The contents of the experimental setup.

Microcosm Reactor Sets	**Composite Sediment from SBY Region**	**Enriched Subculture #4**	**ASM-1**	**ASM-2**
Bioaugmentation	+	+	-	-
Biostimulation	+	-	+ (Autoclaved)	-
Natural Attenuation	+	-	-	+ (Filtered)
Negative Control	+	+ (Filtered)	-	-
Sterile Control	+ (Autoclaved)	-	-	+ (Filtered)

The sediment-to-liquid ratio in the microcosm reactors was adjusted to 3 g:3.5 mL according to the study by Demirtepe and Imamoglu (2019). In this context, all microcosm reactors were prepared to contain 86 g of wet sediment and 100 mL of the corresponding liquid layer. The bioaugmentation, biostimulation, and natural attenuation reactors were established as triplicates, whereas the negative control and sterile reactors were operated in parallels. Microcosm reactors were set up in 250 mL septum-sealed serum bottles under a N_2:CO_2:H_2 (78%:20%:2%) atmosphere in the anaerobic chamber, inside which the 2% hydrogen was considered to dissolve and act as an/the electron donor. 0.2 mL of resazurin solution was added to all reactors as a redox indicator. Once sealed, the microcosm bottles were incubated at 22°C in darkness using a standard incubator. The microcosm reactors were operated for four months, with samples collected five times in total, starting on day zero and at 28-day intervals thereafter. Sampling was conducted within the anaerobic chamber, where approximately 2 g of wet sediment was taken each time and collected in amber bottles. After sampling, the reactors were re-sealed with aluminum caps and returned to the incubator. The samples were first completely frozen at -20°C overnight and then fully dried under vacuum in the Labconco 7753030 freeze dryer.

3.6. Analysis of PCBs and PBDEs

3.6.1. Extraction and Cleanup from Aqueous Enrichment Cultures

As an indirect method, the growth of microorganisms of interest from the phylum *Chloroflexi* was monitored by observing the dehalogenation of PCB-61 and BDE-47 in enrichment cultures. Therefore, their extraction from aqueous culture was done to observe their concentration change in time. For this purpose, 1 mL of culture sample was collected in an amber bottle inside the anaerobic chamber. Upon removal from the chamber, 5 mL of hexane was added, and the bottle was shaken vigorously by hand for 10 seconds, vortexed thoroughly, and shaken again for another 10 seconds. The sample was then centrifuged at 2500 rpm for 5 minutes to achieve clear phase separation. A purification column was prepared using a glass Pasteur pipette containing copper powder and 6% deactivated alumina (1:5 w/w), layered with 2-3 cm of anhydrous sodium sulfate and secured with a piece of glass wool at the bottom. The column was pre-rinsed with 5 mL of hexane. The solvent layer of the centrifuged sample was passed through the column, followed by an additional 1 mL of hexane (Demirtepe 2017). Subsequently, 1 mL of the eluted extract was transferred to a glass test tube, and Mirex internal standard was added to reach a final concentration of 50 ppb. The extract was then concentrated to 100 µL using a stream of high-purity nitrogen. The final sample volume was measured with a syringe and transferred to GC vials with inserts for GC-MS analysis.

3.6.2. Ultrasonic Extraction and Clean-up of PCBs and PBDEs from Sediments

Sediment samples were extracted for PCB and PBDE measurement using the US EPA Method 3550C Ultrasonic Extraction protocol (US EPA 2007). Freeze-dried sediment (~1 g) was combined with 5 g of anhydrous sodium sulfate in 40 mL vials and spiked with surrogate standards PCB-30, PCB-185, and BDE-77. After an overnight soak

in a 30 mL hexane:acetone mixture (1:1 v/v), the samples underwent two 30-minute extractions in an ultrasonic bath with fresh solvent mixture each time. Copper powder was introduced into the extraction solvents to facilitate sulfur removal. The resulting extracts were pooled and concentrated to a volume of 2 to 5 mL using a rotary evaporator. To remove interfering organic compounds, the concentrated, colored extract was treated with concentrated sulfuric acid (1:1.5 v/v) according to US EPA Method 3665A (US EPA 1996). The clear top layer of the extract was further purified by passing it through a column containing 0.5 g of 6% deactivated alumina topped with anhydrous sodium sulfate, eluted with 2 mL of hexane. Finally, the purified extract was concentrated to 1 mL under a high-purity nitrogen stream and spiked with a Mirex internal standard to achieve a final concentration of 50 ppb before being transferred to GC vials.

3.7. Instrumental Analysis

For PCB analysis of the microcosm sediments, the extracts were analyzed with Agilent 8890N gas chromatography coupled with 5977C mass spectrometry (GC-MS). The target compounds were measured in electron ionization (EI) mode using Agilent DB-5MS capillary column (30 m x 0.25 mm x 0.25 μm). The oven program began at 60°C and held for 1 minute, then increased to 170°C at a rate of 20°C/minute and held for 3 minutes, later continued to increase to 200°C at a rate of 3°C/minute and held for 2 minutes, and then to 300°C at a rate of 8°C/minute and held for 3 minutes. The injection volume was 1 μL in pulsed splitless mode, the temperature of the MS transfer was 280°C, the temperature of the ion source was 230°C, and the temperature of the inlet was 250°C. The carrier gas was helium at a flow rate of 1.1 mL/minute.

For PBDE analysis of the microcosm sediments, the extracts were also analyzed with Agilent 8890N gas chromatography coupled with 5977C mass spectrometry (GC-MS). The target compounds were measured in EI mode using Agilent DB-5MS capillary column (15 m x 0.25 mm x 0.10 μm). The oven program started at 50°C, was held for 1 minute, then increased to 150°C at a rate of 25°C/minute, later continued to increase to 200°C at a rate of 3°C/minute, then to 280°C at a rate of 8°C/minute and held for 3 minutes, and next to 300°C at a rate of 8°C/minute and held for 3 minutes to complete.

The injection volume was 1 µL in pulsed splitless mode, the temperature of the MS transfer was 280°C, the temperature of the ion source was 230°C, and the temperature of the inlet was 250°C. The carrier gas was helium at a flow rate of 1 mL/minute.

For PCB-61, the extracts were analyzed with Agilent 8890N GC-5977C MS. The target compounds were measured in EI mode using Agilent DB-5MS capillary column (30 m x 0.25 mm x 0.25 µm). The oven program began at 50°C and held for 1 minute, then increased to 150°C at a rate of 25°C/minute, later continued to increase to 200°C at a rate of 3°C/minute, and then to 280°C at a rate of 8°C/minute. The injection volume was 1 µL in pulsed splitless mode, the temperature of the MS transfer was 280°C, the temperature of the ion source was 230°C, and the temperature of the inlet was 280°C. The carrier gas was helium at a flow rate of 1 mL/minute.

Table 10. The list of PCB congeners and their corresponding mass-to-charge (m/z) values and retention times in GC-MS (SS: Surrogate standard, IS: Internal standard).

PCB Homolog Group	Congener Number	Retention Time (min)	m/z	PCB Homolog Group	Congener Number	Retention Time (min)	m/z
Mono-CBs	#1	8.30	188, 152	Hexa-CBs	#151/82	23.73	360, 290
	#2	9.29	188, 152		#147	24.05	360, 290
	#3	9.36	188. 152		#149	24.26	360, 290
Di-CBs	#4	9.89	222, 152		#146	24.42	360, 290
	#9	10.75	222, 152		#153	25.07	360, 290
	#6	11.07	222, 152		#138	25.76	360, 290
	#8	11.31	222, 152	Hepta-CBs	#179	25.37	394, 326
Tri-CBs	#19	11.96	186, 256		#187	26.29	394, 326
	#18	12.90	186, 256		#183	26.95	394, 326
	#16	13.63	186, 256		#174	27.55	394, 326
	#25	14.65	256, 186		#177	27.72	394, 326
	#28	14.98	256, 186		#173	28.00	394, 326
	#22	15.75	256, 186		#180	28.45	394, 326
Tetra-CBs	#52	16.61	220, 292		#170	29.22	394, 326
	#44	17.54	220, 292	Octa-CBs	#199	29.47	430, 360
	#71	18.07	292, 220		#203	29.59	430, 360
	#67	18.92	292, 220		#195	30.36	430, 360
	#74	19.41	292, 220		#194	30.93	430, 360
	#66/95	19.78	292, 220	Nona-CB	#206	31.78	464, 394
	#56	20.63	292, 220	**Standard Chemicals**	**Standard Name**	**Retention Time (min)**	**m/z**
Penta-CBs	#101	21.16	326, 254				
	#99	21.45	326, 254	SS	PCB-30	12.29	256, 186
	#87	22.67	326, 254	SS	PCB-185	27.35	396, 326
	#110	23.12	326, 254	IS	Mirex	29.43	272, 237
	#118	23.61	326, 254				

Table 11. The list of PBDE congeners and their corresponding mass-to-charge (m/z) values and retention times in GC-MS (SS: Surrogate standard, IS: Internal standard).

PBDE Homolog Group	Congener Number	Retention Time (min)	m/z	Standard Chemicals	Standard Name	Retention Time (min)	m/z
Tri-BDEs	#17	5.51	248, 406	SS	BDE-77	7.81	486, 326
	#28	5.87	248, 408	IS	Mirex	7.51	272, 237
Tetra-BDEs	#47	27.24	326, 486				
	#66	7.47	326, 486				
	#71	7.08	326, 486				
Penta-BDEs	#85	9.13	406, 564				
	#99	8.58	404. 564				
	#100	8.26	406, 564				
Hexa-BDEs	#138	11.00	484, 644				
	#153	10.1	484, 644				
	#154	9.47	484, 644				
Hepta-BDEs	#183	11.86	564, 722				
	#190	12.67	564, 722				
Octa-BDEs	#194	13.93	642, 802				
	#195	14.10	642, 802				
	#196	13.52	642, 802				
	#204	13.22	642, 802				
	#197	13.25	642, 802				
	#198/199/200/203	13.43	642, 802				
	#201	13.14	642, 802				
	#202	13.03	642, 802				
	#205	13.75	642, 802				
Nona-BDEs	#206	14.76	722, 880				
	#207	14.51	722, 880				
	#208	14.42	720, 880				
Deca-BDE	#209	16.05	799, 960				

For BDE-47, the extracts were analyzed with Agilent 8890N GC-5977C MS. The target compounds were measured in EI mode using Agilent DB-5MS capillary column (30 m x 0.25 mm x 0.25 μm). The oven program began at 90°C and held for 1 minute, then increased to 315°C at a rate of 20°C/minute and held for 6 minutes to complete the process. The injection volume was 1 μL in pulsed splitless mode, the temperature of the MS transfer was 280°C, the temperature of the ion source was 300°C, and the temperature of the inlet was 300°C. The carrier gas was helium at a flow rate of 1.5 mL/minute.

The instrumental analyses were initially performed in scan mode to determine the mass-to-charge (m/z) values and retention times. Next, selective ion monitoring (SIM) methods were developed based on these values, and the samples were analyzed in SIM mode. The m/z values and retention times of the target analytes, and surrogate (SS) and internal standard (IS) chemicals are presented for PCBs and PBDEs in Table 10 and Table

11, respectively. The compounds are categorized into homolog groups based on the number of chlorine and bromine atoms in their molecules.

3.8. Quality Assurance/Quality Control

To present the results with precision and accuracy, quality assurance/quality control (QA/QC) protocols were followed. The instrumental analyses were performed with seven or eight points of calibration curves for PCB mixtures, PBDE mixtures, PCB-61, and BDE-47. All calibration curves were obtained with R^2 greater than 0.99 and a relative standard deviation of less than 20%.

The average recoveries of surrogate standards in all microcosm samples were 122.7±21.4% for PCB-30, 114.2±21.2% for PCB-185, and 55.5±19.2% for BDE-77. In every batch of 13 microcosm samples, one blank sample was analyzed. The surrogate recovery in the blank samples were 88.7±34.3% for PCB-30, 82.5±11.4% for PCB-185, and 120.3±7.14% for BDE-77. No surrogate or blank correction was applied to the samples.

3.9. Data Analysis

3.9.1. Dehalogenation Rate Calculation

In studies such as the present one, fitting the degradation rate of congeners over time based on concentration to a rate law is generally challenging. The reason is that all PCB and PBDE congeners simultaneously act as both mother and daughter compounds within different degradation pathways, except deca- and mono-chlorinated/brominated congeners. As a result, the decrease in the total number of halogens per molecular structure with time is considered a more suitable metric for evaluating the degradation rate of PCBs and PBDEs (Abramowicz et al. 1993; Payne, May, and Sowers 2011;

Demirtepe and Imamoglu 2019). This approach is expressed in units such as chlorine atoms removed per biphenyl per day or bromine atoms removed per diphenyl ether per day.

The PCB and PBDE congeners in each sample were measured as ng contaminant/g of dry sediment weight and then converted to nmoles. Then, chlorine per biphenyl (Cl/bp) and bromine per diphenyl ether (Br/dp) content of sediments were calculated using Equation 1.1. The time rate of change was lastly calculated as shown in Equations 1.2 and 1.3. All calculations were based on the conservation of the biphenyl and diphenyl ether moieties and the assumption that coeluting congeners were present in equal proportions. Furthermore, the degradation rates were computed as the difference in the chlorine per biphenyl or bromine per diphenyl ether content between days 0 and 112 divided by the time passed, i.e. 112 days.

$$\text{Chlorine per biphenyl (Cl/bp)} \\ \text{or Bromine per diphenyl ether (Br/dp)} = \frac{\sum_{i=1}^{n} (C_i \times n_i)}{\sum_{i=1}^{n} C_i} \quad (1.1)$$

$$\text{Chlorine removed per biphenyl} \\ \text{per day (Cl/bp/day)} = \frac{(Cl/bp)_{t_1} - (Cl/bp)_{t_2}}{|t_1 - t_2|} \quad (1.2)$$

$$\text{Bromine removed per diphenyl ether} \\ \text{per day (Br/dp/day)} = \frac{(Br/dp)_{t_1} - (Br/dp)_{t_2}}{|t_1 - t_2|} \quad (1.3)$$

where:

C_i: The total concentration of each homolog group *i*, (nmole/g dry weight)

n_i: The total number of chlorines or bromines for each homolog group

t: The time (day)

3.9.2. Ecotoxicological Risk Evaluation

The ecotoxicological risks of target compounds in Aliağa sediments were evaluated with the approach of risk quotient (RQ) calculation (Chen et al. 2024). Chronic and sublethal RQs were calculated by dividing the concentration of target compounds in the sediment by the predicted no-effect concentration (PNEC) (Equations 2.1 and 2.2).

$$RQ = \frac{C_m}{PNEC_{sed}} \quad (2.1)$$

$$PNEC_w = \frac{NOEC \text{ or } LC50}{AF} \quad (2.2)$$

where

C_m: The measured concentration of the target compound (ng/g)

$PNEC_{sed}$: PNEC of target compounds in sediment (ng/g)

$PNEC_w$: PNEC of target compounds in water (ng/L)

NOEC: No observed effect concentration obtained from the US EPA's ECOTOX database (ng/L)

LC50: Lethal concentration 50% obtained from the US EPA's ECOTOX database (ng/L)

AF: Assessment factor (taken as 100 and 1000 depending on the endpoints of NOEC and LC50, respectively).

Since sediment toxicity data were mostly unavailable, $PNEC_w$ was converted into the corresponding $PNEC_{sed}$ values using the sediment-water distribution coefficient (K_d, L/kg) following Equations 2.3 and 2.4.

$$K_d = K_{oc} \times f_{oc} \quad (2.3)$$

$$PNEC_{sed} = PNEC_{w} \times K_{d} \quad (2.4)$$

where

K_{oc}: The organic carbon partition coefficient of the compound (L/kg)

f_{oc}: The fraction of total organic carbon in the microcosm sediment (5.11% by weight)

As a result, risk levels were categorized as minimal ($RQ < 0.01$), low ($0.01 \leq RQ < 0.1$), moderate ($0.1 \leq RQ < 1$), and high ($RQ \geq 1$) (European Commission (EC) 2003).

3.9.3. Statistical Analysis

To investigate if there were any differences in the concentrations of total PCBs and PBDEs with time, analysis of variance within subject design was performed using IBM SPSS 25. The difference was evaluated as significant if $p<0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Environmental DNA Analysis

The aim of this study was to test the bioremediation of the highly contaminated area associated with the ship breaking yards in Aliağa, İzmir. To achieve this, the presence of the indigenous *Chloroflexi*-related microorganisms was first confirmed and subsequently enriched for their potential application in the same environment. Sediment samples were collected not only from the ship breaking area but also from adjacent regions within close proximity to assess the presence and abundance of *Chloroflexi*. As the initial step, environmental DNA analyses were conducted to determine the presence and quantify the population of *Chloroflexi*.

4.1.1. Measurement of DNA Concentration and Purity

The first step of environmental DNA analysis is its isolation from the sediment samples. However, significant challenges were encountered in isolating high-concentration DNA from sediment samples, which might contain inhibitory substances like humic acids, organic matter, and fine particles. These substances were considered to interfere with the DNA extraction such that initial attempts using the commercial soil DNA isolation kit yielded low DNA concentrations below 10 ng/µL. To address this, the method was refined incrementally. Inspired by Bourrain et al. (1999), sonication and thermal shock were introduced, coupled with three freeze-thaw cycles to enhance cell lysis and DNA release. However, these steps yielded unsignificant improvements.

Subsequently, DNA isolation was tested using air-dried sediment instead of wet sediment, but the results remained insufficient. An alternative approach involved mixing 5 mL of wet sediment with 5 mL of distilled water and 2 mL of 10% SDS to disrupt microbial cell membranes (Tsai and Olson 1991). While this protocol improved DNA recovery, the concentrations were still under 10 ng/µL. To ensure the DNA isolation kit was functioning properly, the protocol was tested with a chicken digestate sample, which resulted in a DNA concentration of 40 ng/µL (data not shown), confirming that the sediment matrix was the limiting factor rather than the kit. Further optimization involved increasing the processed sediment volume, starting with two Falcon tubes containing sediment, distilled water, and SDS, then scaling up to three tubes, and finally six tubes. By combining the supernatants from these larger volumes, DNA concentrations suitable for downstream applications were achieved. The concentration and purity of the DNA isolates, measured using the Thermo Scientific NanoDrop 8000 spectrophotometer, along with the A_{260}/A_{280} and A_{260}/A_{230} purity ratios obtained from absorbance at 230 nm, 260 nm, and 280 nm wavelengths, are presented in Table 12. As an indicator of DNA purity, the A_{260}/A_{280} and A_{260}/A_{230} ratios are expected to be above 1.6 and 2.0, respectively. However, the presence of impurities is very common in environmental samples. The A_{260}/A_{280} ratios of sediment samples from Aliağa Bay (AB), Nemrut Bay (NB), and the ship breaking yards (SBY) were found to be within the desired range, and the DNA concentrations were sufficient. Therefore, these values were found to be satisfactory for PCR amplification and AGE.

Table 12. DNA concentrations and purity ratios of samples named by three geographical regions.

Sample Name	**Concentration (ng/µL)**	**A_{260}/A_{280}**	**A_{260}/A_{230}**
AB	20.35	1.87	1.19
NB	28.94	1.87	1.17
SBY	15.66	1.89	3.49

4.1.2. PCR Amplification and Agarose Gel Electrophoresis

All DNA samples were subjected to PCR amplification, followed by AGE for visualization to confirm the presence and size of amplified products. The target segment of the 16S rRNA gene, where the primer pair is expected to bind, is approximately 500 base pairs in length. The DNA ladder used displays DNA fragments ranging from 250 to 1000 base pairs, and band images of all PCR products from the composite sediments of the study regions were obtained (Figure 7). The primers specific to the *Chloroflexi* phylum are not expected to bind to the 16S rRNA genes of other microorganisms. Consequently, no bands were observed in the PCR reaction containing *E. coli* DNA, nor in the negative control (NC) without template DNA, as expected. This step ensured that PCR amplification was successful and allowed for the verification of expected amplicon sizes.

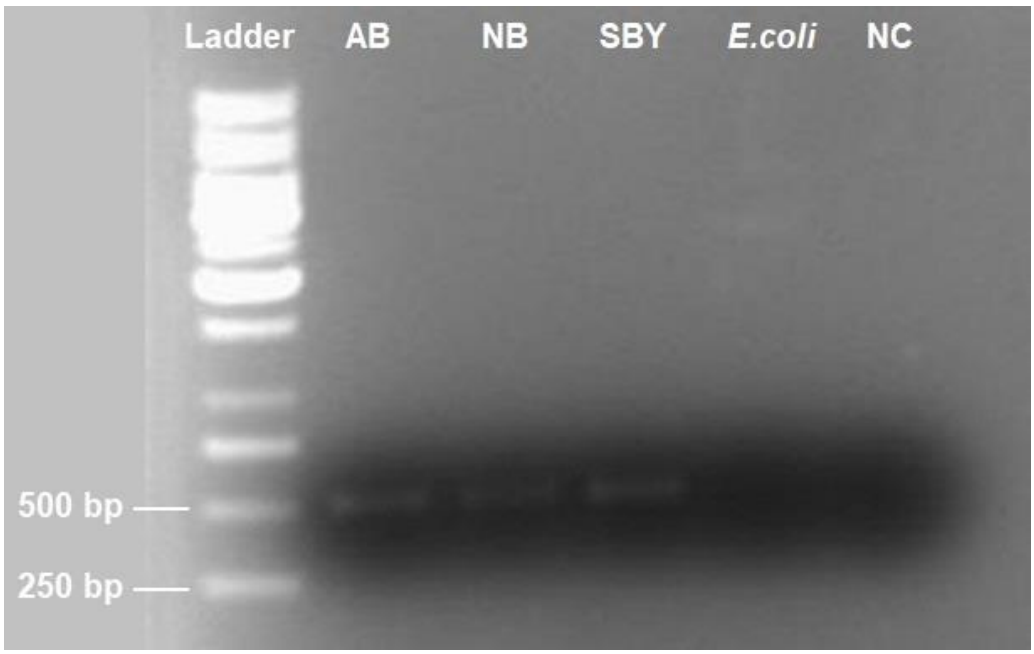

Figure 7. AGE image of PCR products.

4.1.3. Phylogenetic Analysis of 16S rRNA Genes from Environmental Samples

All PCR products showing bands on the AGE were subjected to Sanger sequencing. The resulting query sequences for each geographical region were generated and analyzed using NCBI's BLASTn tool. The AB, NB, and SBY query sequences displayed 95%, 94%, and 97% query coverage and percent identities of 80.38%, 80.52%, and 80.64%, respectively, showing high similarity to *Dehalococcoides mccartyi* 195. The results highlighted matches specifically in the 16S rRNA gene region, with notable overlaps with various clones. These sequences were associated with environmental bacteria and contained phylogenetically conserved gene regions. The BLAST results revealed that the sequences obtained from all three regions exhibited a strong connection to environmental microorganisms, suggesting that these bacteria shared common evolutionary or ecological characteristics.

Sequences generated with forward and reverse primers for each region were also aligned with the complete genome reference sequence of *Dehalococcoides mccartyi* 195 (accession number NC_002936.3) obtained from the Nucleotide database on the NCBI website, using CodonCode Aligner v.11.0.2 software. In Figure 8, the gray NC_002936.3 row at the top represents a region of the reference genome between approximately 250-750 base pairs. In the sequence alignments for each geographical region, blue arrows indicate the binding sites of the forward primers, while red arrows indicate the binding sites of the reverse primers. Additionally, black-and-white blocks represent positions present in the reference genome but are unreadable in the sequencing, while pink, red, and green blocks indicate base variability at their respective positions. The 16S rRNA gene sequences from the three different geographical regions show some differences and mismatches around 700-750 bp but overall exhibit high similarity to the reference genome.

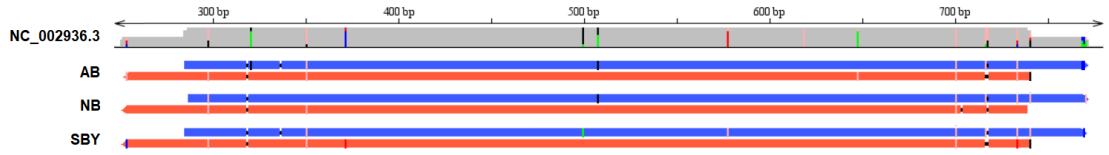

Figure 8. Alignment of 16S rRNA gene sequences with the *Dehalococcoides mccartyi* 195 reference genome.

4.1.4. Absolute Microbial Quantification by Droplet Digital PCR

ddPCR, a method well-suited for microbial abundance studies even at very low DNA concentrations, was applied to quantify the ratio of *Chloroflexi* phylum microorganisms within the total microbial consortium. This analysis was conducted to observe the sample with the highest representation of *Chloroflexi* in the SBY region and to investigate the density of *Chloroflexi*-related microorganisms in all three geographical regions. In a typical ddPCR experiment, the sample is randomly partitioned into at least 13,000 separate droplets, with some containing DNA templates and others containing none or multiple copies of the template. The PCR process amplifies the DNA to the endpoint, and the fluorescent signal within each droplet is monitored. Using QuantaSoft v.1.7.4.0917 software, integrated with the Bio-Rad ddPCR system, the results can be displayed as a one-dimensional graph, where each droplet's fluorescence intensity is plotted against droplet count. Figures 9 and 10 demonstrate the *Chloroflexi* 16S rRNA and total bacterial 16S rRNA genes, respectively. The Y-axis indicates fluorescence intensity in the fluorescein amidite (FAM) channel, which suggests the presence of the target DNA sequence when above a certain threshold. Higher fluorescence values mean that droplets are likely positive for the target DNA, with strong signals indicating the presence of the target gene. The X-axis represents individual droplets analyzed sequentially, providing a count for each droplet and plotting its fluorescence signal. The purple horizontal line represents the fluorescence threshold. Droplets above this threshold (blue) are considered positive for the target DNA and assigned a value of 1, while those below it (gray) are negative and assigned a value of 0.

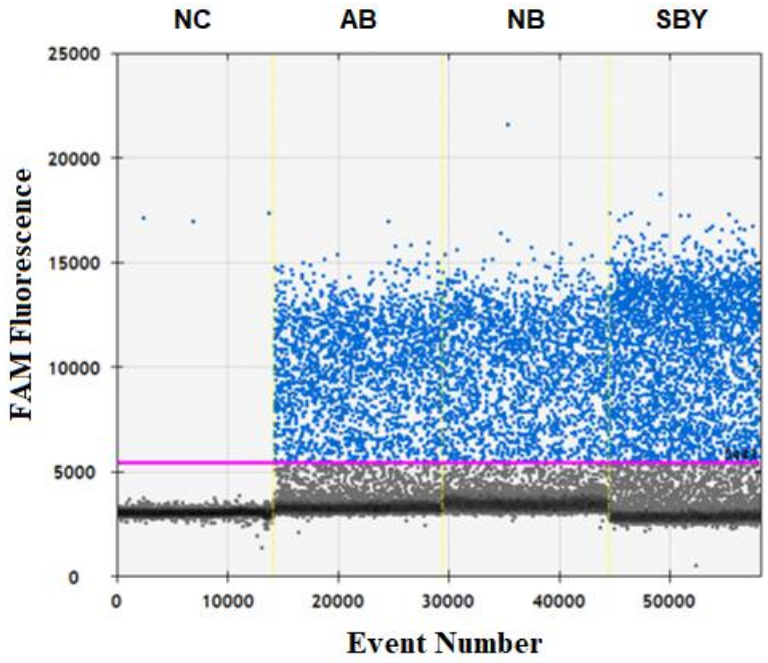

Figure 9. Droplet fluorescence intensity and positive/negative droplet distribution for *Chloroflexi* 16S rRNA gene.

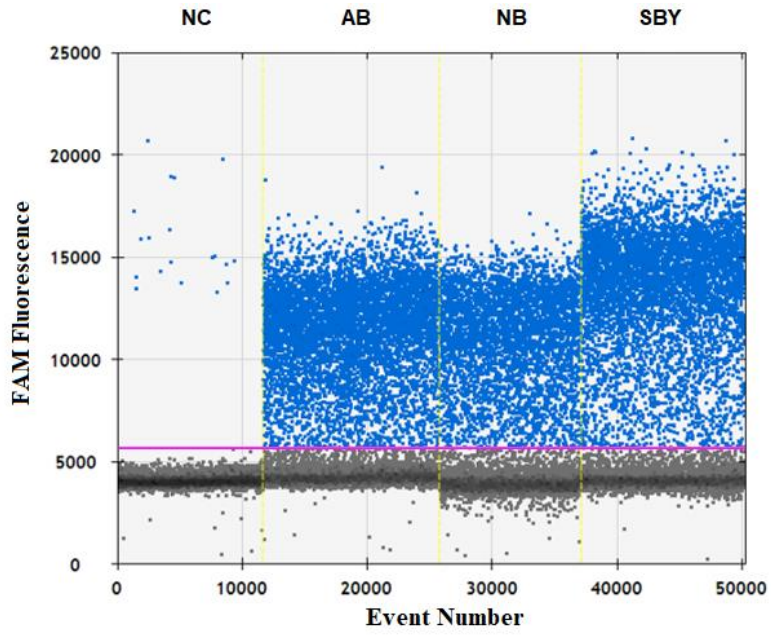

Figure 10. Droplet fluorescence intensity and positive/negative droplet distribution for total bacteria 16S rRNA gene.

After identifying positive droplets based on fluorescence intensity, the software automatically calculates the concentration of each target DNA molecule using a Poisson distribution model. The concentration values obtained from the software are presented in Table 13. The proportion of the *Chloroflexi* phylum within the total bacteria was calculated by dividing the copy number of the *Chloroflexi* 16S rRNA gene by the copy number of the total bacterial 16S rRNA gene and multiplying the result by 100. According to this calculation, the sample with the highest representation of the *Chloroflexi* phylum, at 37.6%, was from the sample collected from the ship breaking yards which supported our hypothesis. Hence, enrichment studies were initiated with SBY sediments to increase the abundance of microorganisms belonging to the *Chloroflexi* phylum, with the aim of better understanding their performance in remediating the sediments contaminated by POPs.

Table 13. Concentrations of *Chloroflexi* and total bacterial 16S rRNA genes determined by ddPCR analysis in geographical regions and proportions of the *Chloroflexi* phylum.

Geographical Region	***Chloroflexi* 16S rRNA Concentration (copies/µL)**	**Total Bacteria 16S rRNA Concentration (copies/µL)**	***Chloroflexi* Ratio (%)**
AB	165	704	23.4
NB	163	518	31.4
SBY	260	691	37.6

4.2. Enrichment Studies

The enrichment procedure of *Chloroflexi*-related microorganisms from SBY region using the sediments collected from there was described in Section 3.4. To monitor the enrichment of *Chloroflexi* in the initial cultures and the following subcultures, the change in the concentration of the added electron acceptors, PCB-61 and BDE-47, was

observed periodically. The changes in PCB-61 and BDE-47 concentration in the initial parallel enrichment cultures, Cultures 1-1 and Cultures 1-2, are illustrated in Figure 11. As seen, there were notable reductions in PCB-61 and BDE-47 concentration at 96% and 81% in both reactors within 30 days, respectively. This indicated that the microorganisms present in the sediment utilized PCB-61 and BDE-47 as electron acceptors.

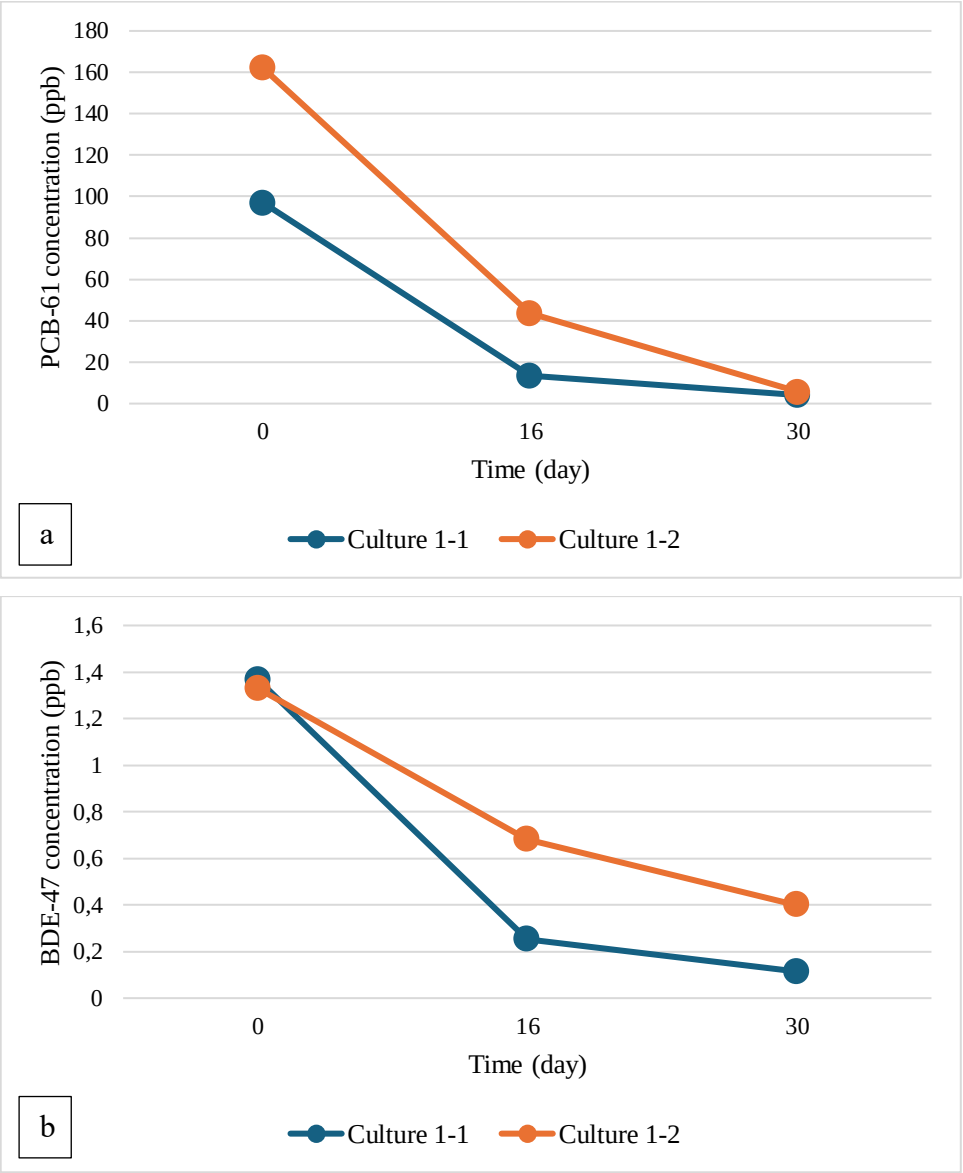

Figure 11. Changes in (a) PCB-61 and (b) BDE-47 concentration in the initial cultures during enrichment studies.

The initial cultures were subcultured following the procedure described in Section 3.4. The PCB-61 and BDE-47 concentration change with time in these subcultures are presented in Figure 12. It was observed that after each subculture was prepared, the cultures required time to adapt to the new environment and reach equilibrium. In some cultures, a temporary increase in concentration or plateau level was observed. After the adaptation process was completed, the cultures began to effectively remove the target compound. Therefore, to more accurately reflect the point at which the actual removal process began for each culture, concentration values at certain time points were designated as the initial concentration. For example, the concentration values on day 0 for Subculture 2 and on days 0 and 7 for Subculture 3 were not considered the initial concentrations of PCB-61 for the respective cultures. For this reason, the concentration ratios with respect to the initial concentration (C/C_0) vs time graphs were used to evaluate the changes in PCB-61 and BDE-47 removal over time, normalizing the data and enhancing comparability. This allowed for a more consistent comparison by eliminating the effects of differences in initial concentrations among different cultures. Additionally, the average PCB-61 and BDE-47 concentrations in each subculture prepared in duplicates were calculated to eliminate any potential differences between the bottles.

The PCB-61 removal performance in Subculture 2 is illustrated in Figure 12a. Upon examining the graph, it is seen that the C/C_0 ratio decreased to 0.04 on day 117, starting from the process that began on day 23. This indicates that 96% of the PCB-61 was successfully removed. Notably, a rapid removal occurred between days 23 and 51, followed by a slowdown. The graph in Figure 12b shows a 44% removal of BDE-47 over 117 days in Subculture 2. The most important reduction occurred between days 31 and 67. There was an overall gradual decline in concentration, indicating effective microbial adaptation and degradation. When examining the PCB-61 removal performance of Subculture 3, a relatively slow removal process was observed at the beginning (Figure 12c). However, an increase in the removal rate was noted from day 46 to day 67, indicating that more than approximately 60% of the PCB-61 was removed during this period. Between days 67 and 80, a slight increase in the C/C_0 ratio was observed, indicating a slowdown in the rate of PCB-61 removal. BDE-47 levels initially increased until day 46 likely due to a lag period in the cultures (Figure 12d). A sharp decrease occurred between days 46 and 67. Similar to PCB-61 removal, a slight increase in the C/C_0 ratio was observed between days 67 and 80, but a total removal of 45% was achieved overall. Moving on to Subculture 4, there was a generally decreasing trend in PCB-61

removal (Figure 12e). Approximately 60% of PCB-61 was removed up to day 21. From day 21 to day 43, the removal rate slowed down but continued, and reached 75%. However, there was an unexpected increase in the C/C_0 ratio which was most likely due to the errors in sample preparation or analytical processes. By day 21, approximately 35% of BDE-47 had been removed (Figure 12f). Between days 21 and 43, the removal rate slowed down but continued and reached 42%. However, the same slight increase in BDE-47 concentration was observed again between days 43 and 64. In conclusion, Subculture 4 was able to remove up to 75% and 42% of PCB-61 and BDE-47 within 64 days, respectively. The concentration of the electron acceptors at 1.6 times the initial concentration was decreased successfully. These results indicated that Subculture 4 became applicable for use in microcosm bioremediation studies.

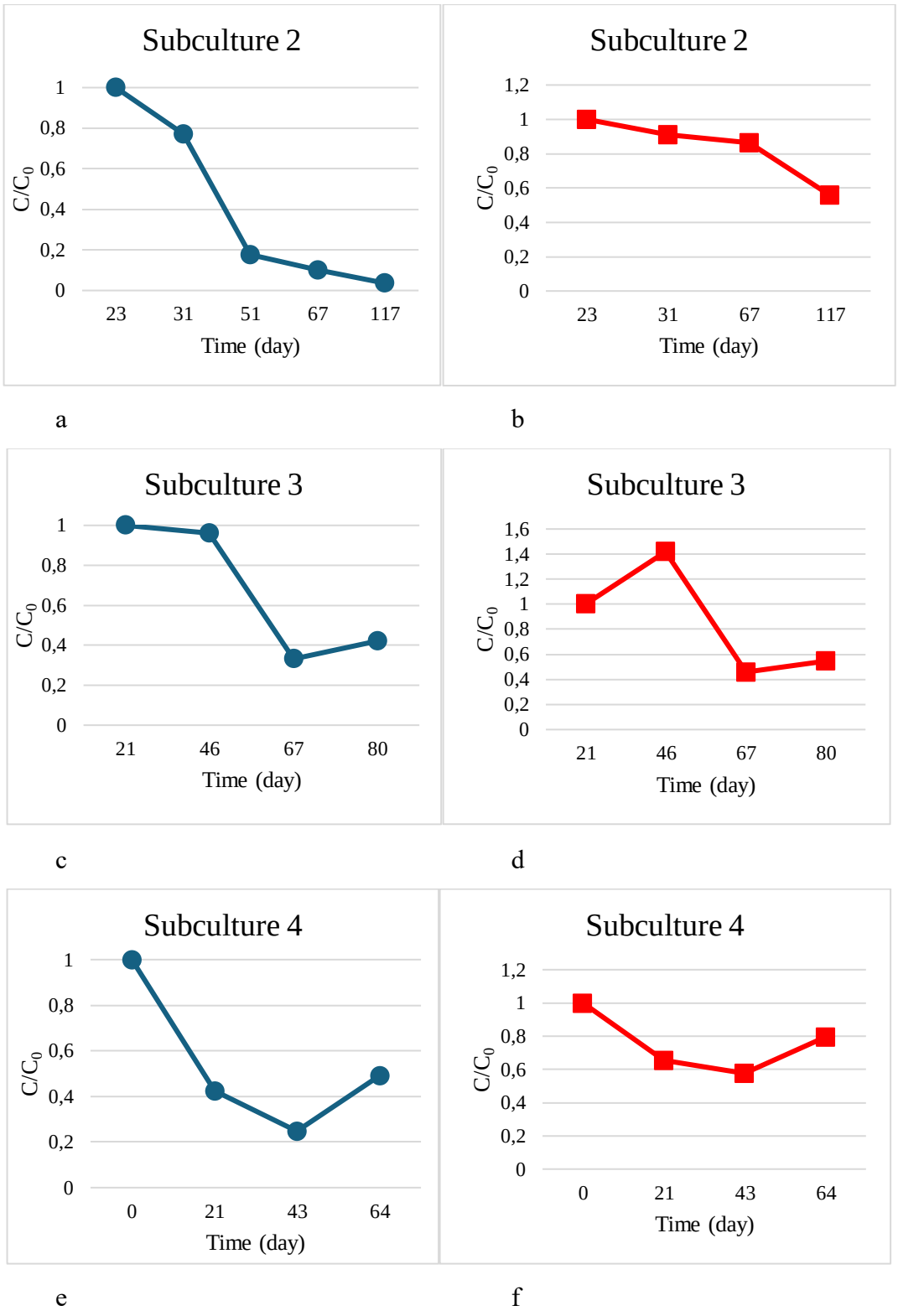

Figure 12. Temporal changes in PCB-61 (blue) and BDE-47 (red) degradation in the Subcultures 2 (a,b), 3 (c,d), and 4 (e,f).

4.3. Sediment Microcosm Studies

4.3.1. Degradation of POPs in Sediments

The temporal trends in total PCB concentrations were monitored at specific time points (days 0, 28, 56, 84, and 112) to evaluate the performance of bioaugmentation (BA), biostimulation (BS), natural attenuation (NA), negative control (NC), and sterile control (SC) microcosm setups. Figure 13 shows the change in the total concentration of PCBs in ng per g dry weight of sediments (dw) over time across the different treatment groups. Both BA and BS sets exhibited similar trends during the first 56 days. However, after that, a noticeable decline in total PCB levels began for both cases. This trend could be attributed to a lag period for microorganisms to adapt to the new conditions. While BA set experienced a 14% decline in PCB concentrations, the decline was %19 for BS set over a 112-day incubation period. NA and NC microcosms showed similar and stable trends up to day 84, and then started to decline resulting in a final degradation of %12 for both setups in 112 days. Hence, it can be speculated that bioaugmentation and biostimulation decreased the lag period for the microorganisms for PCB removal in the sediments. For the SC set, the total PCB concentration exhibited a stable pattern throughout the 112-day incubation period. The total PCB concentrations in the SC set slightly varied, but statistical analysis showed no significant change with time (analysis of variance – within subject design, $F(2,5)=0.03$, $p>0.05$). Since the SC set was prepared by sterilizing with $HgCl_2$ and autoclaved to prevent all microbial activity, the results confirmed that the PCB levels remained the same in the absence of biological activity. The decreases in total PCB concentrations of the active sets were as expected, as anaerobic reductive dechlorination occurred through the removal of chlorine atoms, involving their replacement with hydrogen atoms on the biphenyl ring.

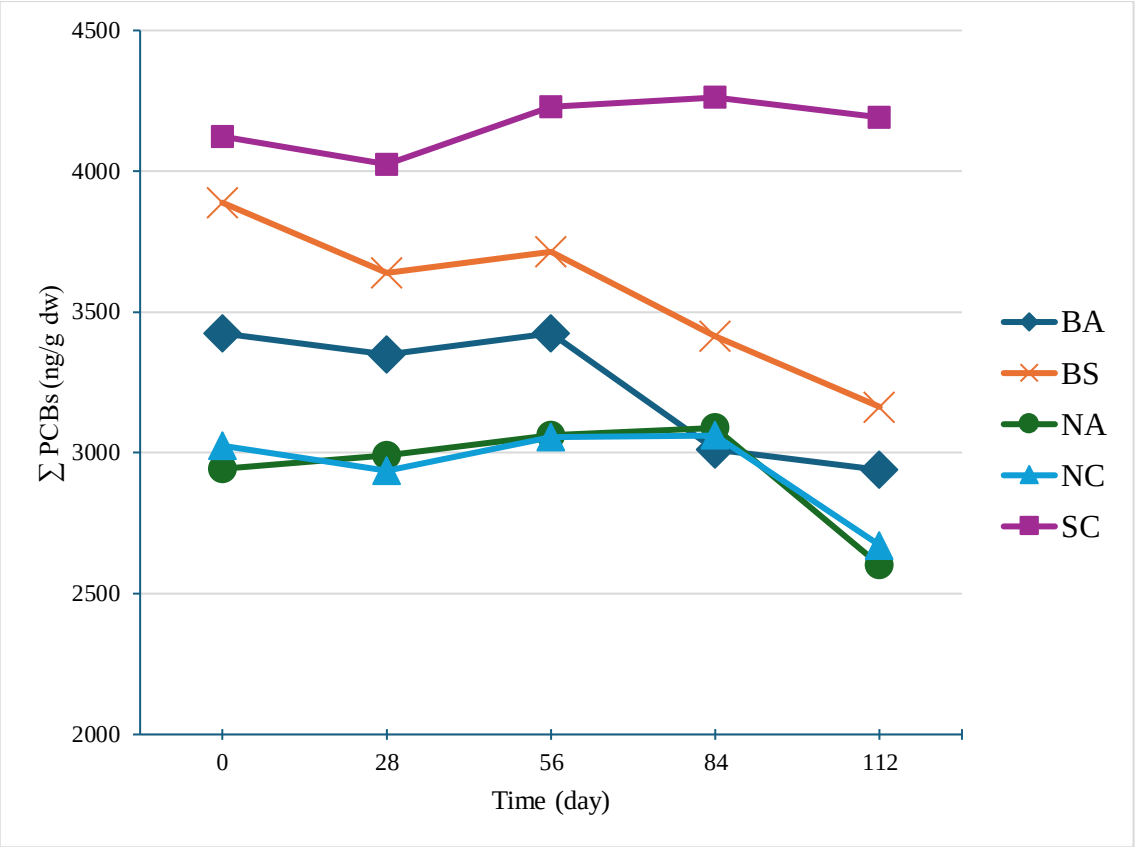

Figure 13. Total PCB concentration with respect to time for each microcosm set. Values represent the average of duplicate and triplicate reactors.

When previous microcosm studies were examined, higher removal percentages were observed than those of this study. Bioaugmentation removed 19.9% of total PCBs after 70 days at 20 °C under rotation in PCB-contaminated marine sediments in Matturro et al. (2016), which was conducted with a *D. mccartyi*-containing enriched culture. In the same study, removal up to 26.7% was reported for NA set (Matturro et al. 2016). These differences suggested that PCB degradation strongly depended on the variations in experimental conditions or the composition of autochthonous microbial communities.

In Krumins et al. (2009), di- to tetra-CBs were increased by 20% following the addition of pentachloronitrobenzene (PCNB) to the Anacostia River sediment microcosms containing weathered PCBs over a 415-day incubation period. By comparison, in the present study, the application of a medium enriched with acetate, yeast extract, vitamins, and minerals resulted in a 4% increase in mono- to tetra-CBs within 112 days. These outcomes emphasized the differences in biostimulatory conditions, such

as the use of haloprimers versus nutrient-enriched media. Additionally, in Krumins et al. (2009), bioaugmentation with a *D. mccartyi*-containing enriched culture led to a 16% increase in di- to tetra-CBs over 415 days. In contrast, in this study, the same enriched culture resulted in only a 3% increase in mono- to tetra-CBs within 112 days.

In addition to PCBs, the temporal trends in PBDE concentrations were also monitored to evaluate the effectiveness of the same microcosm treatments (Figure 14). The total PBDE removal efficiency in the BA set exhibited a continuous decreasing trend at 33% despite a period of reduced removal observed between days 28 and 56. Similarly, the BS set achieved a total removal of 24% with an exponentially decreasing removal rate. For the NA set, the total removal was limited to 8%. The concentration remained stable during the first 28 days, increased between days 28 and 56, and eventually fell below the initial concentration by day 112. The observed increase might be due to the analytical complications in the sediments' extraction. Similar to PCB removal efficiency, it can be concluded that BA and BS treatments decreased the lag period for the microorganisms during PBDE degradation, as well. The NC set showed a 19% reduction in PBDE levels and followed a continuously decreasing trend similar to those observed in the BA and BS sets. On the other hand, the SC set exhibited no statistically significant changes in PBDE concentrations throughout the 112-day incubation period (analysis of variance – within subject design, $F(2,5)=1.61$, $p>0.05$), confirming that the absence of microbial activity prevents PBDE removal. As a result, the reductions in total PBDE concentrations confirmed that the anaerobic reductive debromination was facilitated by the bioremediation techniques applied.

In the study by Demirtepe and Imamoglu (2019), bioaugmentation with *Dehalobium chlorocoercia* strain DF-1 achieved a 40.2% reduction in BDE-209 over 180 days, supported by sodium formate and ethanol as the carbon source and electron donors and incubation at 25°C. In comparison, the present bioaugmentation set, using the *Chloroflexi*-containing enriched culture with sodium acetate and hydrogen, achieved a 15.4% reduction in BDE-209 at 22°C in 112 days. For biostimulation, the earlier study reported the highest reduction of 55.3% while the present biostimulation set achieved an 18.1% reduction. Natural attenuation resulted in a 30.9% reduction in BDE-209 whereas current results showed a lower reduction of 7.8%. Finally, their negative control exhibited a 31.2% reduction. However, the negative control present showed a 5.7% reduction. This comparison emphasized that differences in microbial species, substrate selection, and environmental conditions notably influenced BDE-209 removal efficiency.

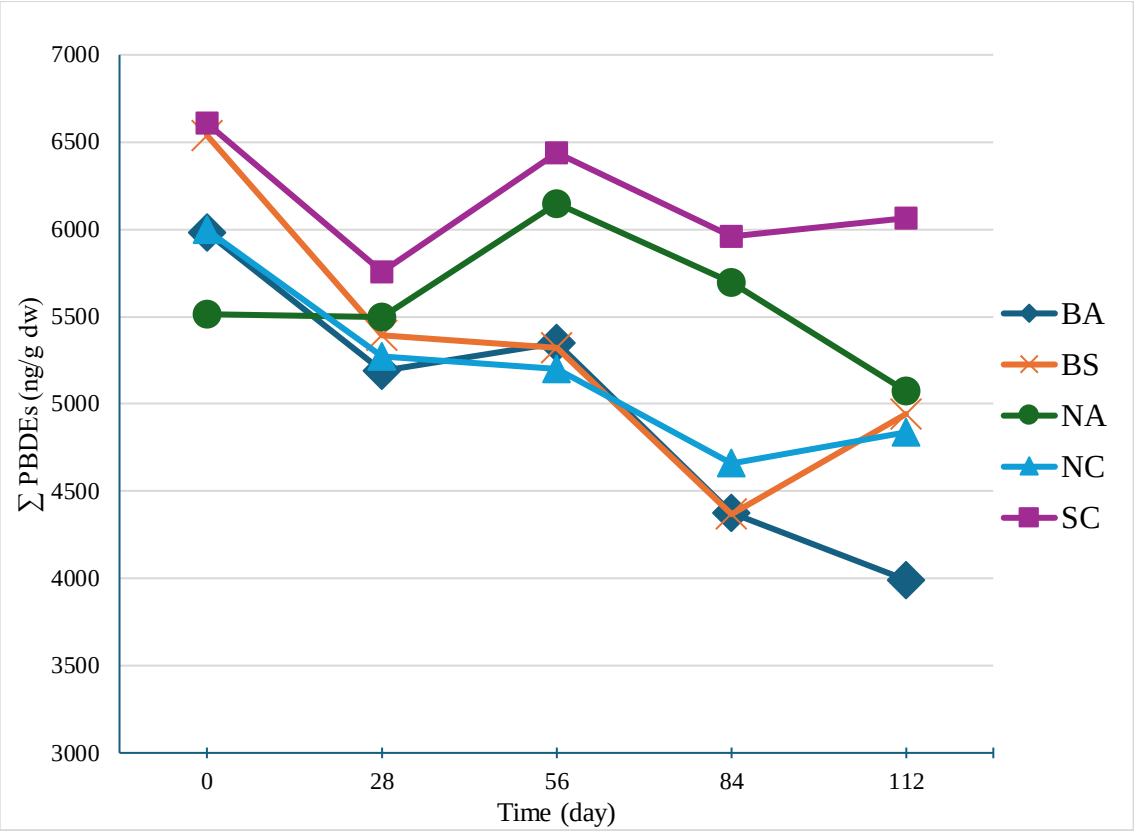

Figure 14. Total PBDE concentration with respect to time for each microcosm set. Values represent the average of duplicate and triplicate reactors.

In another study, Tokarz III et al. (2008) reported a 3.8% reduction in BDE-209 over 300 days when phosphate buffer, methanol, and dextrose were added to the lake sediment microcosms. Besides, they observed decreases of 30% in BDE-47 and 3% in BDE-99 in 240 days. This showed a continuous debromination of lower brominated PBDEs. In comparison, the present biostimulation set achieved a 18% reduction in BDE-209 within 112 days and demonstrated a faster and more effective breakdown. However, in this system, BDE-47 and BDE-99 levels increased by 0.5% and 0.8%, respectively. This suggested that the debromination process had just begun. This difference might have resulted from the substrates, as the earlier study's use of phosphate buffer, methanol, and dextrose likely facilitated further breakdown of lower brominated PBDEs, while sodium acetate and hydrogen proved more effective for initial BDE-209 degradation.

In the study by Pan et al. (2007), the objective was to assess the biostimulatory effect of acetate as a carbon source on the degradation of BDE-47 in contaminated

mangrove sediments. The degradation reached 48% and 76% after 60 and 180 days, respectively. In comparison, the present study found a degradation of BDE-47 at 11% over 112 days in the biostimulation treatment amended with acetate. Nevertheless, it should be noted that the mother congeners of BDE-47 continued to appear while it was also being transformed into tri-BDEs. Hence, a net increase or decrease in BDE47 concentration might not be the result of these microcosm studies.

4.3.2. Change in Concentrations of Homolog Groups

The degradation of PCB and PBDE compounds was also individually assessed based on the changes in the concentrations of the homolog groups. The data provided insights into the temporal trends and the relative performances of each treatment strategy and emphasized the key differences between biologically or nutritionally enhanced and control setups.

The distribution of the PCB homolog groups at day zero is shown in Figure 15 as an average of all sets. Even though the initial concentrations of the groups differed across the treatment sets, their distributions were consistent, as the same composite sediment was used to set up the microcosms. The graph indicated that hexa- and hepta-CBs were dominant, accounting for 27% and 20% of the total amount of PCBs, respectively. These were followed by moderate amounts of octa-, penta-, tetra-, and tri-CBs, with nona-, di-, and mono-CBs present in minimal amounts.

The distribution of the PBDE homolog groups in the BA set at day 0 and day 112 is shown in Figure 16. At day 0, BDE-209 as the sole member of the deca-BDE group was predominantly found at 75% in the sediments, followed by moderate amounts of tetra- and nona-BDEs, and minimal amounts of tri-, penta-, octa-, hexa- and hepta-BDEs. By day 112, the dominance of BDE-209 still continued but decreased to 60% of the total PBDEs, while tetra-BDEs increased from 10% to 12%. Tri-, hexa-, and octa-BDEs each rose to 8%. This indicated the transformation of higher-brominated PBDEs, such as BDE-209, into lower-brominated homolog groups through anaerobic reductive debromination in BA strategy.

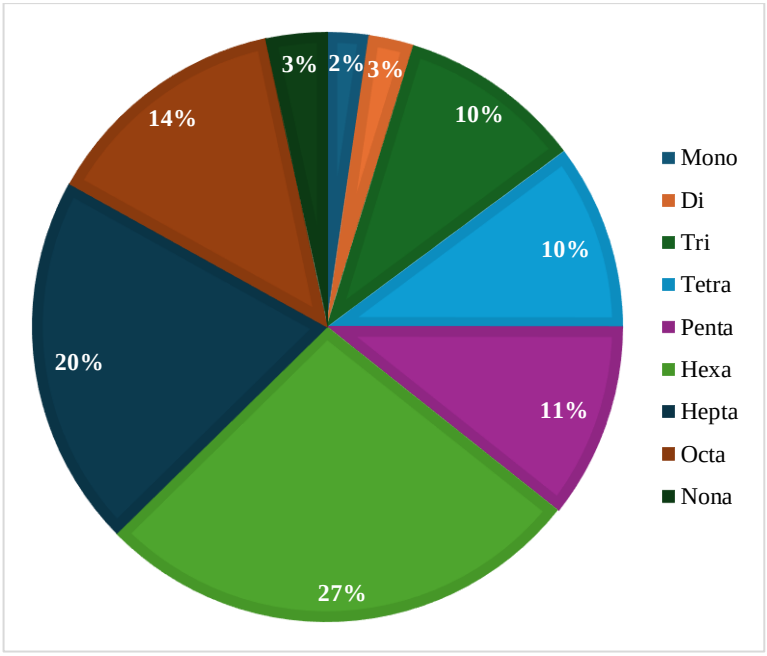

Figure 15. The distribution of PCB homolog groups based on their concentration at day zero.

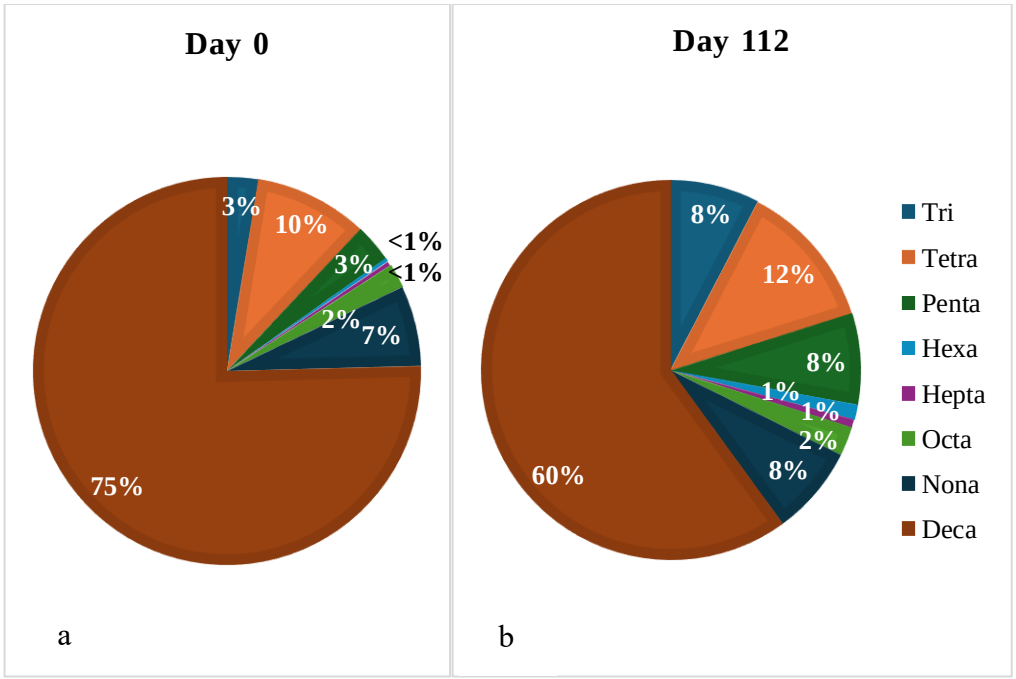

Figure 16. The distribution of PBDE homolog groups in the bioaugmentation set at (a) day 0 and (b) day 112.

The BA treatment showed a visible decline in PCB concentrations across the various homolog groups over the course of 112 days. Figure 17 presents the PCB concentrations in nmol per g dw at five time points. The overall trend demonstrated a remarkable decrease in the concentrations of highly chlorinated PCB groups, such as hexa-, hepta-, and octa-CBs, while the lower chlorinated groups, including mono-, di-, tri-, tetra-, and penta-CBs, exhibited more stable concentrations with slight fluctuations. There was a slight decrease in the concentration of the nona-CB, namely PCB-206, after day 28. While the decrease was particularly noticeable after day 56 for octa-CB group, the hepta-CB group began to show a clear decrease around day 84. The observed trends in the hexa-CB and penta-CB groups provided an interesting comparison between the two. Specifically, the hexa-CB group showed a noticeable decline in the concentrations between day 56 and day 84, followed by another drop between day 84 and day 112. However, this decline could not find a corresponding increase in the penta-CB group. Instead, the penta-CB concentrations increased only between day 84 and day 112. Similarly, the trend in the tetra-CB group between day 28 and day 56 shows relative stability while the tri-CB group experienced an evident increase between day 28 and day 56. Another interesting trend was the decrease in the concentrations of the penta-CB group between days 28 and 56 while the tetra-CB group exhibited stability and the concentrations of tri-CB group increased within the same timeframe. This suggested that the dechlorination of penta-CBs may have still resulted in the formation of tetra-CBs; however, the subsequent dechlorination of tetra-CBs into tri-CBs may have been more pronounced. In addition, the decreases in the tetra- and penta-CB groups between days 56 and 84 could not find a response in tri-CB group. These discrepancies can be explained by the possible transformation to lower chlorinated congeners, which were not measured in the present study due to the lack of relevant reference standard mixtures. In addition, as the transformation shifted toward the production of lower chlorinated PCBs, they might have partitioned into the water phase since the water solubility of these compounds were higher as indicated in Table 1. Therefore, this could have resulted in their loss of track since only the sediments were analyzed in this study. This phenomenon especially explained the absence of observable increases in mono- and di-CB groups, as equilibrium between sediment and water phases might have masked their presence. Lastly, the dechlorination process, in which chlorine atoms were removed one by one, occurred in a concerted manner simultaneously across homolog groups, rather than through strictly sequential pathways.

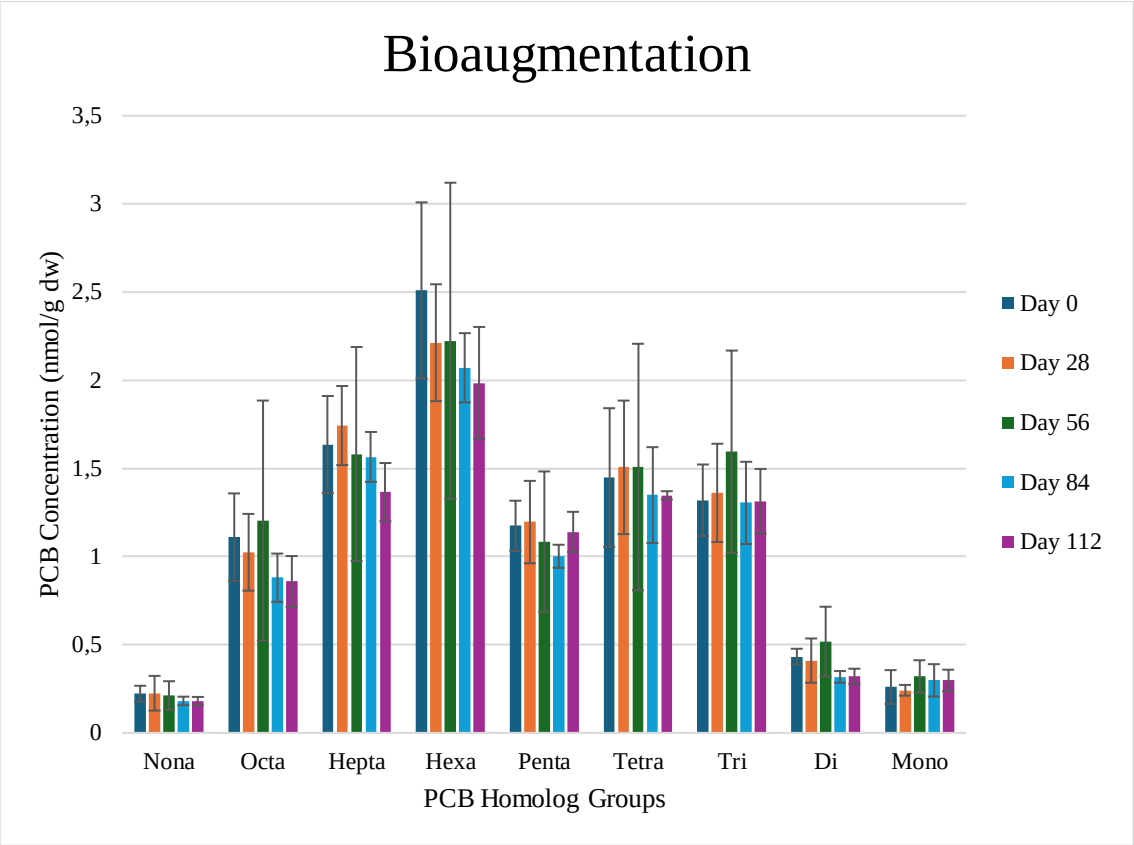

Figure 17. PCB concentrations in bioaugmentation treatment with respect to time for each homolog group.

The BS treatment led to varying trends in PCB concentrations across different homolog groups. The general pattern showed a notable reduction in the concentrations of highly chlorinated PCBs (Figure 18). The concentrations of octa-CBs demonstrated a noticeable decrease between day 0 and day 28, after which they stabilized and remained constant throughout the rest of the study period. Hepta-CBs maintained a stable concentration from day 0 to day 84, followed by a decline between day 84 and day 112. In contrast, hexa-CBs showed a continuous decrease throughout the study. Interestingly, while the decline in hexa-CBs between days 0 and 56 appeared to cause an increase in penta-CBs, the subsequent decline in hexa-CBs after day 56 did not correspond to a proportional increase in penta-CBs. Instead, this could be explained by the increase in tetra-CBs observed between days 56 and 112. This suggested that the decline in hexa-CBs may not have directly reflected in penta-CBs but instead manifested as an increase in tetra-CBs over a delayed time period. Similarly, the decline in penta-CBs between days

56 and 112 was only matched by an increase in tetra-CBs at day 112. This pattern could be explained by a phenomenon referred to as retarded degradation, in which the transformation of one homolog group became apparent in the next time interval. Tri-CBs showed an overall decline. However, the decrease in tetra-CBs between day 56 and day 84 and the increase between day 84 and day 112 corresponded to the pattern seen in tri-CBs, where an increase occurred between day 56 and day 84, followed by a decrease from day 84 to day 112. For di-CBs, a slight increase was observed at day 56, followed by a decrease. Finally, mono-CBs demonstrated a more stable trend throughout the experimental period, with minor fluctuations but no significant changes. The last two trends could be attributed to factors such as the subsequent increase in the solubility of these compounds or the transformation into those that were not measured in this study.

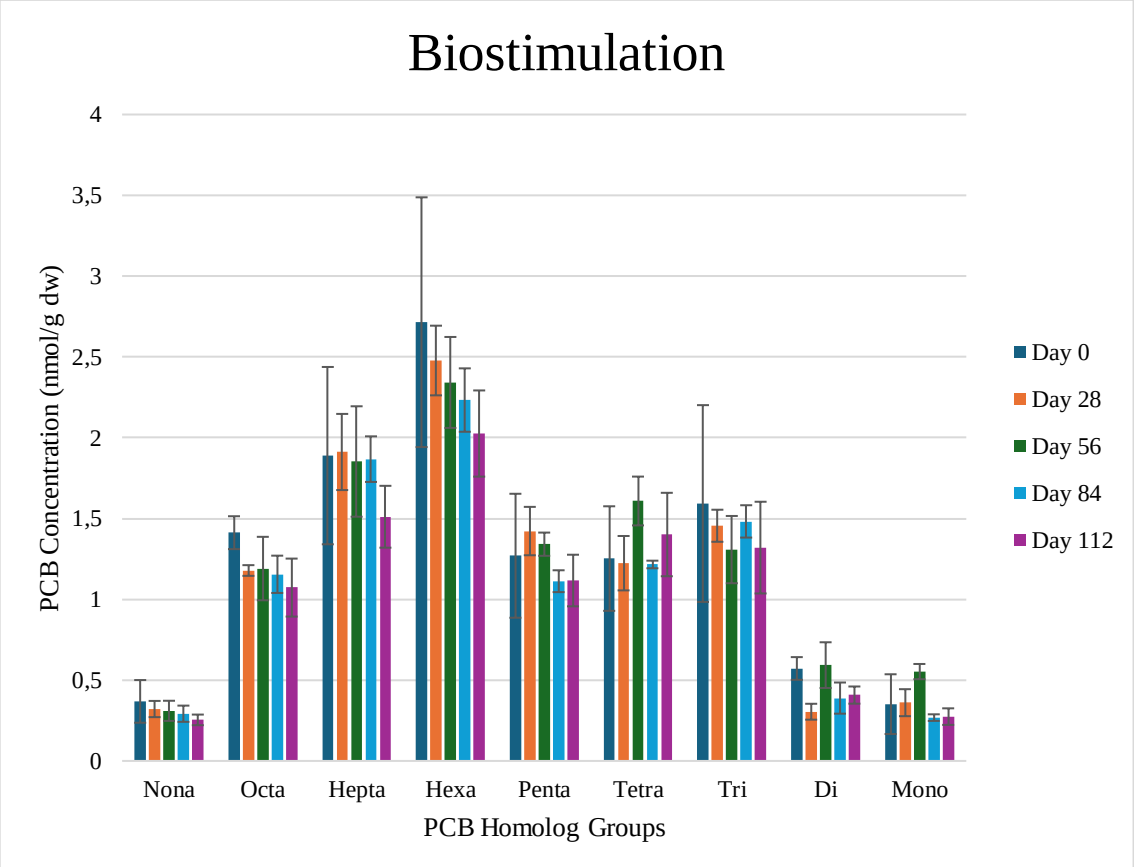

Figure 18. PCB concentrations in biostimulation treatment with respect to time for each homolog group.

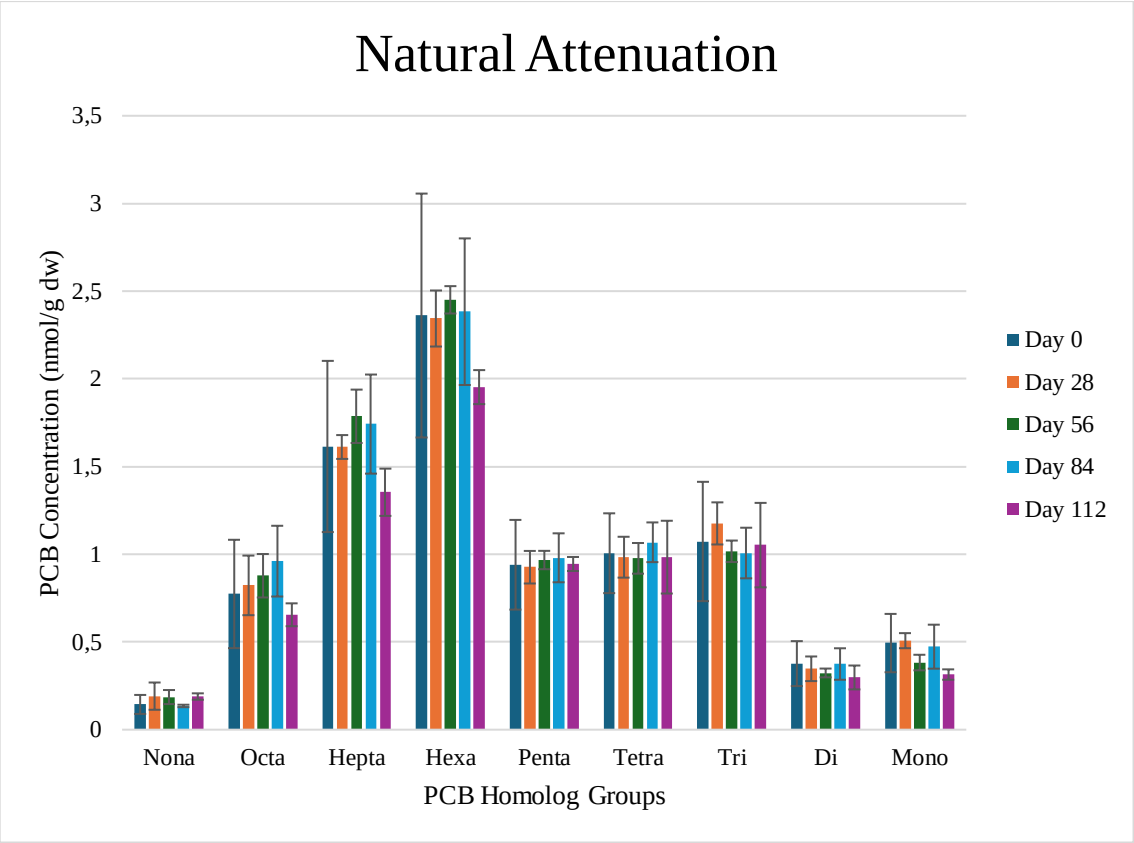

Figure 19. PCB concentrations in natural attenuation microcosms with respect to time for each homolog group.

In the NA set, when homolog groups were examined, there was no significant change in the lower chlorinated tri-, tetra-, and penta-CB groups (Figure 19). Mono- and di-CBs groups also remained stable throughout the study. Specifically, the pseudo-increase in octa-, hepta-, and hexa-CBs could be attributed to the possible contribution of unmeasured nonachlorinated PCBs, such as PCB-207 and PCB-208, as mother congeners. Only PCB-206 was measured in the nona-CB group in this study, and it demonstrated a stable trend. Additionally, decachlorinated PCB-209, which has commonly detected in environmental samples, may have also contributed to this transformation (Megson et al. 2023; Saba and Boehm 2011).

In Matturro et al. (2016)'s study, NA resulted in a 32.5% reduction of hexachlorinated PCB-153 and a 23.8% reduction of heptachlorinated PCB-180 within 70 days. In contrast, in our study, the hexa-CB group showed only a 1.71% reduction, and the hepta-CB group exhibited a 1.24% reduction over 112 days. These differences can be

attributed to the experimental conditions again, as Matturro et al. (2016) incubated the microcosms at 20 °C under rotation, whereas in our study, incubation was performed without rotation at 22 °C. These differences emphasized the fundamental effect of experimental conditions on the activity of autochthonous microbial communities.

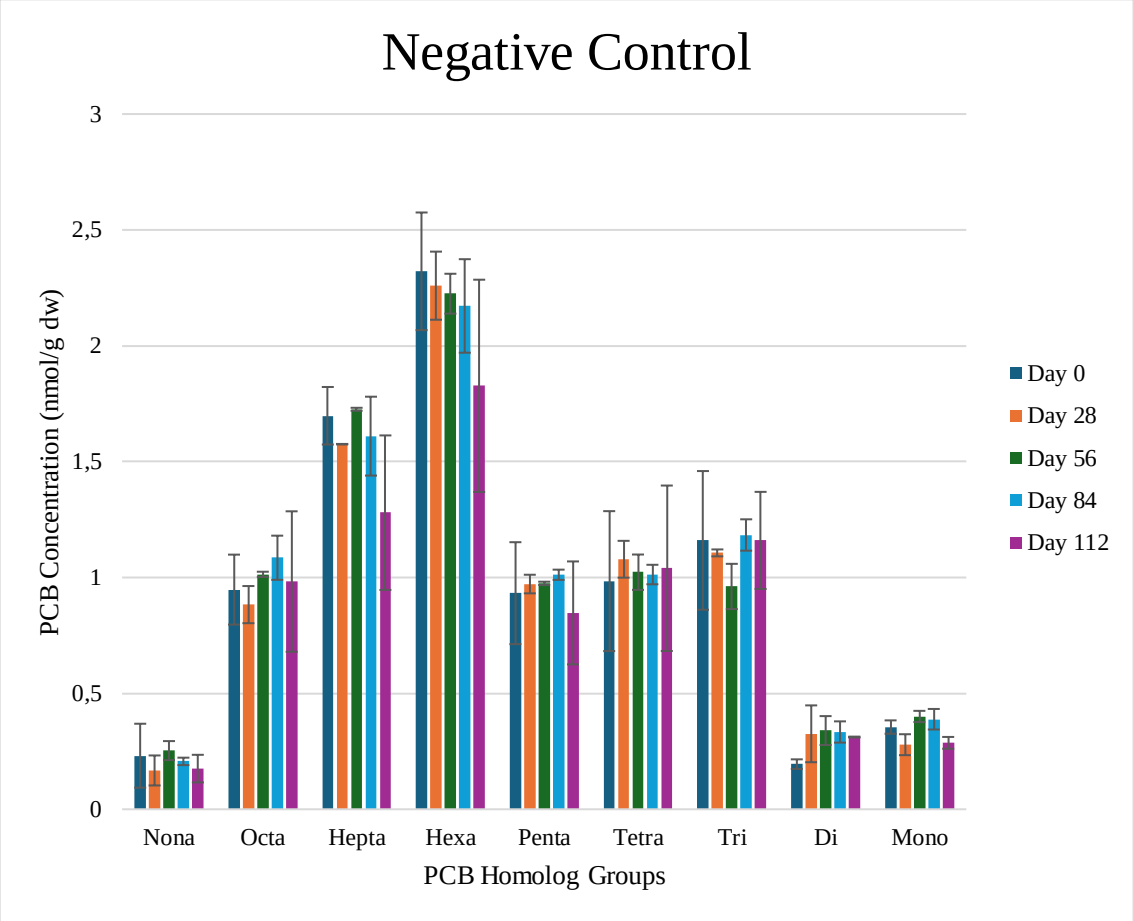

Figure 20. PCB concentrations in the negative control set with respect to time for each homolog group.

In the NC set, octa-CBs exhibited fluctuations but no significant difference was observed between day 0 and day 112 (Figure 20). Hepta-CB group showed fluctuations during the first 84 days but experienced a notable decrease in concentration by day 112. Hexa-CBs exhibited a gradual decrease over the first 84 days, which may have contributed to the gradual increase in penta-CBs during the same period. However, the larger decrease observed in hexa-CBs until day 112 was not reflected in penta-CB group.

Tetra-CBs maintained a stable trend throughout the experimental period, while tri-CBs showed a decrease until day 56, followed by an increase up to day 112. This unexpected result may have been caused by an analytical error or simply by averaging the results of only two reactors. Nonetheless, the initial decrease in tri-CBs during the first 56 days may have explained the increase in di-CBs during the same period. In conclusion, while reductions were observed in highly chlorinated hexa- and hepta-CBs, the concentration changes in lower chlorinated PCBs (except for tri-CBs) remained more stable. This suggested that lower chlorinated PCBs might be more affected by analytical errors or other factors.

Overall, the significant reductions observed in highly chlorinated PCB groups in the BA and BS sets demonstrated their capacity to degrade hexa-, hepta-, and octa-CB groups effectively. In the BA set, the presence of microorganisms emphasized that this effect originated from biological mechanisms, whereas in the BS set, the micronutrients provided in the growth medium were found to play an important role by enhancing the activity of native microbial communities. The growth medium in the BS set facilitated the highest PCB removal (19%) by supplying essential micronutrients. In contrast, the limitation or depletion of micronutrients over time in the BA set may have reduced microbial efficiency; however, it still achieved a higher removal (14%) compared to the NA and NC sets. In the BA set, a synergistic interaction might have occurred between the enriched culture microorganisms added and the native microorganisms in the sediment. The enriched culture microorganisms might have produced intermediate products that served as an energy source for the native microorganisms, and vice versa. Both the NA and NC sets showed similar removal efficiencies (12%). However, the used-up growth medium added to the NC set did not appear to provide any advantages to the native microorganisms and may even have had adverse effects due to the potential presence of inhibitory byproducts. These findings focused on the critical importance of nutrient and energy supplementation in PCB degradation. Specifically, supporting microbial activity with micronutrients emerged as a key strategy for enhancing the degradation of highly chlorinated PCB groups, offering an effective approach to PCB degradation in sediments.

When the changes in the concentration of PBDE homolog groups in BA treatment were considered, it was seen that deca-BDE (BDE-209) showed a continuous decrease and indicated that the debromination process was actively ongoing (Figure 21). Nona-BDEs exhibited both increases and decreases, as increases occurred during the conversion of deca-BDE to nona-BDEs, and decreases occurred when nona-BDEs transformed into

lower brominated forms. Octa-BDEs displayed a similar trend and demonstrated that they were both formed and subsequently converted into lower brominated homologs.

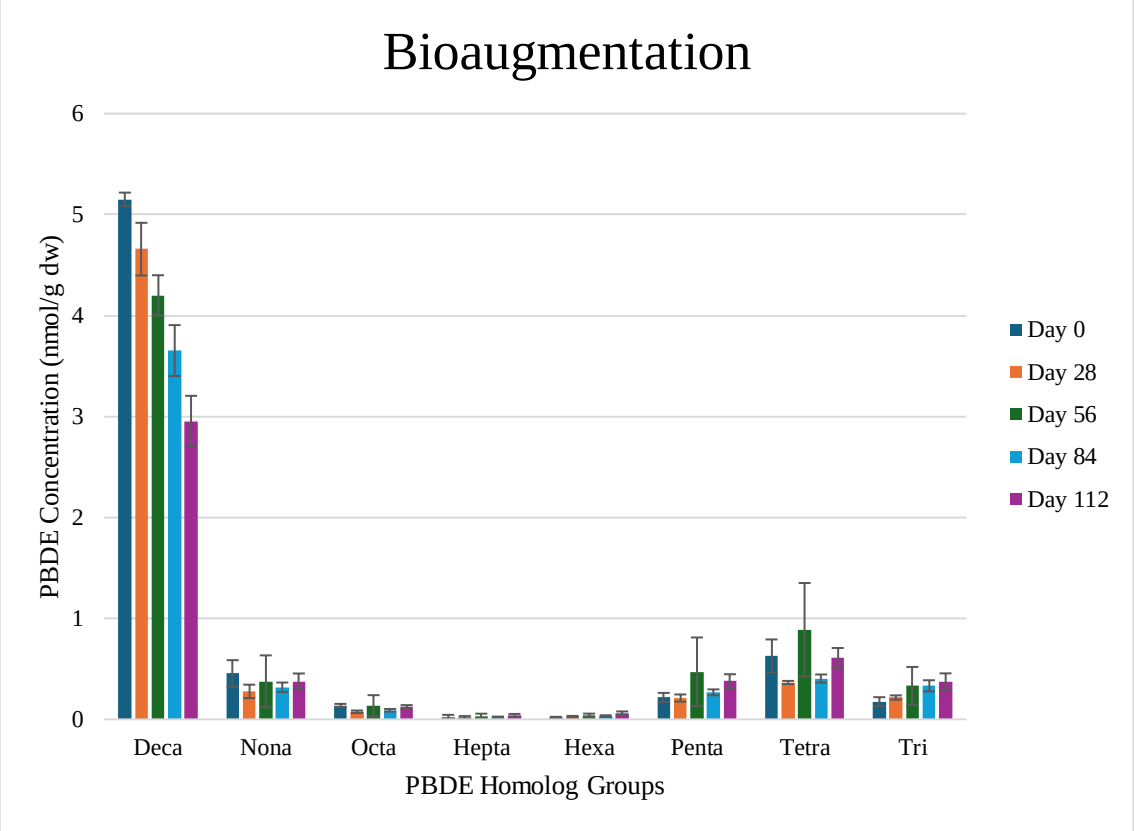

Figure 21. PBDE concentrations in the bioaugmentation set with respect to time for each homolog group.

Following the previous paragraph, although hexa- and hepta-BDEs could not be clearly and distinctly tracked, a notable increase in hexa-BDEs was observed on day 112. The reason might have been that the inability to monitor the concentration changes of hepta-BDEs such as BDE-184 and BDE-191, as well as the hexa-BDEs such as BDE-128, which had the potential to be formed but were not measured in this study (Tokarz III et al. 2008; Huang, Chang, and Lee 2014). The only hepta- and hexa-BDEs measured here were PCB-183 and -190, and PCB-138, -153, and -154. Penta- and tetra-BDEs showed fluctuations. This suggested that transformation processes were continuing through these homologs. Tri-BDEs exhibited a continuous increase which indicated that

the debromination process was in the early stages and lower brominated homologs were beginning to accumulate. Since mono- and di-BDEs were not measured, the transformation of higher brominated PBDEs into these homologs could not be tracked. However, it is likely that the two measured congeners within the tri-BDE group (BDE-17 and BDE-28) were formed, and higher brominated PBDEs may have been transformed into other tri-BDEs that could not be measured. Additionally, as mentioned for the PCB degradation results, lower brominated PBDEs might have partitioned into the water phase since their water solubility increased as the transformation progressed toward their formation.

In the study by Xu et al. (2022), the penta-BDEs (BDE-47, -99, and -100) were converted to diphenyl ether with 90% efficiency within 60 days through bioaugmentation using *D. mccartyi* strain CG1 in sediment microcosms incubated at 30°C. In present study, only BDE-85, -99, and -100 were analyzed, and a 5% increase was observed in 112 days. This increase could be attributed to the reductive debromination of hexa- and higher-brominated PBDEs into penta-BDEs, as their total reduction was calculated as 13%. Additionally, the observed increases in tri- and tetra-BDE levels (5% and 3%, respectively) indicated that the debromination process in present treatment was in its early stages. However, mono- and di-BDEs as well as diphenyl ether were not measured in this study, which limited the ability to fully assess the extent of debromination into lower-brominated products.

In the BS treatment, BDE-209 showed a continuous decrease, while nona-BDEs and octa-BDEs exhibited both increases and decreases (Figure 22). This indicated the transformation of deca-BDE into nona-BDEs, followed by the conversion of nona-BDEs into octa-BDEs and subsequently into lower brominated forms. Hexa- and hepta-BDEs showed notable increases, particularly on day 112. This suggested that transformations from higher homologs were ongoing. Penta-BDEs, despite fluctuations, displayed an overall increasing trend, whereas the trend for tetra-BDEs continued with alternating decreases and increases. Tri-BDEs consistently showed an increase which confirmed that the debromination process was actively progressing and lower brominated homologs were accumulating. Overall, debromination processes were actively occurring under biostimulation strategy.

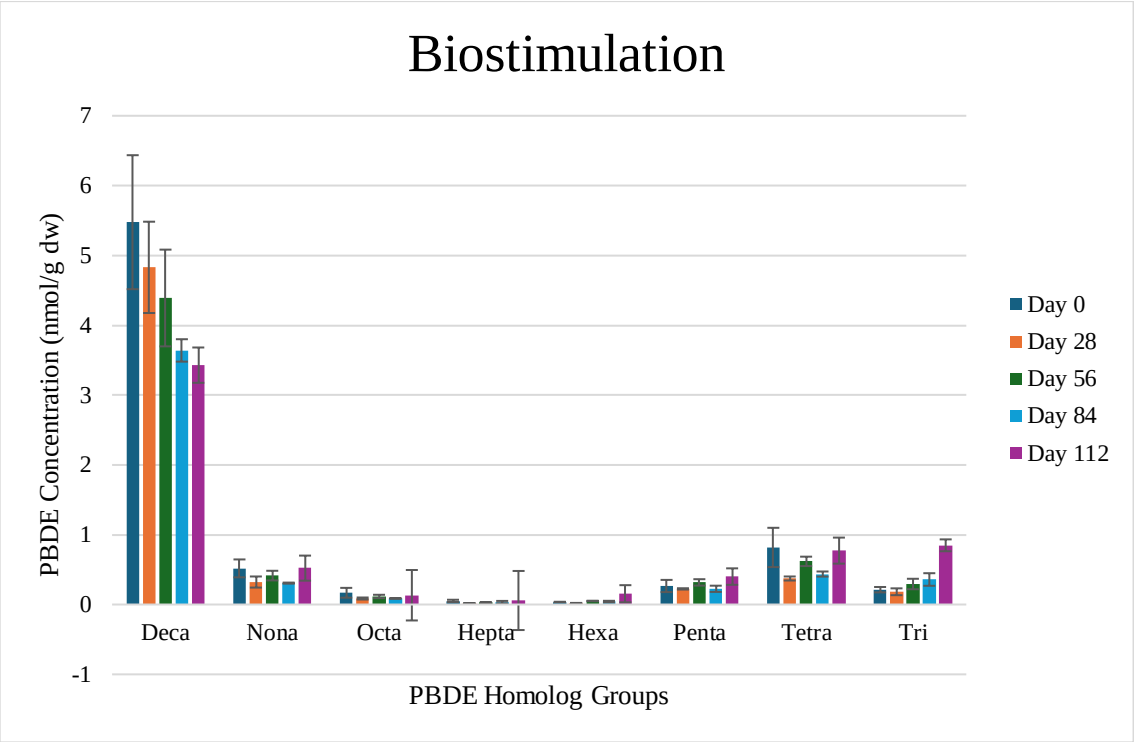

Figure 22. PBDE concentrations in the biostimulation set with respect to time for each homolog group.

In the NA set, the deca-BDE remained stable between days 0 and 56 but showed a decreasing trend afterwards (Figure 23). Nona-BDEs increased and reached the highest level on day 56, and it was followed by a decline. Octa- and hexa-BDEs exhibited similar trends. Hepta-BDEs increased between days 28 and 84 and then began to decrease. This indicated that the transformation process was continuing. Penta-BDEs showed a steady increase. It suggested that they accumulated as intermediate products in the transformation process. Tetra-BDEs displayed fluctuations with alternating increases and decreases, indicating that transformation into those were still occurring.

Although the extent of the decreases in deca-BDE varied between days, an overall decline was observed in the NC set (Figure 24). Nona-BDEs initially decreased until day 56 and then increased, showing similarities with the BA and BS sets. For octa-BDEs and hepta-BDEs, no remarkable transformation or increase was observed. Hexa-BDEs showed a minimal increase until day 112. Penta-BDEs displayed a decrease until day 56, and then an increase until day 112. Tetra-BDEs exhibited an overall decrease until day 84, but an increase was observed on day 112, resembling the BS set. This suggested that

penta-BDEs and tetra-BDEs accumulated and would continue to be formed as intermediate products later. As tri-BDEs remained stable, this indicated that the transformation did not progress to the level of tri-BDEs or that the accumulation of tri-BDEs under negative control conditions was very limited.

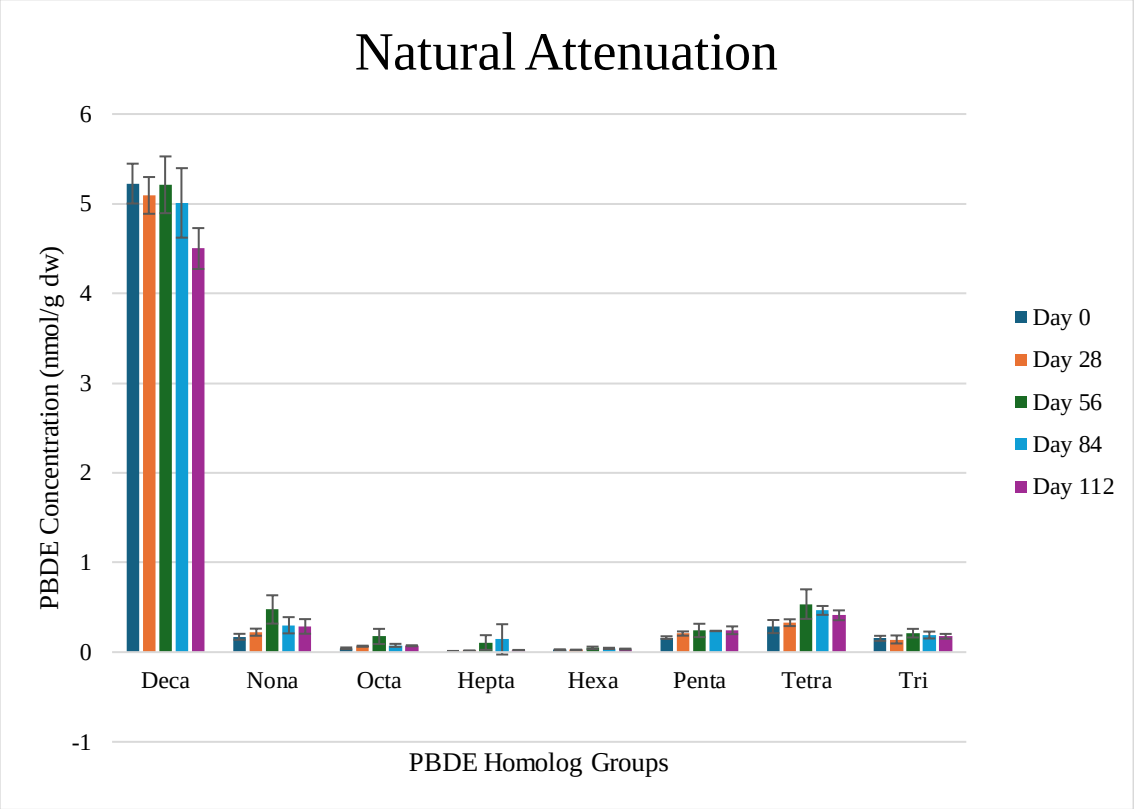

Figure 23. PBDE concentrations in the natural attenuation set with respect to time for each homolog group.

The removal of PCBs and PBDEs was examined under different microcosm strategies, and BA and BS demonstrated the highest efficiency in their removal. Therefore, the combined removal of PCBs and PBDEs as a mixture revealed the superior performance of BA and BS strategies driven by enhanced biological activity. The BA strategy benefited from the addition of enriched microbial cultures, which facilitated the transformation of both pollutants through synergistic interactions. On the other hand, BS treatment effectively stimulated native microbial communities by providing essential nutrients. NA and NC sets exhibited limited removal efficiencies. Hence, it emphasized

that the importance of biological enhancement and nutrient supplementation in achieving effective degradation of PCB and PBDE mixtures in contaminated sediments.

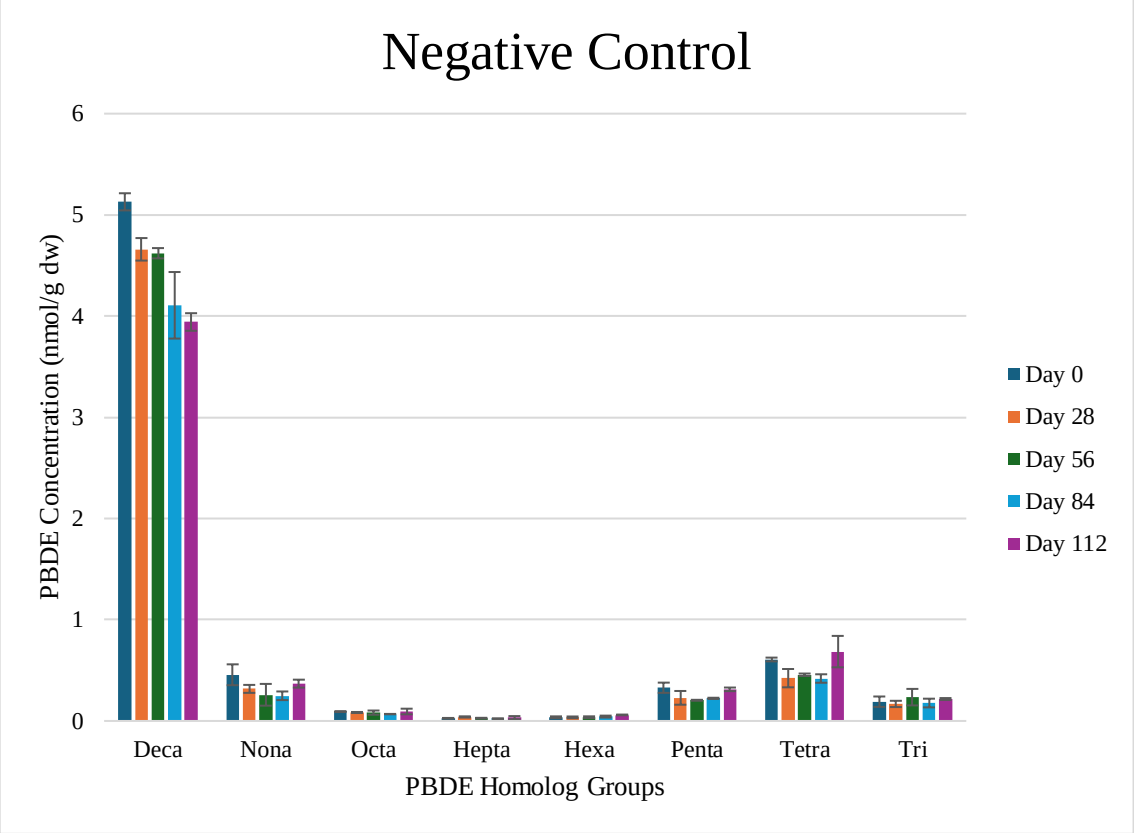

Figure 24. PBDE concentrations in the negative control set with respect to time for each homolog group.

4.3.3. Anaerobic Dehalogenation Rates

The calculated PCB degradation rates (Cl/bp/day) across the experimental setups were as follows: NC (0.00130) > BA (0.00124) > BS (0.00085) > SC (0.00053) > NA (0.00020). In the NC set, the observation of the highest rate was noteworthy despite the limited nutrient and energy availability for microorganisms. This suggested that physical mechanisms, such as partitioning into the aqueous phase, may have played a more dominant role, contributing to the reduced Cl/bp at day 112. Then, as mentioned before,

the synergy observed in the BA set might have enhanced the PCB degradation rate. However, the depletion of micronutrients over time or potential competition between the enriched culture and native microorganisms could have resulted in a rate lower than that of the NC set. Significant reductions were observed in the concentrations of especially highly chlorinated PCB groups, but this process may have progressed at a slower pace. The lower rate in the BS set compared to the NC and BA sets indicated that, although micronutrient supplementation supported microbial activity, its effects might have been distributed over time. Lastly, the SC and NA sets, as expected, demonstrated the limited impact of abiotic mechanisms and natural conditions through their lowest observed rates.

The comparison of the degradation rates observed in this study and in previous studies is presented in Table 14. In such degradation studies, either the weathered (i.e. aged, POP-contaminated) sediments are used or the sediments are spiked (i.e. artificially contaminated) with POPs in the laboratory. In few studies, weathered PCB contaminated sediments, like this study, were tested either with or without any amendments (Magar et al. 2005; Payne, May, and Sowers 2011). Weathered Lake Hartwell sediments without any amendments showed very close degradation rate to this study's natural attenuation set (Magar et al. 2005), while Baltimore Harbor sediments with bioaugmentation yielded three times higher rate than this study (Payne et al. 2011). On the other hand, sediments spiked with various Aroclor mixtures in the laboratory demonstrated much higher degradation rates, even without any amendments (Demirtepe et al. 2015; Fagervold et al. 2011). This discrepancy might be due to aging of sediments, in which contaminants are more strongly sorbed onto the sediments, resulting in lower bioavailability of the contaminants in the weathered sediments. Nevertheless, introduction of microorganisms with dehalorespiring capability and micronutrients led to the increase in the degradation rate in this study and in the previous studies.

The calculated PBDE degradation rates (Br/dp/day) across the experimental setups were as follows: BS (0.00945) > BA (0.00741) > NC (0.00291) > NA (0.00279) > SC (0.00270). These results differed from the PCB degradation rates in which NA demonstrated the lowest rate. Here, BS exhibited the highest degradation rate, potentially due to the enhancement of microbial activity through micronutrient supply. Interestingly, the NA rate was comparable to NC which suggested that abiotic and natural microbial processes were less efficient for PBDE removal compared to that of PCBs. The increased rate observed in the BA set suggested that the enriched culture played a notable role in the PBDE degradation process, even though its impact was less pronounced compared to

the enhancement achieved through nutrient supplementation in the BS set. The relatively lower degradation rate in the SC set confirmed the limited impact of abiotic factors. These observations emphasized the importance of biostimulation for PBDE degradation.

Table 14. The PCB degradation rates in sediments as chlorine per biphenyl removed per day using varying experimental conditions.

Experimental Conditions	**Rate (Cl/bp/day)**	**Reference**
Bioaugmentation with *Chloroflexi* of weathered Aliağa sediments	0.00124	This study
Biostimulation with micronutrients of weathered Aliağa sediments	0.00085	This study
Weathered Aliağa sediments without any amendment	0.00020	This study
Spiked Baltimore Harbor sediments without any amendment	0.00430	Demirtepe et al. 2015
Spiked Baltimore Harbor sediments biostimulation with fatty acids	0.0036	Kjellerup et al. 2014
Spiked Baltimore Harbor sediments bioaugmented with four species	0.0059	Fagervold et al. 2011
Spiked Baltimore Harbor sediments without any amendment	0.0047	Fagervold et al. 2011
Weathered Lake Hartwell sediments without any amendment	0.000258	Magar et al. 2005
Weathered Baltimore Harbor sediments bioaugmentation with *Dehalobium chlorocoercia*	0.0041	Payne, May, and Sowers 2011

Table 15 provides a comparison of the PBDE degradation rates observed in this study with those reported in previous research. The findings of this study showed strong similarities to those reported by Demirtepe and Imamoglu (2019). Both studies indicated that bioaugmentation and biostimulation significantly improved PBDE degradation, with biostimulation through nutrient supplementation typically achieving higher degradation rates than bioaugmentation. Additionally, natural attenuation setups consistently yielded the lowest degradation rates. While sewage sludge demonstrated a degradation rate of

0.0065 Br/bp/day, which was comparable to the BA rate observed in present study (0.00741 Br/bp/day), the BS setup in this study achieved a higher rate of 0.00945 Br/bp/day (Xu, Zhao, and He 2024). This suggested that nutrient supplementation in weathered sediments can provide a more significant enhancement in degradation rates than natural microbial activity within sewage sludge.

Table 15. The PBDE degradation rates in sediments and sewage sludge as bromine per diphenyl ether removed per day under different experimental conditions.

Experimental Conditions	Rate (Br/dp/day)	Reference
Biostimulation with micronutrients of weathered Aliağa sediments	0.00945	This study
Bioaugmentation with *Chloroflexi* of weathered Aliağa sediments	0.00741	This study
Weathered Aliağa sediments without any amendment	0.00279	This study
Weathered Aliağa sediments with spent growth medium	0.00291	This study
Bioaugmentation with *Dehalobium chlorocoercia* strain DF-1 of spiked Ankara sediments	0.0011	Demirtepe and Imamoglu 2019
Biostimulation with micronutrients of spiked Ankara sediments	0.0012	Demirtepe and Imamoglu 2019
Spiked Ankara sediments without any amendment	0.0009	Demirtepe and Imamoglu 2019
Nutrient amendments to sewage sludge from Singapore, China and USA	0.0065	Xu, Zhao, and He 2024

4.3.4. Possible Dehalogenation Pathways

A dechlorination pathway was proposed for the bioremediation strategies tested in this study, focusing on the stepwise transformation of highly chlorinated PCBs into less chlorinated congeners. The proposed pathway began with the dechlorination of PCB-180

(2,2',3,4,4',5,5'-heptachlorobiphenyl) (Figure 25). This compound have undergone transformation into two distinct congeners, i.e. PCB-153 (2,2',4,4',5,5'-hexachlorobiphenyl) and PCB-138 (2,2',3,4,4',5'-hexachlorobiphenyl). Subsequently, PCB-153 was further dechlorinated to PCB-101 (2,2',4,5,5'-pentachlorobiphenyl) and PCB-99 (2,2',4,4',5-pentachlorobiphenyl). PCB-138 was dechlorinated into PCB-99 and PCB-87 (2,2',3,4,5'-pentachlorobiphenyl). Finally, PCB-101 continued its transformation, resulting in PCB-52 (2,2',5,5'-tetrachlorobiphenyl).

The formation of lower chlorinated congeners followed different patterns in BA and BS treatments. As shown in Figure 26, the bioaugmentation strategy revealed distinct dechlorination pathways occurring over specific time intervals. Between days 28 and 56, PCB-180 underwent a significant transformation into PCB-138. This was evidenced by a sharp decline in PCB-180 concentrations during this period. At the same time, PCB-101 showed a marked decrease. It was further dechlorinated into PCB-52. Later in the study, from days 84 to 112, PCB-153 began to dechlorinate into PCB-101. During the same interval, PCB-138 branched into two distinct pathways. It was transformed into PCB-87 and PCB-99. The enriched microbial activity played a significant role in driving these changes.

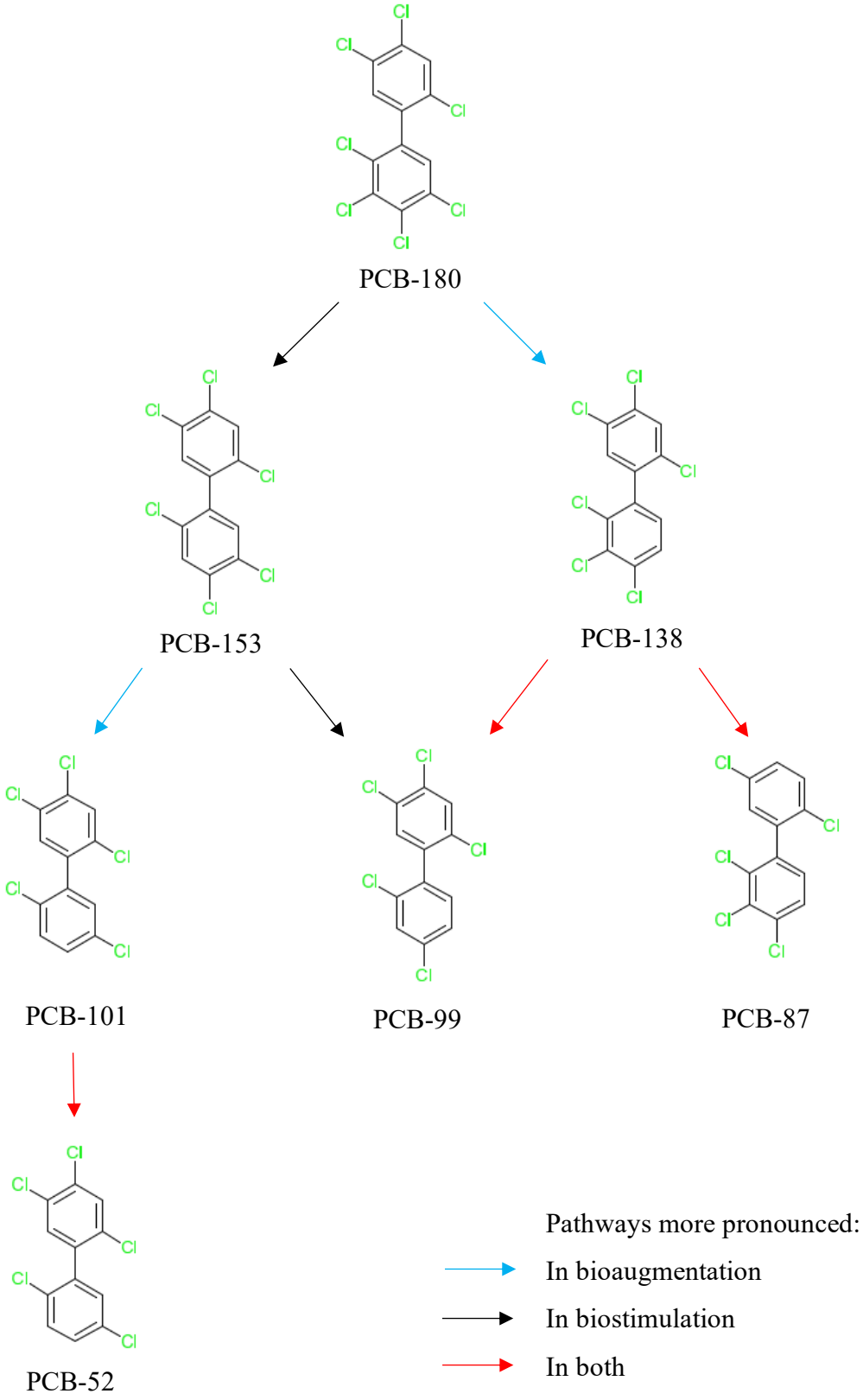

Figure 25. Proposed dechlorination pathway for PCB congeners under BA and BS strategies.

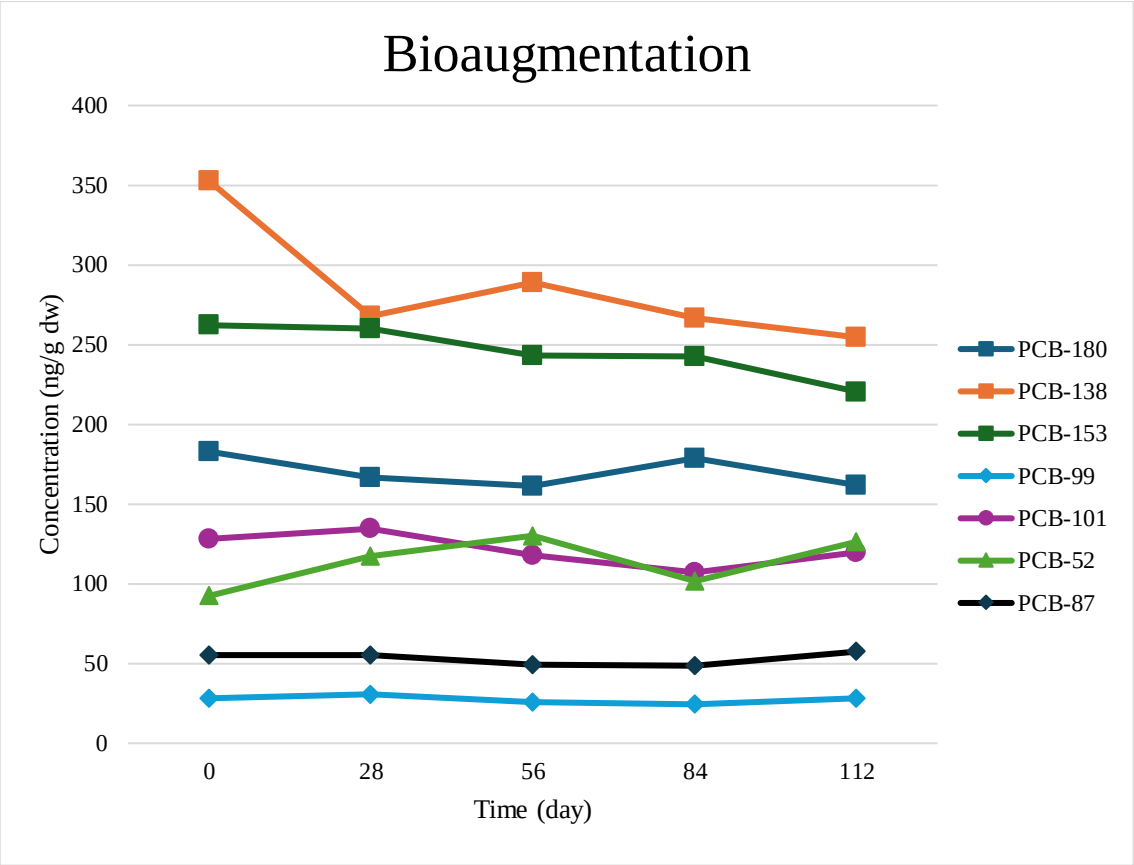

Figure 26. Concentration changes of PCB congeners over time under the BA strategy.

As shown in Figure 27, the BS treatment also exhibited distinct dechlorination pathways. PCB-180 was transformed into PCB-153 between days 84 and 112, as the mother decreased, the product congener increased. From days 0 to 28, PCB-153 was dechlorinated into PCB-99. During the same period, PCB-138 also contributed to the production of PCB-99. PCB-138 followed an additional pathway and was dechlorinated into PCB-87 during two separate intervals: days 28 to 56 and days 84 to 112. Finally, PCB-101 was dechlorinated into PCB-52 between days 84 and 112. These time-specific trends highlighted the sequential and dynamic nature of PCB dechlorination under the BA and BS strategies.

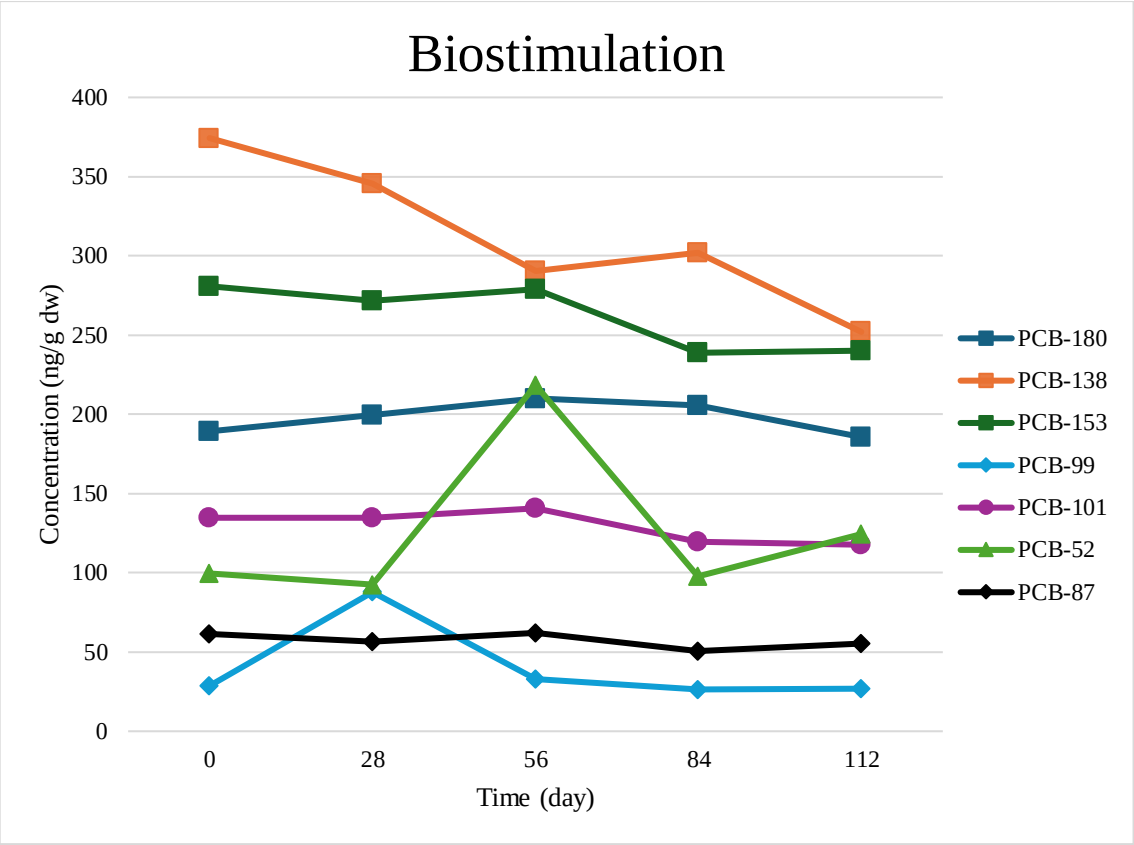

Figure 27. Concentration changes of PCB congeners over time under the BS treatment.

Two debromination pathways were proposed for the BA and BS strategies tested in this study by focusing on the stepwise transformation of highly brominated PBDEs into less brominated congeners. First proposed pathway examined the debromination of BDE-209 (2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether) which transformed into three daughter congeners comprising of the nona-BDE group such as BDE-206 (2,2′,3,3′,4,4′,5,5′,6-nonabromodiphenyl ether), BDE-207 (2,2′,3,3′,4,4′,5,6,6′-nonabromodiphenyl ether), and BDE-208 (2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether). Figure 28 illustrates the debromination pathway of interest. Figures 29 and 30 shows the changes in the concentrations of the nona-BDE congeners during the BA and BS processes, respectively. Between days 0 and 28, the concentrations of all congeners decreased in both the BA and BS sets. This situation suggested that the transformation of BDE-209 did not fully start yet. By day 56, BDE-207 becomes dominant over the other two congeners in the BA set, while both BDE-207 and BDE-206 become dominant over BDE-208 in the BA set. Starting from day 84, the formation rate of BDE-207 showed a

more notable increase compared to the other congeners. The observed trend suggested that BDE-207 was becoming the dominant product during the later stages of the debromination process.

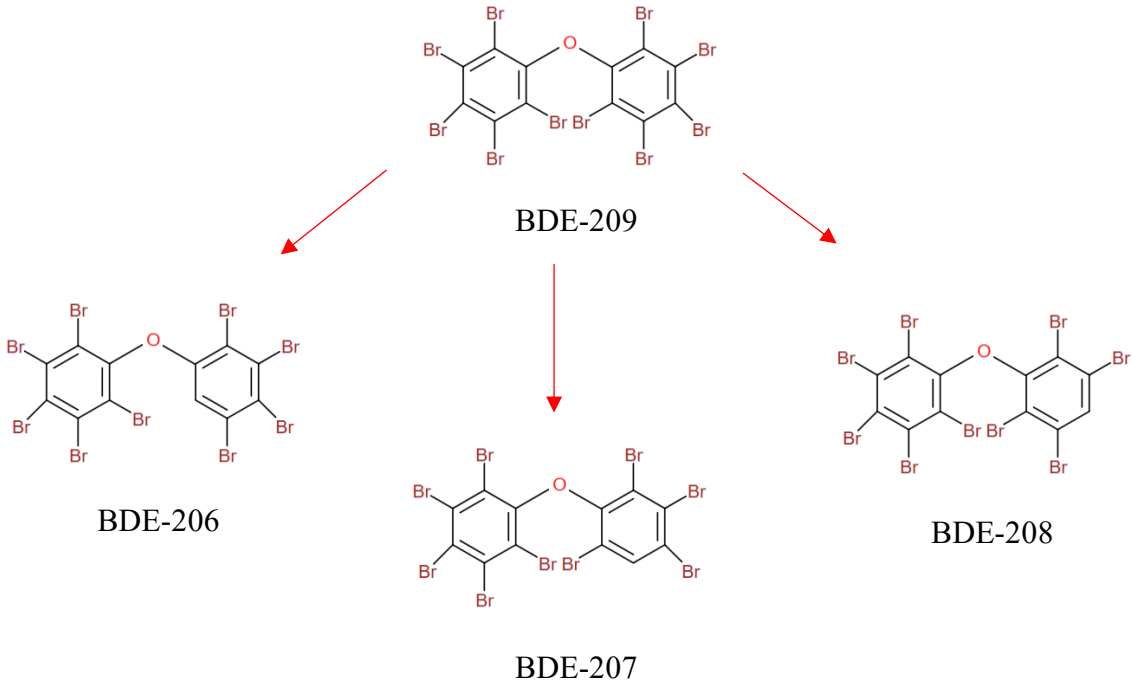

Figure 28. Proposed debromination pathway of BDE-209 under BA and BS strategies. Red arrows represent that the reactions occur in both sets.

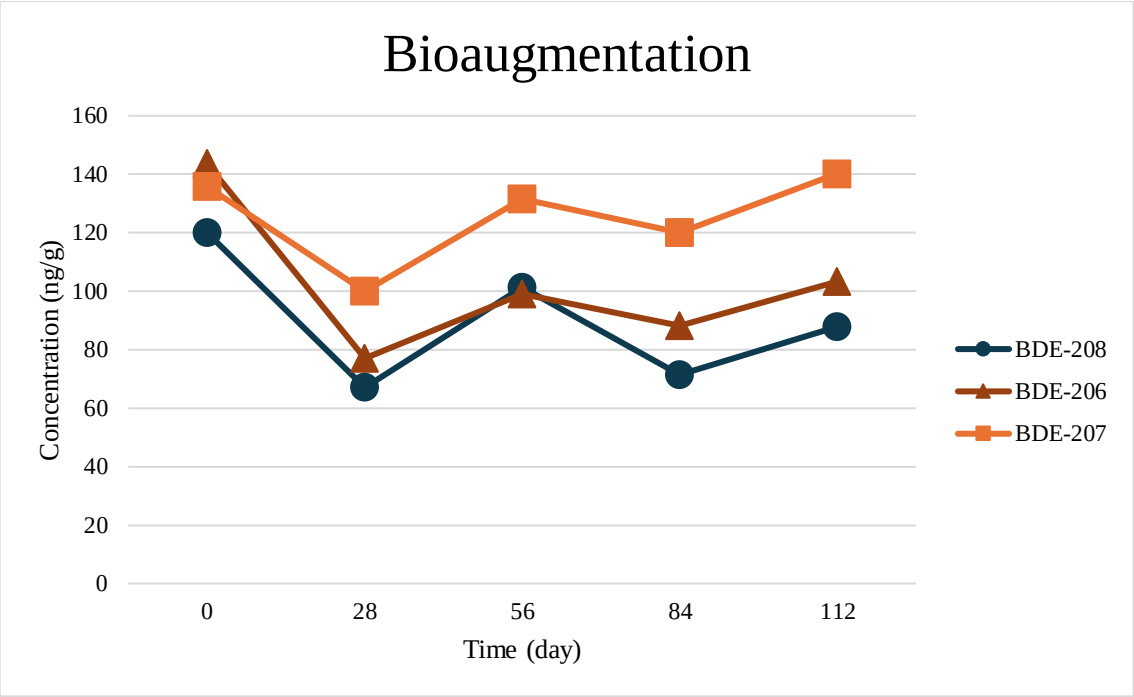

Figure 29. Concentration changes of nona-BDE congeners during the BA process.

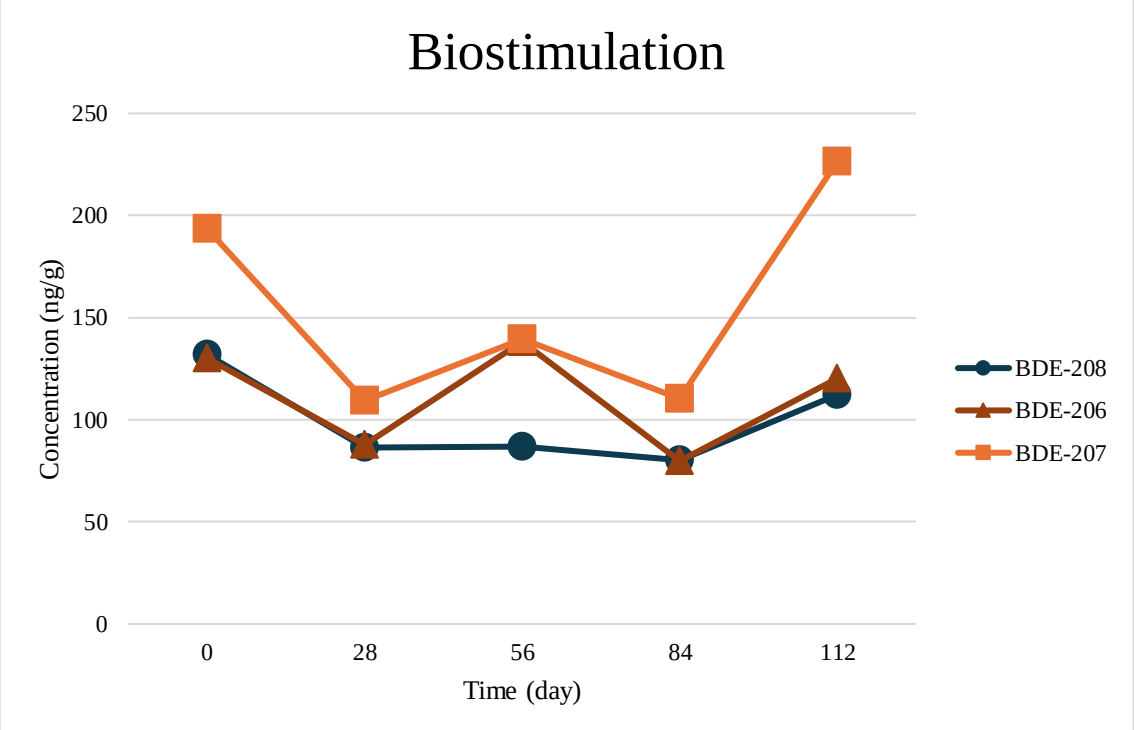

Figure 30. Concentration changes of nona-BDE congeners during the BS process.

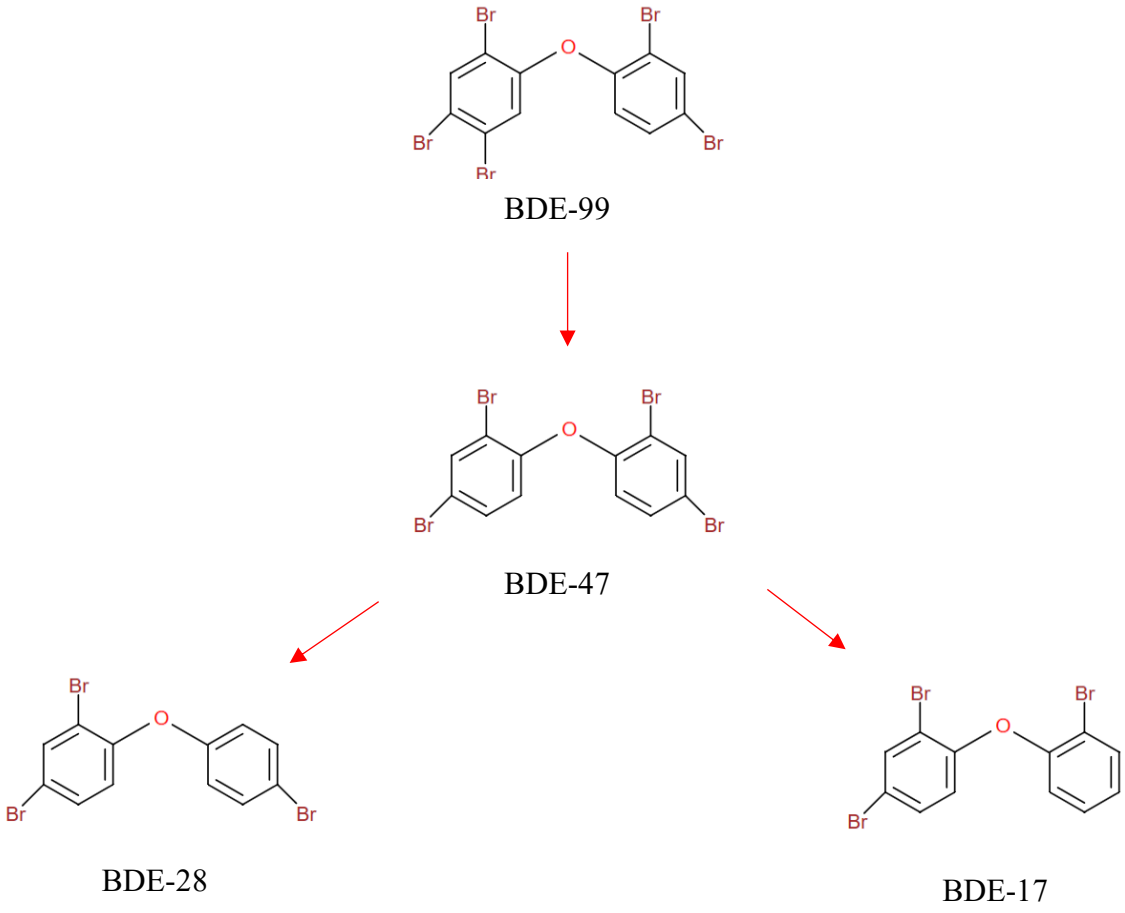

Figure 31. Proposed debromination pathway of BDE-99 under BA and BS strategies. Red arrows indicate that the reactions occur in both BA and BS sets.

The second proposed debromination pathway began with the debromination of BDE-99 (2,2',4,4',5-pentabromodiphenyl ether) into BDE-47 (2,2',4,4'-tetrabromodiphenyl ether) (Figure 31). Then, this compound has undergone transformation into two distinct congeners such as BDE-28 (2,4,4'-tribromodiphenyl ether) and BDE-17 (2,2',4-tribromodiphenyl ether). In both the BA and BS sets, BDE-47 followed similar trends and remained dominant in the sediments (Figures 32 and 33). BDE-28 showed different behaviors between the two strategies. In the BA set, its concentration increased until day 84, and then it stabilized. This suggested that it might have reached its peak. In contrast, BDE-28 continued to increase throughout the 112-day period in the BS set. BDE-17 also displayed varying trends between the sets.

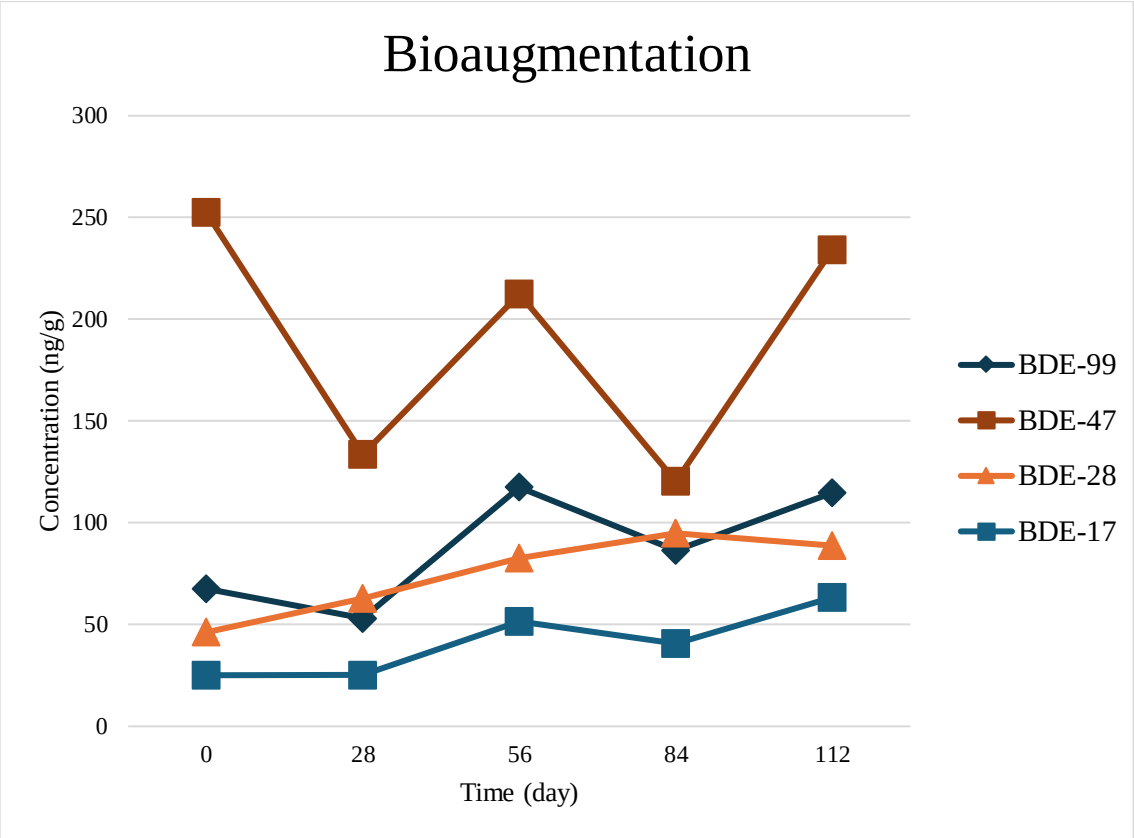

Figure 32. Concentration changes of PBDE congeners over time under the BA treatment.

In the BA set, its concentration remained stable from days 0 to 28, then increased until day 112. Meanwhile, in the BS set, it remained stable until day 84, and then increased between days 84 and 112. The difference in the trends between BDE-28 and BDE-17 indicated that BDE-28 could be a more favorable product. In the BA strategy, BDE-28 appeared to become the end product by day 112, as its concentration stabilized, while BDE-17 continued to increase and could still be transforming. In the BS strategy, the increase in BDE-28 continued. It suggested that it could also become an end product, although this has not been achieved within the 112-day experimental period yet. Additionally, the production of BDE-17 was ongoing, and its transformation could continue.

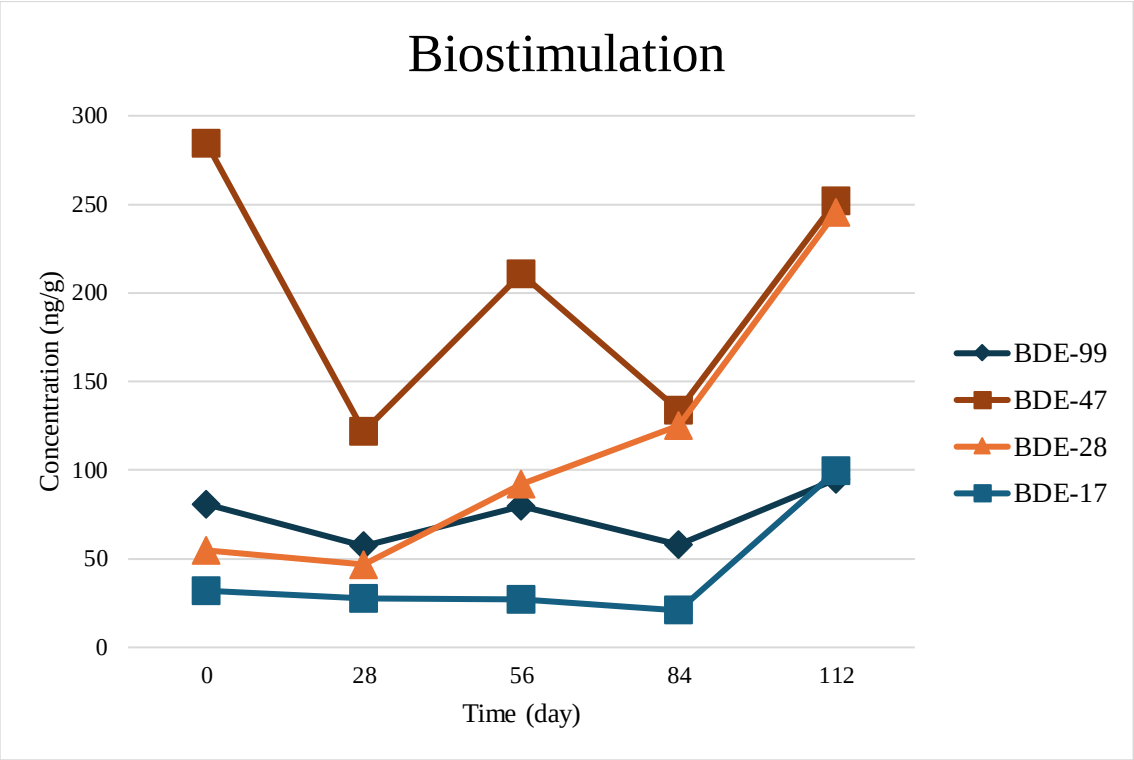

Figure 33. Concentration changes of PBDE congeners over time under the BS treatment.

4.3.5. Ecotoxicological Risk Evaluation

In this study, six indicator PCBs (PCB-28, PCB-52, PCB-101, PCB-153, PCB-138, and PCB-180) commonly employed by international agencies to assess the magnitude of total PCB contamination in environmental studies were selected to evaluate their ecotoxicological risk. Based on the risk quotient (RQ) calculations mentioned in Section 3.9.2, the values across four treatments over five time points revealed varying ecotoxicological risks (Table 16). PCB-28 remained within the moderate risk category throughout the study, with slight decreases and increases in RQ values over time for all microcosms. This was an expected result since PCB-28 is a tri-CB, hence its formation from tetra-CBs led to the increase in its concentration. Overall, between days 0 and 112, RQ of PCB-28 decreased in all but BS microcosm. PCB-52 consistently exhibited high risk across all treatments, with the highest RQ observed in the BS treatment on day 56. Similar to PCB-28, PCB-52 is a tetra-CB, which might have formed while degradation

progressed. As a result, RQ of PCB-52 increased between days zero and 112 for all microcosms. PCB-101, PCB-153 and PCB-180 demonstrated low risk, and an overall decrease in RQ was observed in all microcosms. PCB-138 presented moderate risk across all treatments, with RQ values gradually decreasing over time. Overall, PCB-52 stood out as the most concerning congener due to its high RQ values, particularly under BS. BA and BS strategies generally showed higher RQ values compared to NA and NC sets since degradation produced lower chlorinated congeners in higher amounts in BS and BA sets.

Table 16. Ecotoxicological risk assessment of indicator PCBs over time across different treatments (Green: Minimal risk, yellow: low risk, orange: moderate risk, and red: high risk).

		Risk Quotient				
Compound	**Treatment**	**Day 0**	**Day 28**	**Day 56**	**Day 84**	**Day 112**
PCB-28	**BA**	0.738	0.745	0.744	0.726	0.694
	BS	0.674	0.701	0.598	0.583	0.671
	NA	0.565	0.599	0.563	0.527	0.484
	NC	0.621	0.597	0.514	0.627	0.545
PCB-52	**BA**	2.199	2.789	3.097	2.424	3.009
	BS	2.363	2.202	5.195	2.326	2.964
	NA	1.863	1.841	1.908	2.202	2.049
	NC	1.711	1.832	1.926	1.592	2.170
PCB-101	**BA**	0.037	0.039	0.034	0.031	0.035
	BS	0.039	0.039	0.041	0.034	0.034
	NA	0.034	0.033	0.034	0.034	0.029
	NC	0.032	0.031	0.033	0.032	0.027
PCB-153	**BA**	0.090	0.090	0.084	0.084	0.076
	BS	0.097	0.094	0.096	0.082	0.083
	NA	0.096	0.091	0.093	0.097	0.078
	NC	0.089	0.087	0.083	0.086	0.073
PCB-138	**BA**	0.180	0.137	0.148	0.137	0.130
	BS	0.192	0.177	0.149	0.155	0.129
	NA	0.158	0.161	0.172	0.149	0.122
	NC	0.169	0.165	0.161	0.145	0.122
PCB-180	**BA**	0.033	0.030	0.029	0.032	0.029
	BS	0.034	0.035	0.037	0.037	0.033
	NA	0.032	0.032	0.036	0.035	0.028
	NC	0.035	0.030	0.036	0.034	0.024

The ecotoxicological risks of six PBDE congeners (BDE-28, -47, -99, -100, -153, and -209) were also evaluated based on their RQ values across four treatments over five time points (Table 17). BDE-28 showed moderate risk in all treatments until day 84, and reached a high-risk level between days 84 and 112 only in the BS treatment due to its accumulation as the end-product, which was observable in Figure 22. BDE-47 was initially categorized within the moderate risk in BA, BS and NC treatments on day 0, while NA showed low risk for the compound. Over time, the RQ values of BDE-47 exhibited slight fluctuations. These results were parallel with the fluctuations in the degradation trends of the related sets. As transformations occurred, BDE-47 might have formed and degraded. BDE-99 and BDE-100 exhibited moderate risk across all treatments. BA and BS showed slightly higher values than NA and NC for BDE-100, since its formation was more pronounced in the BA and BS sets. BDE-153 emerged as a critical congener in this study, and showed significant variations. There was a remarkable increase in ecotoxicological risk, particularly in the BS treatment at the end of the experiment. In the BA treatment, the RQ values remained moderate. NA showed an increase to the high risk level between days 56 and 84, but then it returned to the moderate risk. Therefore, NA conditions stabilized BDE-153 in the absence of microbial activity. NC maintained consistently high RQ values after day 28. Even though BDE-209 remained the moderate risk levels, it showed a consistent decrease in ecotoxicological risk across all treatments. This emphasized its effective debromination over time, especially in the BA and BS treatments, where microbial activity significantly enhanced its degradation efficiency.

Overall, the ecotoxicological risk evaluation emphasized the need for microcosm studies to understand the possible results of applying varying bioremediation strategies, as they might increase the risk although a general decrease in the POPs concentration was observed. Hence, bioremediation strategies should be applied with caution not to cause a higher ecotoxicological risk in the aquatic environment.

Table 17. Ecotoxicological risk assessment of indicator PBDEs over time across different treatments (Green: Minimal risk, yellow: low risk, orange: moderate risk, and red: high risk).

		Risk Quotient				
Compound	**Treatment**	**Day 0**	**Day 28**	**Day 56**	**Day 84**	**Day 112**
BDE-28	**BA**	0.477	0.648	0.854	0.979	0.918
	BS	0.563	0.483	0.952	1.291	2.537
	NA	0.509	0.434	0.685	0.607	0.523
	NC	0.586	0.548	0.785	0.511	0.698
BDE-47	**BA**	0.186	0.098	0.156	0.089	0.172
	BS	0.209	0.090	0.155	0.098	0.186
	NA	0.082	0.080	0.134	0.112	0.100
	NC	0.145	0.100	0.106	0.099	0.171
BDE-99	**BA**	0.420	0.331	0.732	0.539	0.716
	BS	0.502	0.357	0.496	0.361	0.592
	NA	0.289	0.424	0.484	0.469	0.489
	NC	0.630	0.399	0.342	0.421	0.605
BDE-100	**BA**	0.126	0.140	0.384	0.125	0.220
	BS	0.162	0.152	0.186	0.152	0.299
	NA	0.107	0.125	0.155	0.151	0.158
	NC	0.215	0.169	0.143	0.156	0.205
BDE-153	**BA**	0.386	0.426	0.833	0.654	0.834
	BS	1.054	0.519	0.992	0.897	4.666
	NA	0.682	0.760	1.454	1.014	0.855
	NC	0.956	1.051	1.047	1.251	1.225
BDE-209	**BA**	0.405	0.366	0.330	0.287	0.232
	BS	0.430	0.379	0.345	0.286	0.269
	NA	0.410	0.400	0.409	0.393	0.353
	NC	0.403	0.366	0.363	0.323	0.310

CHAPTER 5

CONCLUSION

This study investigated the effectiveness of three bioremediation strategies that can be applied to PCB and PBDE contaminated sediments collected from Aliağa coastal region in İzmir, Türkiye. The collected sediments were known to be influenced by the ship breaking operations for almost fifty years. Laboratory scale microcosms were established in such a way that the bioaugmentation, biostimulation, and natural attenuation strategies were mimicked, together with their control sets. This experimental design allowed for a comparison of the introduction of various amendments into the sediments regarding the reduction in concentration of PCBs and PBDEs, their degradation rates and pathways, and the ecotoxicological risks they pose.

For the bioaugmentation strategy, the indigenous microorganisms of the ship breaking yard sediments were targeted to be enriched and utilized. As a first step, the presence and abundance of the *Chloroflexi* phylum, capable of reductive dehalogenation of halogenated organic compounds like PCBs and PBDEs, were required to be confirmed. To do that, the sediments from the ship breaking yard area and the close vicinity were investigated, and the results were compared. The environmental DNA analyses were conducted following isolation, conventional PCR, AGE, and Sanger sequencing procedures. Additionally, to determine the ratio of *Chloroflexi* related microorganisms to the total microbial consortia, ddPCR was applied. As a result, the ship breaking yard sediments were found to possess 37.6% abundance of *Chloroflexi* related microorganisms. Hence, the enrichment studies were initiated with the ship breaking yard sediments and supplemented artificial seawater medium. The medium also contained the electron acceptors PCB-61 and BDE-47 to be utilized by microorganisms under anaerobic conditions. The extent of enrichment upon microbial growth was monitored by the reduction in PCB-61 and BDE-47 concentrations in the initial and follow-up subcultures. Microbial growth was achieved in the fourth subculture, so it was utilized in the bioaugmentation treatment.

The total PCB and PBDE concentrations in the sediments were reduced by 14% and 33% in the bioaugmentation set in 112 days, respectively. Comparatively, biostimulation set, involving the artificial seawater medium containing micronutrients, achieved a 19% and 24% reduction in total PCBs and PBDEs, respectively. Hence, the highest reduction in PCBs and PBDEs was observed in different bioremediation strategies, indicating the varying effects of the amendments on different contaminants. Natural attenuation set consistently showed little decrease in both contaminants, with an 84 day lag period for the adaptation of the microorganisms. The negative control set, established to observe the absence of microorganisms in the culture medium, demonstrated a total PCB reduction percentage close to natural attenuation but much higher total PBDE reduction than natural attenuation. The higher reduction percentage might be due to haloprimer effect of the electron acceptors, i.e. PCB-61 and BDE-47, in the medium. Sterile control sets exhibited stable concentrations of POPs throughout the incubation period, proving the sole effect of biological activity in the other sets.

The concentrations of homolog groups were also monitored during the incubation period. Higher halogenated PCBs and PBDEs were transformed into the lower halogenated ones as a result of anaerobic reductive dehalogenation. For PCBs and PBDEs, not all tri-, tetra-, and penta- congeners could be measured, which limited the direct observation of the increase in the concentrations of lower homolog groups. Additionally, the increased water solubilities of the lower halogenated compounds might have resulted in the partitioning of them into the water phase. Nevertheless, as dehalogenation proceeded, the concentrations of hexa- to deca- congeners decreased.

The degradation rates were calculated based on chlorine per biphenyl and bromine per diphenyl ether contents over the course of 112 days. For PCBs, the highest rates were observed for the negative control and bioaugmentation sets. This suggested that physical mechanisms, such as partitioning into the aqueous phase, might have played a more dominant role, contributing to the reduced Cl/bp at day 112. Then, the synergy among the added and native consortia observed in the BA set might have enhanced the PCB degradation rate. The lower rate in the BS set compared to the NC and BA sets indicated that, although micronutrient supplementation supported microbial activity, its effects might have been distributed over time. When compared to the literature studies, comparable rates to the degradation in historically contaminated sediments were noteworthy. Artificially spiked sediments showed higher rates, supporting the claim that they did not necessarily represent the real environmental systems. For PBDEs, BS

exhibited the highest degradation rate, potentially due to the enhancement of microbial activity through micronutrient supply. Also, the rate observed in the BA set suggested that the enriched culture played a notable role in the PBDE degradation process.

Possible degradation mechanisms were proposed for PCB-180, BDE-209, and BDE-99. Except for BDE-209, other congeners were also products of higher halogenated compounds. Hence, the fluctuating concentrations were observed. Although a net difference between BA and BS sets in the degradation was not clearly observable, for some congeners the interpretation was more certainly possible. For example, BDE-207 was found to be the dominant product in both BA and BS, while BDE-208 was the least preferential product of the BA set.

Ecotoxicological risks of PCBs and PBDEs were also evaluated upon the indicator congeners. For PCBs, especially PCB-52 exhibited consistently high risk throughout the experiment, likely due to its formation rate surpassing its removal rate. PCB-28 and PCB-138 showed moderate risk while low risk was observed in the rest of the congeners with gradual decreases in both categories. PCB-28, however, did not appear to accumulate significantly, as its formation rate might have been lower than its removal rate. For PBDEs, the risk of BDE-28 escalated to high in BS while BDE-47 fluctuated but mostly remained within moderate risk. BDE-153 exhibited notable high and moderate risk variations, while the risk of BDE-209 consistently decreased. Since BA and BS treatments produced lower halogenated congeners more, they resulted in higher risk values than NA and NC treatments overall. These results emphasized the need for a careful assessment of the outcomes of bioremediation strategies as they might lead to higher ecotoxicological risks. Nevertheless, in the natural sediment systems, aerobic conditions prevail in the first few cm's of the surface. Hence, the congeners with higher solubility, such as PCB-28 and PCB-52, can get solubilized and eventually partitioned into pore water, enabling them to be transported to aerobic zones. In such zones, aerobic microorganisms could potentially degrade these compounds. While anaerobic dehalogenation could lead to the increased toxicity of PCB-52, aerobic mechanisms that might become active later could be a critical pathway for the remediation and complete degradation of PCBs.

Overall, this study provided crucial information on the degradation performance, the end-products, and the resulting risks posed by the bioremediation strategies that can be applicable to POPs contaminated sediments. The findings are promising for the development of an efficient bioremediation strategy and can be used by policy-makers when the regulations enforce the management of contaminated sites.

Future studies should explore the complete list of congeners to better understand the fate of POPs in the sediments. Researchers would also focus on several strategies to expand the understanding of PCB and PBDE bioremediation. The composition and dynamics of microbial communities during BA and BS can be followed using molecular techniques targeting functional genes, such as reductive dehalogenases, to identify key players in biodegradation. Using pure cultures of specific microbial species could also help identify crucial pathways and enzymes involved in transformation. Additionally, the effects of environmental factors, such as temperature and nutrient levels, can be investigated to optimize conditions for microbial activity. Another promising approach could involve exploring alternative carbon sources and electron donors/acceptors. Extending the incubation period could provide insights into the long-term efficiency of bioremediation. Furthermore, the larger scale experimental systems should be operated to better represent the natural environmental conditions, where both aerobic and anaerobic conditions are simulated. Additionally, the sustainability of these strategies can be evaluated by a combined analysis of the environmental, economic, and social impacts.

REFERENCES

Abramowicz, Daniel A. 1995. “Aerobic and Anaerobic PCB Biodegradation in the Environment.” *Environmental Health Perspectives* 103 (5): 97–99. https://doi.org/ 10.1289/ehp.95103s497.

Abramowicz, Daniel A., Michael J. Brennan, Heidi M. Van Dort, and Edith L. Gallagher. 1993. “Factors Influencing the Rate of Poly Chlorinated Biphenyl Dechlorination in Hudson River Sediments.” *Environmental Science and Technology* 27 (6): 1125–1131. https://doi.org/10.1021/es00043a011.

Adams, Godleads O., Prekeyi T. Fufeyin, Samson E. Okoro, and Igelenyah Ehinomen. 2015. “Bioremediation, Biostimulation and Bioaugmention: A Review.” *International Journal of Environmental Bioremediation & Biodegradation* 3 (1): 28–39. https://doi.org/10.12691/ijebb-3-1-5.

Agency for Toxic Substances and Disease Registry (ATSDR). 2000. “Toxicological Profile for Polychlorinated Biphenyls (PCBs).” Atlanta, GA. Accessed January 5, 2025. https://www.atsdr.cdc.gov/toxprofiles/tp17.pdf.

Agency for Toxic Substances and Disease Registry (ATSDR). 2004. “Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers.” Accessed January 5, 2025. https://www.govinfo.gov/content/pkg/govpub-he20-purl-gpo31608/pdf/govpub-he20-purl-gpo31608.pdf.

Alaee, Mehran. 2003. “An Overview of Commercially Used Brominated Flame Retardants, Their Applications, Their Use Patterns in Different Countries/Regions and Possible Modes of Release.” *Environment International* 29 (6): 683–689. https://doi.org/10.1016/S0160-4120(03)00121-1.

Alexander, David E. 1999. “Bioaccumulation, Bioconcentration, Biomagnification.” *Environmental Geology*, 1 (2): 43–44. https://doi.org/10.1007/1-4020-4494-1_31.

Atlas, Ronald M., and Jim Philp. 2005. *Bioremediation: Applied Microbial Solutions for Real-world Environmental Cleanup*. ASM Press.

Aydin, Yagmur M., Melik Kara, Yetkin Dumanoglu, Mustafa Odabasi, and Tolga Elbir. 2014. “Source Apportionment of Polycyclic Aromatic Hydrocarbons (PAHs) and

Polychlorinated Biphenyls (PCBs) in Ambient Air of an Industrial Region in Turkey." *Atmospheric Environment* 97 (11): 271–285. https://doi.org/10.1016/j.atmosenv.2014.08.032.

Ballschmiter, Karlheinz, and Manfred Zell. 1980. "Analysis of Polychlorinated Biphenyls (PCB) by Glass Capillary Gas Chromatography - Composition of Technical Aroclor- and Clophen-PCB Mixtures." *Fresenius' Zeitschrift Für Analytische Chemie* 302 (1): 20–31. https://doi.org/10.1007/bf00469758.

Bedard, Donna L., Heidi Van Dort, and Kim A. Deweerd. 1998. "Brominated Biphenyls Prime Extensive Microbial Reductive Dehalogenation of Aroclor 1260 in Housatonic River Sediment." *Applied and Environmental Microbiology* 64 (5): 1786–1795. https://doi.org/10.1128/aem.64.5.1786-1795.1998.

Bedard, Donna L., and Ralph J. May. 1996. "Characterization of the Polychlorinated Biphenyls in the Sediments of Woods Pond: Evidence for Microbial Dechlorination of Aroclor 1260 in Situ." *Environmental Science and Technology* 30 (1): 237–245. https://doi.org/10.1021/es950262e.

Bedard, Donna L., and John F. Quensen III. 1995. "Microbial Reductive Dechlorination of Polychlorinated Biphenyls." In *Microbial Transformation and Degradation of Toxic Organic Chemicals*, edited by Lily Y. Young and Ceng E. Cerniglia, 127–216. New York: Wiley-Liss Inc.

Bedard, Donna L., Kirsti M. Ritalahti, and Frank E. Löffler. 2007. "The Dehalococcoides Population in Sediment-Free Mixed Cultures Metabolically Dechlorinates the Commercial Polychlorinated Biphenyl Mixture Aroclor 1260." *Applied and Environmental Microbiology* 73 (8): 2513–2521. https://doi.org/10.1128/aem.02909-06.

Bedard, Donna L. 2008. "A Case Study for Microbial Biodegradation: Anaerobic Bacterial Reductive Dechlorination of Polychlorinated Biphenyls-from Sediment to Defined Medium." *Annual Review of Microbiology* 62 (10): 253–270. https://doi.org/10.1146/annurev.micro.62.081307.162733.

Berges, John A., Daniel J. Franklin, and Paul J. Harrison. 2001. "Evolution of an Artificial Seawater Medium: Improvements in Enriched Seawater, Artificial Water over the Last Two Decades." *Journal of Phycology* 37 (6): 1138–1145. https://doi.org/10.1046/j.1529-8817.2001.01052.x.

Bock, Karl Walter. 2017. “From Dioxin Toxicity to Putative Physiologic Functions of the Human Ah Receptor in Homeostasis of Stem/Progenitor Cells.” *Biochemical Pharmacology* 123 (1): 1–7. https://doi.org/10.1016/J.BCP.2016.06.015.

Borja, Josephine, Donna M. Taleon, Joseph Auresenia, and Susan Gallardo. 2005. “Polychlorinated Biphenyls and Their Biodegradation.” *Process Biochemistry* 40 (6): 1999–2013. https://doi.org/10.1016/j.procbio.2004.08.006.

Bossert, Ingeborg D., Max M. Häggblom, and Lily Y. Young. 2004. “Microbial Ecology of Dehalogenation.” In *Dehalogenation: Microbial Processes and Environmental Applications*, 33–52. Springer, Boston, MA.

Bourrain, Muriel, Wafa Achouak, Vincent Urbain, and Thierry Heulin. 1999. “DNA Extraction from Activated Sludges.” *Current Microbiology* 38 (6): 315–319. https://doi.org/10.1007/PL00006809.

Breivik, Knut, Andy Sweetman, Jozef M. Pacyna, and Kevin C. Jones. 2007. “Towards a Global Historical Emission Inventory for Selected PCB Congeners — A Mass Balance Approach: 3. An Update.” *Science of The Total Environment* 377 (2–3): 296–307. https://doi.org/10.1016/j.scitotenv.2007.02.026.

Brown, John F., Robert E. Wagner, Donna L. Bedard, Michael J. Brennan, James C. Carnahan, and Ralph J. May. 1984. “PCB Transformations in Upper Hudson Sediments.” In *Northeastern Environmental Science* , 167–179. Troy, NY .

Bursian, Steven J., John L. Newsted, and Matthew J. Zwiernik. 2011. “Polychlorinated Biphenyls, Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans.” In *Reproductive and Developmental Toxicology*, 543–567. Academic Press.

Chang, Bea-Ven, Wen G. Liu, and Shaw Y. Yuan. 2001. “Microbial Dechlorination of Three PCB Congeners in River Sediment.” *Chemosphere* 45 (6–7): 849–856. https://doi.org/10.1016/s0045-6535(01)00106-0.

Chen, Juan, Chao Wang, Ying Pan, Shazia S. Farzana, and Nora F. Y. Tam. 2018. “Biochar Accelerates Microbial Reductive Debromination of 2,2′,4,4′-Tetrabromodiphenyl Ether (BDE-47) in Anaerobic Mangrove Sediments.” *Journal of Hazardous Materials* 341 (1): 177–186. https://doi.org/10.1016/j.jhazmat.2017.07.063.

Chen, Juan, Hai C. Zhou, Chao Wang, Chun Q. Zhu, and Nora F. Y. Tam. 2015. “Short-Term Enhancement Effect of Nitrogen Addition on Microbial Degradation and

Plant Uptake of Polybrominated Diphenyl Ethers (PBDEs) in Contaminated Mangrove Soil." *Journal of Hazardous Materials* 300 (12): 84–92. https://doi.org/10.1016/j.jhazmat.2015.06.053.

Chen, Yulin, Yang Tan, Yuying Feng, Tao Dong, Chunxia Jiang, Chen Wang, Yuyi Yang, and Zulin Zhang. 2024. "Selected Legacy and Emerging Organic Contaminants in Sediments of China's Yangtze – the World's Third Longest River: Response to Anthropogenic Activities." *Environmental Pollution* 346 (4): 123608-123619. https://doi.org/10.1016/j.envpol.2024.123608.

Chiarenzelli, Jeffrey R., Ronald J. Scrudato, and Michele L. Wunderlich. 1997. "Volatile Loss of PCB Aroclors from Subaqueous Sand." *Environmental Science & Technology* 31 (2): 597–602. https://doi.org/10.1021/es960555n.

Cho, Young-Cheol, Ellen B. Ostrofsky, Roger C. Sokol, Robert C. Frohnhoefer, and G-Yull Rhee. 2002. "Enhancement of Microbial PCB Dechlorination by Chlorobenzoates, Chlorophenols and Chlorobenzenes." *FEMS Microbiology Ecology* 42 (1): 51–58. https://doi.org/10.1111/j.1574-6941.2002.tb00994.x.

Cutter, Leah A., Joy E. M. Watts, Kevin R. Sowers, and Harold D. May. 2001. "Identification of a Microorganism That Links Its Growth to the Reductive Dechlorination of 2,3,5,6-Chlorobiphenyl." *Environmental Microbiology* 3 (11): 699–709. https://doi.org/10.1046/j.1462-2920.2001.00246.x.

Darnerud, Per O., Gunnar S. Eriksen, Torkell Jóhannesson, Poul B. Larsen, and Matti Viluksela. 2001. "Polybrominated Diphenyl Ethers: Occurrence, Dietary Exposure, and Toxicology." *Environmental Health Perspectives* 109 (1): 49–68. https://doi.org/10.1289/ehp.01109s149.

Das, Nilanjana, and Preethy Chandran. 2010. "Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview." *Biotechnology Research International* 2011 (9): 941-954. https://doi.org/10.4061/2011/941810.

Davies, Rebecca, and Enmin Zou. 2012. "Polybrominated Diphenyl Ethers Disrupt Molting in Neonatal Daphnia Magna." *Ecotoxicology* 21 (5): 1371–1380. https://doi.org/10.1007/S10646-012-0891-6.

Demirtepe, Hale, and Ipek Imamoglu. 2019. "Degradation of Decabromodiphenyl Ether (BDE-209) in Microcosms Mimicking Sediment Environment Subjected to Comparative Bioremediation Strategies." *Journal of Environmental Management* 233 (3): 120–130. https://doi.org/10.1016/j.jenvman.2018.11.144.

Demirtepe, Hale, Birthe V. Kjellerup, Kevin R. Sowers, and Ipek Imamoglu. 2015. "Evaluation of PCB Dechlorination Pathways in Anaerobic Sediment Microcosms Using an Anaerobic Dechlorination Model." *Journal of Hazardous Materials* 296 (10): 120–127. https://doi.org/10.1016/j.jhazmat.2015.04.033.

Deweerd, Kim A., and Donna L. Bedard. 1999. "Use of Halogenated Benzoates and Other Halogenated Aromatic Compounds to Stimulate the Microbial Dechlorination of PCBs." *Environmental Science and Technology* 33 (12): 2057–2063. https://doi.org/10.1021/es9812498.

Ding, Chang, Matthew J. Rogers, Kun L. Yang, and Jianzhong He. 2017. "Loss of the SsrA Genome Island Led to Partial Debromination in the PBDE Respiring *Dehalococcoides mccartyi* Strain GY50." *Environmental Microbiology* 19 (7): 2906–2915. https://doi.org/10.1111/1462-2920.13817.

Eljarrat, Ethel, and Damià Barceló. 2004. "Toxicity Potency Assessment of Persistent Organic Pollutants in Sediments and Sludges." *Handbook of Environmental Chemistry* 5 (1): 99–140. https://doi.org/10.1007/B97177.

El-Shahawi, Mohammad S., Ahmed Hamza, Abdulaziz S. Bashammakh, and Wejdan T. Al-Saggaf. 2010. "An Overview on the Accumulation, Distribution, Transformations, Toxicity and Analytical Methods for the Monitoring of Persistent Organic Pollutants." *Talanta* 80 (5): 1587–1597. https://doi.org/10.1016/j.talanta.2009.09.055.

Erickson, Bruce D., and Frank J. Mondello. 1992. "Nucleotide Sequencing and Transcriptional Mapping of the Genes Encoding Biphenyl Dioxygenase, a Multicomponent Polychlorinated-Biphenyl-Degrading Enzyme in Pseudomonas Strain LB400." *Journal of Bacteriology* 174 (9): 2903–2912. https://doi.org/10.1128/jb.174.9.2903-2912.1992.

Erickson, Mitchell D. 2001. "Introduction: PCB Properties, Uses, Occurrence, and Regulatory History." In *PCBs: Recent Advances in Environmental Toxicology and Health Effects*, edited by Larry W. Robertson and Larry G. Hansen, 11–30. The University Press of Kentucky.

European Commission. 2003. "Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances, Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market Part III," Accessed December 21, 2024.

https://op.europa.eu/en/publication-detail/-/publication/212940b8-3e55-43f8-8448-ba258d0374bb.

Fagervold, Sonja K., Harold D. May, and Kevin R. Sowers. 2007. "Microbial Reductive Dechlorination of Aroclor 1260 in Baltimore Harbor Sediment Microcosms Is Catalyzed by Three Phylotypes within the Phylum *Chloroflexi*." *Applied and Environmental Microbiology* 73 (9): 3009–3018. https://doi.org/10.1128/aem.02958-06.

Fagervold, Sonja K., Joy E. M. Watts, Harold D. May, and Kevin R. Sowers. 2011. "Effects of Bioaugmentation on Indigenous PCB Dechlorinating Activity in Sediment Microcosms." *Water Research* 45 (13): 3899–3907. https://doi.org/10.1016/j.watres.2011.04.048.

Fan, Guangping, Long Cang, Helena I. Gomes, and Dongmei Zhou. 2016. "Electrokinetic Delivery of Persulfate to Remediate PCBs Polluted Soils: Effect of Different Activation Methods." *Chemosphere* 144 (2): 138–147. https://doi.org/10.1016/j.chemosphere.2015.08.074.

Federal Remediation Technologies Roundtable (FRTR). 1990. Accessed August 15, 2024. https://www.frtr.gov/.

Federal Remediation Technologies Roundtable (FRTR). 2021. "Ex Situ Physical/Chemical Treatment for Soil, Sediment, Bedrock and Sludge." Accessed August 30, 2024. https://www.frtr.gov/matrix2/section3/3_5.html.

Fromme, Hermann, Wolfgang Körner, Nabil Shahin, Antonia Wanner, Michael Albrecht, Sigrun Boehmer, Harun Parlar, Richard Mayer, Bernhard Liebl, and Gabriele Bolte. 2009. "Human Exposure to Polybrominated Diphenyl Ethers (PBDE), as Evidenced by Data from a Duplicate Diet Study, Indoor Air, House Dust, and Biomonitoring in Germany." *Environment International* 35 (8): 1125–1135. https://doi.org/10.1016/j.envint.2009.07.003.

Galceran, Maria T., Francisco J. Santos, Josep Caixach, and Josep Rivera. 1993. "PCBs and Chlorinated Pesticides in Shellfish of a Deltaic Environment." *Chemosphere* 27 (7): 1183–1200. https://doi.org/10.1016/0045-6535(93)90166-3.

Gerecke, Andreas C., Paul C. Hartmann, Norbert V. Heeb, Hans-Peter E. Kohler, Walter Giger, Peter Schmid, Markus Zennegg, and Martin Kohler. 2005. "Anaerobic Degradation of Decabromodiphenyl Ether." *Environmental Science and Technology* 39 (4): 1078–1083. https://doi.org/10.1021/es048634j.

Ghiorse, William C., and John T. Wilson. 1988. “Microbial Ecology of the Terrestrial Subsurface.” *Advances in Applied Microbiology* 33 (1): 107–172. https://doi.org/10.1016/s0065-2164(08)70206-5.

Gomes, Helena I., Lisbeth M. Ottosen, Alexandra B. Ribeiro, and Celia Dias-Ferreira. 2015. “Treatment of a Suspension of PCB Contaminated Soil Using Iron Nanoparticles and Electric Current.” *Journal of Environmental Management* 151 (3): 550–555. https://doi.org/10.1016/j.jenvman.2015.01.015.

Gribble, Gordon W. 2023. “Naturally Occurring Organohalogen Compounds—A Comprehensive Review.” *Progress in the Chemistry of Organic Natural Products* 121 (1): 1–546. https://doi.org/10.1007/978-3-031-26629-4.

Hardy, Marcia L. 2002. “A Comparison of the Properties of the Major Commercial PBDPO/PBDE Product to Those of Major PBB and PCB Products.” *Chemosphere* 46 (5): 717–728. https://doi.org/10.1016/s0045-6535(01)00236-3.

He, Jianzhong, Kristin R. Robrock, and Lisa Alvarez-Cohen. 2006. “Microbial Reductive Debromination of Polybrominated Diphenyl Ethers (PBDEs).” *Environmental Science and Technology* 40 (14): 4429–4434. https://doi.org/10.1021/es052508d.

Hellar-Kihampa, Harieth, Karolien De Wael, Esther Lugwisha, Govindan Malarvannan, Adrian Covaci, and René Van Grieken. 2013. “Spatial Monitoring of Organohalogen Compounds in Surface Water and Sediments of a Rural–Urban River Basin in Tanzania.” *Science of The Total Environment* 447 (3): 186–197. https://doi.org/10.1016/j.scitotenv.2012.12.083.

Hendrickson, Edwin R., Jo A. Payne, Roslyn M. Young, Mark G. Star, Michael P. Perry, Stephen Fahnestock, David E. Ellis, and Richard C. Ebersole. 2002. “Molecular Analysis of *Dehalococcoides* 16S Ribosomal DNA from Chloroethene-Contaminated Sites throughout North America and Europe.” *Applied and Environmental Microbiology* 68 (2): 485-495. https://doi.org/10.1128/aem.68.2.485-495.2002.

Hermanson, Mark H., and Glenn W. Johnson. 2007. “Polychlorinated Biphenyls in Tree Bark near a Former Manufacturing Plant in Anniston, Alabama.” *Chemosphere* 68 (1): 191–198. https://doi.org/10.1016/j.chemosphere.2006.11.068.

Hinzman, Robert L. 1993. “Second Report on the Oak Ridge Y-12 Plant Biological Monitoring and Abatement Program for East Fork Poplar Creek.” Environmental Sciences Division for the US Department of Energy. Accessed January 5, 2025. https://inis.iaea.org/records/2e91d-1t059.

Hofer, Bernd, Silke Backhaus, and Kenneth N. Timmis. 1994. "The Biphenyl/Polychlorinated Biphenyl-Degradation Locus (*bph*) of *Pseudomonas* Sp. LB400 Encodes Four Additional Metabolic Enzymes." *Gene* 144 (1): 9–16. https://doi.org/10.1016/0378-1119(94)90196-1.

Holma-Suutari, Anniina, Päivi Ruokojärvi, Alexander A. Komarov, Dmitry A. Makarov, Vladimir V. Ovcharenko, Alexander N. Panin, and Hannu Kiviranta. 2016. "Biomonitoring of Selected Persistent Organic Pollutants (PCDD/Fs, PCBs and PBDEs) in Finnish and Russian Terrestrial and Aquatic Animal Species." *Environmental Sciences Europe* 28 (1): 1–10. https://doi.org/10.1186/s12302-016-0071-z.

Hong, Sang Hee, Narayanan Kannan, Yongnu Jin, Jong Ho Won, Gi Myung Han, and Won Joon Shim. 2010. "Temporal Trend, Spatial Distribution, and Terrestrial Sources of PBDEs and PCBs in Masan Bay, Korea." *Marine Pollution Bulletin* 60 (10): 1836–1841. https://doi.org/10.1016/j.marpolbul.2010.05.023.

Huang, Huang-Wen, Bea-Ven Chang, and Ching-Chang Lee. 2014. "Reductive Debromination of Decabromodiphenyl Ether by Anaerobic Microbes from River Sediment." *International Biodeterioration & Biodegradation* 87 (2): 60–65. https://doi.org/10.1016/j.ibiod.2013.10.011.

Hussein, Zahra S., Nashwa Hamido, Ahmad K. Hegazy, Mohamed A. El-Dessouky, Nermen H. Mohamed, and Gehan Safwat. 2022. "Phytoremediation of Crude Petroleum Oil Pollution: A Review" *Egyptian Journal of Botany* 62 (3): 611–640. https://doi.org/10.21608/ejbo.2022.136551.1980.

Iseki, Kazuo, Miwako Takahashi, Eduard Bauerfeind, and Chi S. Wong. 1981. "Effects of Polychlorinated Biphenyls (PCBs) on a Marine Plankton Population and Sedimentation in Controlled Ecosystem Enclosures." *Marine Ecology Progress Series* 5 (5): 207–214. https://doi.org/10.3354/meps005207.

Jaward, Foday M., Nick J. Farrar, Tom Harner, Andrew J. Sweetman, and Kevin C. Jones. 2004. "Passive Air Sampling of PCBs, PBDEs, and Organochlorine Pesticides Across Europe." *Environmental Science and Technology* 38 (1): 34–41. https://doi.org/10.1021/es034705n.

Jones, Kevin C., and Pim de Voogt. 1999. "Persistent Organic Pollutants (POPs): State of the Science." *Environmental Pollution* 100 (1–3): 209–221. https://doi.org/10.1016/s0269-7491(99)00098-6.

Kara, Melik, Yetkin Dumanoglu, Hasan Altiok, Tolga Elbir, Mustafa Odabasi, and Abdurrahman Bayram. 2015. “Spatial Variation of Trace Elements in Seawater and Sediment Samples in a Heavily Industrialized Region.” *Environmental Earth Sciences* 73 (1): 405–421. https://doi.org/10.1007/s12665-014-3434-z.

Kargar, Navid, Golnar Matin, Amir Abbas Matin, and Hasan Baha Buyukisik. 2017. “Biomonitoring, Status and Source Risk Assessment of Polycyclic Aromatic Hydrocarbons (PAHs) Using Honeybees, Pine Tree Leaves, and Propolis.” *Chemosphere* 186 (11): 140–150. https://doi.org/10.1016/j.chemosphere.2017.07.127.

Karickhoff, Samuel W., David S. Brown, and Trudy A. Scott. 1979. “Sorption of Hydrophobic Pollutants on Natural Sediments.” *Water Research* 13 (3): 241–248. https://doi.org/10.1016/0043-1354(79)90201-x.

Kelly, Barry C., Michael G. Ikonomou, Joel D. Blair, Anne E. Morin, and Frank A.P.C. Gobas. 2007. “Food Web-Specific Biomagnification of Persistent Organic Pollutants.” *Science* 317 (5835): 236–239. https://doi.org/10.1126/science.1138275.

Key, Peter B., Jennifer Hoguet, Katy W. Chung, John J. Venturella, Paul L. Pennington, and Michael H. Fulton. 2009. “Lethal and Sublethal Effects of Simvastatin, Irgarol, and PBDE-47 on the Estuarine Fish, Fundulus Heteroclitus.” *Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes* 44 (4): 379–382. https://doi.org/10.1080/03601230902801083.

Khairy, Mohammed, Emily Brault, Rebecca Dickhut, Karin C. Harding, Tero Harkonen, Olle Karlsson, Kristina Lehnert, Jonas Teilmann, and Rainer Lohmann. 2021. “Bioaccumulation of PCBs, OCPs and PBDEs in Marine Mammals From West Antarctica.” *Frontiers in Marine Science* 8 (12): 768715-768729. https://doi.org/10.3389/fmars.2021.768715.

Khan, Faisal I., Tahir Husain, and Ramzi Hejazi. 2004. “An Overview and Analysis of Site Remediation Technologies.” *Journal of Environmental Management* 71 (2): 95–122. https://doi.org/10.1016/j.jenvman.2004.02.003.

Kim, Joseph J., Kelsey Delisle, Tanya M. Brown, Farida Bishay, Peter S. Ross, and Marie Noël. 2022. “Characterization and Interpolation of Sediment Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Resident Killer Whale Habitat along the Coast of British Columbia, Canada.” *Environmental Toxicology and Chemistry* 41 (9): 2139–2151. https://doi.org/10.1002/etc.5404.

Kimbara, Kazuhide. 2005. “Minireview Recent Developments in the Study of Microbial Aerobic Degradation of Polychlorinated Biphenyls” *Microbes and Environments* 20 (3): 127–134. https://doi.org/10.1264/jsme2.20.127.

Kjellerup, Birthe V., Courtney Naff, Sarah J. Edwards, Upal Ghosh, Joel E. Baker, and Kevin R. Sowers. 2014. “Effects of Activated Carbon on Reductive Dechlorination of PCBs by Organohalide Respiring Bacteria Indigenous to Sediments.” *Water Research* 52 (5): 1–10. https://doi.org/10.1016/j.watres.2013.12.030.

Kodavanti, Prasada R. S., and Bommanna G. Loganathan. 2017. “Organohalogen Pollutants and Human Health.” In *International Encyclopedia of Public Health* 359–366. Elsevier.

Krumins, Valdis, Joong-Wook Park, Eun-Kyeu Son, Lisa A. Rodenburg, Lee J. Kerkhof, Max M. Häggblom, and Donna E. Fennell. 2009. “PCB Dechlorination Enhancement in Anacostia River Sediment Microcosms.” *Water Research* 43 (18): 4549–4558. https://doi.org/10.1016/j.watres.2009.08.003.

La Guardia, Mark J., Robert C. Hale, and Ellen Harvey. 2006. “Detailed Polybrominated Diphenyl Ether (PBDE) Congener Composition of the Widely Used Penta-, Octa-, and Deca-PBDE Technical Flame-Retardant Mixtures.” *Environmental Science & Technology* 40 (20): 6247–6254. https://doi.org/10.1021/es060630m.

Labbé, Diane, James Garnon, and Peter C. K. Lau. 1997. “Characterization of the Genes Encoding a Receptor-Like Histidine Kinase and a Cognate Response Regulator from a Biphenyl/Polychlorobiphenyl-Degrading Bacterium, Rhodococcus Sp. Strain M5.” *Journal of Bacteriology* 179 (8): 2772–2776. https://doi.org/10.1128/jb.179.8.2772-2776.1997.

Lammel, Gerhard, Ondřej Audy, Athanasios Besis, Christos Efstathiou, Kostas Eleftheriadis, Jiři Kohoutek, Petr Kukučka, et al. 2015. “Air and Seawater Pollution and Air-Sea Gas Exchange of Persistent Toxic Substances in the Aegean Sea: Spatial Trends of PAHs, PCBs, OCPs and PBDEs.” *Environmental Science and Pollution Research International* 22 (15): 11301–11313. https://doi.org/10.1007/s11356-015-4363-4.

Lauby-Secretan, Béatrice, Dana Loomis, Yann Grosse, Fatiha El Ghissassi, Véronique Bouvard, Lamia Benbrahim-Tallaa, Neela Guha, et al. 2013. “Carcinogenicity of Polychlorinated Biphenyls and Polybrominated Biphenyls.” *The Lancet Oncology* 14 (4): 287–288. https://doi.org/10.1016/s1470-2045(13)70104-9.

Lee, Lip K., and Jianzhong He. 2010. "Reductive Debromination of Polybrominated Diphenyl Ethers by Anaerobic Bacteria from Soils and Sediments." *Applied and Environmental Microbiology* 76 (3): 794–802. https://doi.org/10.1128/aem.01872-09.

Li, An, Karl J. Rockne, Neil Sturchio, Wenlu Song, Justin C. Ford, and Hua Wei. 2009. "PCBs in Sediments of the Great Lakes – Distribution and Trends, Homolog and Chlorine Patterns, and in Situ Degradation." *Environmental Pollution* 157 (1): 141–147. https://doi.org/10.1016/j.envpol.2008.07.014.

Löffler, Frank E., James R. Cole, Kirsti M. Ritalahti, and James M. Tiedje. 2004. "Diversity of Dechlorinating Bacteria." In *Dehalogenation: Microbial Processes and Environmental Applications*, 53–87. Springer Science & Business Media.

Lohmann, Rainer, Knut Breivik, Jordi Dachs, and Derek Muir. 2007. "Global Fate of POPs: Current and Future Research Directions." *Environmental Pollution* 150 (1): 150–165. https://doi.org/10.1016/j.envpol.2007.06.051.

Luo, Si, Shaogui Yang, Yingang Xue, Feng Liang, and Cheng Sun. 2011. "Two-Stage Reduction/Subsequent Oxidation Treatment of 2,2',4,4'-Tetrabromodiphenyl Ether in Aqueous Solutions: Kinetic, Pathway and Toxicity." *Journal of Hazardous Materials* 192 (3): 1795–1803. https://doi.org/10.1016/j.jhazmat.2011.07.015.

Lv, Yanhui, Xiaofeng Cao, Haiying Jiang, Wenjing Song, Chuncheng Chen, and Jincai Zhao. 2016. "Rapid Photocatalytic Debromination on TiO_2 with In-Situ Formed Copper Co-Catalyst: Enhanced Adsorption and Visible Light Activity." *Applied Catalysis B: Environmental* 194 (10): 150–156. https://doi.org/10.1016/j.apcatb.2016.04.053.

Mackay, Donald, Wan-Ying Shiu, Kuo-Ching Ma, and Sum Chi Lee. 2006. "Polychlorinated Biphenyls (PCBs)." In *Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals*, II:1479–2063. CRC Press.

Magar, Victor S., Richard C. Brenner, Glenn W. Johnson, and John F. Quensen. 2005. "Long-Term Recovery of PCB-Contaminated Sediments at the Lake Hartwell Superfund Site: PCB Dechlorination. 2. Rates and Extend." *Environmental Science and Technology* 39 (10): 3548–3554. https://doi.org/10.1021/ES0486216.

Masai, Eiji, Akihiro Yamada, Judith M. Healy, Takashi Hatta, Kazuhide Kimbara, Masao Fukuda, and Kazuo Yano. 1995. "Characterization of Biphenyl Catabolic Genes

of Gram-Positive Polychlorinated Biphenyl Degrader Rhodococcus Sp. Strain RHA1." *Applied and Environmental Microbiology* 61 (6): 2079–2085. https://doi.org/10.1128/aem.61.6.2079-2085.1995.

Matturro, Bruna, Carla Ubaldi, Paola Grenni, Anna Barra Caracciolo, and Simona Rossetti. 2016. "Polychlorinated Biphenyl (PCB) Anaerobic Degradation in Marine Sediments: Microcosm Study and Role of Autochthonous Microbial Communities." *Environmental Science and Pollution Research* 23 (13): 12613–12623. https://doi.org/10.1007/s11356-015-4960-2.

May, Harold D., Greg S. Miller, Birthe V. Kjellerup, and Kevin R. Sowers. 2008. "Dehalorespiration with Polychlorinated Biphenyls by an Anaerobic Ultramicrobacterium." *Applied and Environmental Microbiology* 74 (7): 2089–2094. https://doi.org/10.1128/aem.01450-07.

Megson, David, Guuske P. Tiktak, Steve Shideler, Mike Dereviankin, Lacey Harbicht, and Courtney D. Sandau. 2023. "Source Apportionment of Polychlorinated Biphenyls (PCBs) Using Different Receptor Models: A Case Study on Sediment from the Portland Harbor Superfund Site (PHSS), Oregon, USA." *Science of The Total Environment* 872 (5): 162231-162243. https://doi.org/10.1016/j.scitotenv.2023.162231.

Meijer, Sandra N., Wendy A. Ockenden, Andrew J. Sweetman, Knut Breivik, Joan O. Grimalt, and Kevin C. Jones. 2003. "Global Distribution and Budget of PCBs and HCB in Background Surface Soils: Implications for Sources and Environmental Processes." *Environmental Science and Technology* 37 (4): 667–672. https://doi.org/10.1021/es025809l.

Mellor, Claire L., Fabian P. Steinmetz, and Mark T. D. Cronin. 2016. "The Identification of Nuclear Receptors Associated with Hepatic Steatosis to Develop and Extend Adverse Outcome Pathways." *Critical Reviews in Toxicology* 46 (2): 138–152. https://doi.org/10.3109/10408444.2015.1089471.

Ministry of Environment Urbanization and Climate Change. 2010. "Regulation on Control of Soil Contamination and Contaminated Sites Due to Point Sources." Accessed August 25, 2024. https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=14026&MevzuatTur=7&MevzuatTertip=5

Mondello, Frank J. 1989. "Cloning and Expression in Escherichia Coli of Pseudomonas Strain LB400 Genes Encoding Polychlorinated Biphenyl Degradation." *Journal of Bacteriology* 171 (3): 1725–1732. https://doi.org/10.1128/jb.171.3.1725-1732.1989.

Monosson, Emily. 1999. “Reproductive and Developmental Effects of PCBs in Fish: A Synthesis of Laboratory and Field Studies.” In *Reviews in Toxicology*, 25–75. Arizona Health Sciences Library.

Mulligan, Catherine N., Masaharu Fukue, and Yoshio Sato. 2009. “Natural Recovery of Contaminated Sediments.” In *Sediments Contamination and Sustainable Remediation*, 109-134. CRC Press.

Muzyed, Shareef K.I., Filiz Kucuksezgin, and Nalan Tuzmen. 2017. “Persistent Organochlorine Residues in Fish and Sediments Collected from Eastern Aegean Coast: Levels, Occurrence and Ecological Risk.” *Marine Pollution Bulletin* 119 (2): 247–252. https://doi.org/10.1016/j.marpolbul.2017.04.040.

Nakari, Tarja, and Sami Huhtala. 2008. “Comparison of Toxicity of Congener-153 of PCB, PBB, and PBDE to *Daphnia magna*.” *Ecotoxicology and Environmental Safety* 71 (2): 514–518. https://doi.org/10.1016/j.ecoenv.2007.10.012.

Neşer, Gökdeniz, Aynur Kontas, Deniz Ünsalan, Esin Uluturhan, Oya Altay, Enis Darilmaz, Filiz Küçüksezgin, Nermin Tekoğul, and Funda Yercan. 2012. “Heavy Metals Contamination Levels at the Coast of Aliağa (Turkey) Ship Recycling Zone.” *Marine Pollution Bulletin* 64 (4): 882–887. https://doi.org/10.1016/j.marpolbul.2012.02.006.

Odabasi, Mustafa, Yetkin Dumanoglu, Melik Kara, Hasan Altiok, Tolga Elbir, and Abdurrahman Bayram. 2017a. “Spatial Variation of PAHs and PCBs in Coastal Air, Seawater, and Sediments in a Heavily Industrialized Region.” *Environmental Science and Pollution Research* 24 (15): 13749–13759. https://doi.org/10.1007/s11356-017-8991-8.

Odabasi, Mustafa, Yetkin Dumanoglu, Melik Kara, Hasan Altiok, Tolga Elbir, and Abdurrahman Bayram. 2017b. “Polychlorinated Naphthalene (PCN) Emissions from Scrap Processing Steel Plants with Electric-Arc Furnaces.” *Science of the Total Environment* 574 (1): 1305–1312. https://doi.org/10.1016/j.scitotenv.2016.08.028.

Odabasi, Mustafa, Ezgi Ozgunerge Falay, Gizem Tuna, Hasan Altiok, Melik Kara, Yetkin Dumanoglu, Abdurrahman Bayram, Doganay Tolunay, and Tolga Elbir. 2015. “Biomonitoring the Spatial and Historical Variations of Persistent Organic Pollutants (POPs) in an Industrial Region.” *Environmental Science and Technology* 49 (4): 2105–2114. https://doi.org/10.1021/es506316t.

O'Driscoll, Kieran, Jill Robinson, Wen-Son Chiang, Yang-Yih Chen, Ruey-Chy Kao, and Rory Doherty. 2016. "The Environmental Fate of Polybrominated Diphenyl Ethers (PBDEs) in Western Taiwan and Coastal Waters: Evaluation with a Fugacity-Based Model." *Environmental Science and Pollution Research International* 23 (13): 13222–13234. https://doi.org/10.1007/s11356-016-6428-4.

Ohtsubo, Yoshiyuki, Toshiaki Kudo, Masataka Tsuda, and Yuji Nagata. 2004. "Strategies for Bioremediation of Polychlorinated Biphenyls." *Applied Microbiology and Biotechnology* 65 (8): 250–258. https://doi.org/10.1007/s00253-004-1654-y.

Oremland, Ronald S., Diane Newman, Brian W. Kail, and John F. Stolz. 2001. "Bacterial Respiration of Arsenate and Its Significance in the Environment." In *Environmental Chemistry of Arsenic*, 273-295. Marcel Dekker, New York.

Pakdeesusuk, Usarat, David L. Freedman, Cindy M. Lee, and John T. Coates. 2003. "Reductive Dechlorination of Polychlorinated Biphenyls in Sediment from the Twelve Mile Creek Arm of Lake Hartwell, South Carolina, USA ." In *Environmental Toxicology and Chemistry*, 1214–1220. Clemson University.

Pakdeesusuk, Usarat, Cindy M. Lee, John T. Coates, and David L. Freedman. 2005. "Assessment of Natural Attenuation via in Situ Reductive Dechlorination of Polychlorinated Biphenyls in Sediments of the Twelve Mile Creek Arm of Lake Hartwell, SC." *Environmental Science & Technology* 39 (4): 945–952. https://doi.org/10.1021/es0491228.

Pan, Jing, Yong Liang Yang, Qing Xu, Da Zhou Chen, and Dan Li Xi. 2007. "PCBs, PCNs and PBDEs in Sediments and Mussels from Qingdao Coastal Sea in the Frame of Current Circulations and Influence of Sewage Sludge." *Chemosphere* 66 (10): 1971–1982. https://doi.org/10.1016/j.chemosphere.2006.07.070.

Pan, Ying, Juan Chen, Haichao Zhou, Siu G. Cheung, and Nora F.Y. Tam. 2020. "Degradation of BDE-47 in Mangrove Sediments with Amendment of Extra Carbon Sources." *Marine Pollution Bulletin* 153 (4): 110972-110981. https://doi.org/10.1016/j.marpolbul.2020.110972.

Pan, Ying, Pui-Ying Leung, Yuan Y. Li, Juan Chen, Richard Y. C. Kong, and Nora F. Y. Tam. 2021. "Enhancement Effect of Nanoscale Zero-Valent Iron Addition on Microbial Degradation of BDE-209 in Contaminated Mangrove Sediment." *The Science of the Total Environment* 781 (8): 146702-146710. https://doi.org/10.1016/j.scitotenv.2021.146702.

Payne, Rayford B., Harold D. May, and Kevin R. Sowers. 2011. “Enhanced Reductive Dechlorination of Polychlorinated Biphenyl Impacted Sediment by Bioaugmentation with a Dehalorespiring Bacterium.” *Environmental Science and Technology* 45 (20): 8772–8779. https://doi.org/10.1021/es201553c.

Pazi, Idil, L. Tolga Gonul, Filiz Kucuksezgin, Gulsen Avaz, Leyla Tolun, Aydın Unluoglu, Yakup Karaaslan, et al. 2017. “Potential Risk Assessment of Metals in Edible Fish Species for Human Consumption from the Eastern Aegean Sea.” *Marine Pollution Bulletin* 120 (1–2): 409–413. https://doi.org/10.1016/j.marpolbul.2017.05.004.

Péloquin, Luc, and Charles W. Greer. 1993. “Cloning and Expression of the Polychlorinated Biphenyl-Degradation Gene Cluster from Arthrobacter M5 and Comparison to Analogous Genes from Gram-Negative Bacteria.” *Gene* 125 (1): 35–40. https://doi.org/10.1016/0378-1119(93)90742-l.

Pessah, Isaac N., Pamela J. Lein, Richard F. Seegal, and Sharon K. Sagiv. 2019. “Neurotoxicity of Polychlorinated Biphenyls and Related Organohalogens.” *Acta Neuropathologica* 138 (3): 363–387. https://doi.org/10.1007/s00401-019-01978-1.

Pieper, Dietmar H. 2005. “Aerobic Degradation of Polychlorinated Biphenyls.” *Applied Microbiology and Biotechnology* 67 (4): 170–191. https://doi.org/10.1007/s00253-004-1810-4.

Qiu, Mengde, Xingjuan Chen, Daiyong Deng, Jun Guo, Guoping Sun, Bixian Mai, and Meiying Xu. 2012. “Effects of Electron Donors on Anaerobic Microbial Debromination of Polybrominated Diphenyl Ethers (PBDEs).” *Biodegradation* 23 (6): 351–361. https://doi.org/10.1007/s10532-011-9514-9.

Quensen III, John F., James M. Tiedje, and Stephen A. Boyd. 1988. “Reductive Dechlorination of Polychlorinated Biphenyls by Anaerobic Microorganisms from Sediments.” *Science* 242 (4879): 752–754. https://doi.org/10.1126/science.242.4879.752.

Robrock, Kristin R., Mehmet Coelhan, David L. Sedlak, and Lisa Alvarez-Cohen. 2009. “Aerobic Biotransformation of Polybrominated Diphenyl Ethers (PBDEs) by Bacterial Isolates.” *Environmental Science and Technology* 43 (15): 5705–5711. https://doi.org/10.1021/es900411k.

Robrock, Kristin R., Peter Korytár, and Lisa Alvarez-Cohen. 2008. “Pathways for the Anaerobic Microbial Denomination of Polybrominated Diphenyl Ethers.”

Environmental Science and Technology 42 (8): 2845–2852. https://doi.org/10.1021/es0720917.

Rysavy, Jason P., Tao Yan, and Paige J. Novak. 2005. "Enrichment of Anaerobic Polychlorinated Biphenyl Dechlorinators from Sediment with Iron as a Hydrogen Source." *Water Research* 39 (4): 569–578. https://doi.org/10.1016/j.watres.2004.11.009.

Saba, Tarek, and Paul D. Boehm. 2011. "Congener-Based Analysis of the Weathering of PCB Aroclor 1242 in Paper Mill Sludge." *Chemosphere* 82 (9): 1321–1328. https://doi.org/10.1016/j.chemosphere.2010.12.011.

Safe, Stephen H. 1994. "Polychlorinated Biphenyls (PCBs): Environmental Impact, Biochemical and Toxic Responses, and Implications for Risk Assessment." *Critical Reviews in Toxicology* 24 (2): 87–149. https://doi.org/10.3109/10408449409049308.

Sanders, Gordon, John Hamilton-Taylor, and Kevin C. Jones. 1996. "PCB and PAH Dynamics in a Small Rural Lake." *Environmental Science and Technology* 30 (10): 2958–2966. https://doi.org/10.1021/es9509240.

Shih, Yang-hsin, His-Ling Chou, and Yu-Huei Peng. 2012. "Microbial Degradation of 4-Monobrominated Diphenyl Ether with Anaerobic Sludge." *Journal of Hazardous Materials* 213–214 (4): 341–346. https://doi.org/10.1016/j.jhazmat.2012.02.009.

Sivey, John D., and Cindy M. Lee. 2007. "Polychlorinated Biphenyl Contamination Trends in Lake Hartwell, South Carolina (USA): Sediment Recovery Profiles Spanning Two Decades." *Chemosphere* 66 (10): 1821–1828. https://doi.org/10.1016/j.chemosphere.2006.09.013.

Song, Mengke, Chunling Luo, Fangbai Li, Longfei Jiang, Yan Wang, Dayi Zhang, and Gan Zhang. 2015. "Anaerobic Degradation of Polychlorinated Biphenyls (PCBs) and Polychlorinated Biphenyls Ethers (PBDEs), and Microbial Community Dynamics of Electronic Waste-Contaminated Soil." *Science of the Total Environment* 502 (1): 426–433. https://doi.org/10.1016/j.scitotenv.2014.09.045.

Sowers, Kevin R., and Harold D. May. 2013. "In Situ Treatment of PCBs by Anaerobic Microbial Dechlorination in Aquatic Sediment: Are We There Yet?" *Current Opinion in Biotechnology* 24 (3): 482–488. https://doi.org/10.1016/j.copbio.2012.10.004.

Stiborova, Hana, Jana Vrkoslavova, Petra Lovecka, Jana Pulkrabova, Petra Hradkova, Jana Hajslova, and Katerina Demnerova. 2015. "Aerobic Biodegradation of Selected Polybrominated Diphenyl Ethers (PBDEs) in Wastewater Sewage Sludge." *Chemosphere* 118 (1): 315–321. https://doi.org/10.1016/j.chemosphere.2014.09.048.

Stolz, John F., and Ronald S. Oremland. 1999. "Bacterial Respiration of Arsenic and Selenium." *FEMS Microbiology Reviews* 23 (5): 615–627. https://doi.org/10.1111/j.1574-6976.1999.tb00416.x.

Strong, Peter J., and Joanna E. Burgess. 2008. "Treatment Methods for Wine-Related and Distillery Wastewaters: A Review." *Bioremediation Journal* 12 (2): 70–87. https://doi.org/10.1080/10889860802060063.

Suedel, Burton C., Tom M. Dillon, and William H. Benson. 1997. "Subchronic Effects Of Five Di-Ortho Pcb Congeners On Survival, Growth And Reproduction In The Fathead Minnow Pimephales Promelas." *Environmental Toxicology and Chemistry* 16 (7): 1526–1532. https://doi.org/10.1002/etc.5620160729.

Sun, Bingbing, Yuanan Hu, and Hefa Cheng. 2020. "Microwave-Induced Degradation as a Novel Treatment for Destruction of Decabromodiphenyl Ether Sorbed on Porous Minerals." *Chemical Engineering Journal* 391 (6): 123550-123572. https://doi.org/10.1016/j.cej.2019.123550.

Sun, Chunyan, Jincai Zhao, Hongwei Ji, Wanhong Ma, and Chuncheng Chen. 2012. "Photocatalytic Debromination of Preloaded Decabromodiphenyl Ether on the TiO_2 Surface in Aqueous System." *Chemosphere* 89 (4): 420–425. https://doi.org/10.1016/j.chemosphere.2012.05.076.

Tang, Xianjin, Muhammad Z. Hashmi, Biao Zeng, Jiawen Yang, and Chaofeng Shen. 2015. "Application of Iron-Activated Persulfate Oxidation for the Degradation of PCBs in Soil." *Chemical Engineering Journal* 279 (10): 673–680. https://doi.org/10.1016/j.cej.2015.05.059.

Terzaghi, Elisa, Melissa Morselli, Elisabetta Zanardini, Cristiana Morosini, Giuseppe Raspa, and Antonio Di Guardo. 2018. "Improving the SoilPlusVeg Model to Evaluate Rhizoremediation and PCB Fate in Contaminated Soils." *Environmental Pollution* 241 (10): 1138–1145. https://doi.org/10.1016/j.envpol.2018.06.039.

Thompson, Ian P., Christopher J. van der Gast, Lena Ciric, and Andrew C. Singer. 2005. "Bioaugmentation for Bioremediation: The Challenge of Strain Selection."

Environmental Microbiology 7 (7): 909–915. https://doi.org/10.1111/j.1462-2920.2005.00804.x.

Tokarz III, John A., Mi-Youn Ahn, June Leng, Timothy R. Filley, and Loring Nies. 2008. "Reductive Debromination of Polybrominated Diphenyl Ethers in Anaerobic Sediment and a Biomimetic System." *Environmental Science & Technology* 42 (4): 1157–1164. https://doi.org/10.1021/es071989t.

Tsai, Yu-Li, and Betty H. Olson. 1991. "Rapid Method for Direct Extraction of DNA from Soil and Sediments." *Applied and Environmental Microbiology* 57 (4): 1070–1074. https://doi.org/10.1128/aem.57.4.1070-1074.1991.

Tue, Nguyen M., Shin Takahashi, Go Suzuki, Tomohiko Isobe, Pham H. Viet, Yuso Kobara, Nobuyasu Seike, Gan Zhang, Agus Sudaryanto, and Shinsuke Tanabe. 2013. "Contamination of Indoor Dust and Air by Polychlorinated Biphenyls and Brominated Flame Retardants and Relevance of Non-Dietary Exposure in Vietnamese Informal e-Waste Recycling Sites." *Environment International* 51 (1): 160–167. https://doi.org/10.1016/j.envint.2012.11.006.

Tyagi, Meenu, Maria M. R. da Fonseca, and Carla C. C. R. de Carvalho. 2011. "Bioaugmentation and Biostimulation Strategies to Improve the Effectiveness of Bioremediation Processes." *Biodegradation* 22 (4): 231–241. https://doi.org/10.1007/s10532-010-9394-4.

United Nations Environment Programme (UNEP). 2001. "Stockholm Convention on Persistent Organic Pollutants." Stockholm. Accessed August 29, 2024. https://treaties.un.org/pages/viewdetails.aspx?src=treaty&mtdsg_no=xxvii-15&chapter=27.

US EPA. 1994. "Final Record of Decision for the Sangamo Weston/Twelve Mile Creek/Lake Hart-Well PCB Contamination Superfund Site Operable Unit Two, Pickens, Pickens County, South Carolina." Athens, GA. Accessed September 10, 2024. https://semspub.epa.gov/work/hq/187247.pdf.

US EPA. 1996. "Method 3665A: Sulfuric Acid/Permanganate Cleanup." Accessed October 9, 2024. https://www.epa.gov/sites/default/files/2015-12/documents/3665a.pdf.

US EPA. 2007. "Method 3550C: Ultrasonic Extraction." Accessed October 9, 2024. https://www.epa.gov/sites/default/files/2015-12/documents/3550c.pdf.

US EPA. 2010. “An Exposure Assessment of Polybrominated Diphenyl Ethers.” Accessed December 21, 2024. http://www.epa.gov/ncea.

US EPA. 2014. “Technical Fact Sheet – Polybrominated Diphenyl Ethers (PBDEs) and Polybrominated Biphenyls (PBBs).” Accessed December 21, 2024. https://19january2017snapshot.epa.gov/sites/production/files/2014-03/documents/ffrrofactsheet_contaminant_perchlorate_january2014_final_0.pdf.

US EPA. 2024a. “Comptox Chemicals Dashboard.” Accessed November 8, 2024. https://comptox.epa.gov/dashboard.

US EPA. 2024b. “ECOTOX Knowledgebase.” Accessed December 17, 2024. https://cfpub.epa.gov/ecotox.

Varadhan, Srinivasa A., Amid P. Khodadoust, and Richard C. Brenner. 2011. “Effect of Biostimulation on the Microbial Community in PCB-Contaminated Sediments through Periodic Amendment of Sediment with Iron.” *Journal of Industrial Microbiology and Biotechnology* 38 (10): 1691–1707. https://doi.org/10.1007/s10295-011-0959-y.

Wang, Shaorui, Yan Wang, Wenrui Lei, Yingtao Sun, Yujie Wang, Chunling Luo, and Gan Zhang. 2015. “Simultaneous Enhanced Removal of Cu, PCBs, and PBDEs by Corn from e-Waste-Contaminated Soil Using the Biodegradable Chelant EDDS.” *Environmental Science and Pollution Research* 22 (22): 18203–18210. https://doi.org/10.1007/s11356-015-5045-y.

Wania, Frank, and Donald Mackay. 1996. “Tracking the Distribution of Persistent Organic Pollutants.” *Environmental Science & Technology* 30 (9): 390-396. https://doi.org/10.1021/es962399q.

Wen, Sheng, Fang-Xing Yang, Yan Gong, Xiao-Ling Zhang, Yang Hui, Jing-Guang Li, Ai-Ling Liu, Yong-Ning Wu, Wen-Qing Lu, and Ying Xu. 2008. “Elevated Levels of Urinary 8-Hydroxy-2′-Deoxyguanosine in Male Electrical and Electronic Equipment Dismantling Workers Exposed to High Concentrations of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans, Polybrominated Diphenyl Ethers, and Polychlorinated Biphenyls.” *Environmental Science and Technology* 42 (11): 4202–4207. https://doi.org/10.1021/es800044m.

Wheeler, Michael A., Veit Rothhammer, and Francisco J. Quintana. 2017. “Control of Immune-Mediated Pathology via the Aryl Hydrocarbon Receptor.” *Journal of Biological Chemistry* 292 (30): 12383–12389. https://doi.org/10.1074/jbc.r116.767723.

Wiegel, Juergen, and Qingzhong Wu. 2000. “Microbial Reductive Dehalogenation of Polychlorinated Biphenyls.” *FEMS Microbiology Ecology* 32 (1): 1–15. https://doi.org/10.1111/j.1574-6941.2000.tb00693.x.

Wilson, John T. 2011. “An Approach for Evaluating the Progress of Natural Attenuation in Groundwater.” U.S. EPA Ground Water and Ecosystems Restoration Division. Accessed September 9, 2024. https://nepis.epa.gov/exe/zypdf.cgi?dockey=p100dpoe.pdf.

Winchell, Lloyd J., and Paige J. Novak. 2008. “Enhancing Polychlorinated Biphenyl Dechlorination in Fresh Water Sediment with Biostimulation and Bioaugmentation.” *Chemosphere* 71 (1): 176–182. https://doi.org/10.1016/j.chemosphere.2007.10.021.

World Health Organization (WHO). 1994. “Brominated Diphenyl Ethers.” International Programme on Chemical Safety. Environmental Health Criteria 162. Accessed December 15, 2024. https://www.inchem.org/documents/ehc/ehc/ehc162.htm

Wolin, Erica A., Meyer J. Wolin, and Ralph S. Wolfe. 1963. “Formation of Methane by Bacterial Extracts.” *The Journal of Biological Chemistry* 238 (8): 2882–2886. https://doi.org/10.1016/s0021-9258(18)67912-8.

Wu, Juan, Yunqiang Yi, Yuqing Li, Zhanqiang Fang, and Eric P. Tsang. 2016. “Excellently Reactive Ni/Fe Bimetallic Catalyst Supported by Biochar for the Remediation of Decabromodiphenyl Contaminated Soil: Reactivity, Mechanism, Pathways and Reducing Secondary Risks.” *Journal of Hazardous Materials* 320 (12): 341–349. https://doi.org/10.1016/j.jhazmat.2016.08.049.

Wu, Ming-Hong, Jing-Cheng Pei, Ming Zheng, Liang Tang, Yang-Yang Bao, Ben-Tuo Xu, Rui Sun, Yan-Feng Sun, Gang Xu, and Jian-Qiu Lei. 2015. “Polybrominated Diphenyl Ethers (PBDEs) in Soil and Outdoor Dust from a Multi-Functional Area of Shanghai: Levels, Compositional Profiles and Interrelationships.” *Chemosphere* 118 (1): 87–95. https://doi.org/10.1016/j.chemosphere.2014.06.022.

Wu, Qingzhong, Donna L. Bedard, and Juergen Wiegel. 1997. “Temperature Determines the Pattern of Anaerobic Microbial Dechlorination of Aroclor 1260 Primed by 2,3,4,6-Tetrachlorobiphenyl in Woods Pond Sediment.” *Applied and Environmental Microbiology* 63 (12): 4818–4825. https://doi.org/10.1128/aem.63.12.4818-4825.1997.

Wu, Qingzhong, Charles E. Milliken, Patrick G. Meier, Joy E. M. Watts, Kevin R. Sowers, and Harold D. May. 2002. "Dechlorination of Chlorobenzenes by a Culture Containing Bacterium DF-1, a PCB Dechlorinating Microorganism." *Environmental Science and Technology* 36 (15): 3290–3294. https://doi.org/10.1021/es0158612.

Wu, Zhineng, Wei Han, Miaomiao Xie, Min Han, Yao Li, and Yingying Wang. 2019. "Occurrence and Distribution of Polybrominated Diphenyl Ethers in Soils from an E-Waste Recycling Area in Northern China." *Ecotoxicology and Environmental Safety* 167 (1): 467–475. https://doi.org/10.1016/j.ecoenv.2018.10.029.

Xu, Guofang, Hung L. Ng, Chen Chen, Siyan Zhao, and Jianzhong He. 2022. "Efficient and Complete Detoxification of Polybrominated Diphenyl Ethers in Sediments Achieved by Bioaugmentation with Dehalococcoides and Microbial Ecological Insights." *Environmental Science and Technology* 56 (12): 8008–8019. https://doi.org/10.1021/acs.est.2c00914.

Xu, Guofang, Hung L. Ng, Chen Chen, Matthew J. Rogers, and Jianzhong He. 2024. "Combatting Multiple Aromatic Organohalide Pollutants in Sediments by Bioaugmentation with a Single Dehalococcoides." *Water Research* 255 (5): 121447-121459. https://doi.org/10.1016/j.watres.2024.121447.

Xu, Guofang, Siyan Zhao, Chen Chen, Xuejie Zhao, Rajaganesan Ramaswamy, and Jianzhong He. 2022. "Dehalogenation of Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls Catalyzed by a Reductive Dehalogenase in Dehalococcoides Mccartyi Strain MB." *Environmental Science & Technology* 56 (7): 4039–4049. https://doi.org/10.1021/acs.est.1c05170.

Xu, Guofang, Siyan Zhao, and Jianzhong He. 2024. "Underexplored Organohalide-Respiring Bacteria in Sewage Sludge Debrominating Polybrominated Diphenyl Ethers." *Environmental Science & Technology* 58 (30): 13348–13357. https://doi.org/10.1021/acs.est.4c00906.

Yang, Chu-Wen, Huang-Wen Huang, Wei-Liang Chao, and Bea-Ven Chang. 2015. "Bacterial Communities Associated with Aerobic Degradation of Polybrominated Diphenyl Ethers from River Sediments." *Environmental Science and Pollution Research* 22 (5): 3810–3819. https://doi.org/10.1007/s11356-014-3626-9.

Ye, Mao, Mingming Sun, Jinzhong Wan, Guodong Fang, Huixin Li, Feng Hu, Xin Jiang, and Fredrick O. Kengara. 2015. "Evaluation of Enhanced Soil Washing Process with Tea Saponin in a Peanut Oil-Water Solvent System for the Extraction of PBDEs/PCBs/PAHs and Heavy Metals from an Electronic Waste Site Followed

by Vetiver Grass Phytoremediation." *Journal of Chemical Technology and Biotechnology* 90 (11): 2027–2035. https://doi.org/10.1002/jctb.4512.

Yılmaz, Atilla, Burak Karacık, Sevil D. Yakan, Bernhard Henkelmann, Karl-Werner Schramm, and Oya S. Okay. 2016. "Organic and Heavy Metal Pollution in Shipbreaking Yards." *Ocean Engineering* 123 (9): 452–457. https://doi.org/10.1016/j.oceaneng.2016.06.036.

Yu, Ying-Xin, Shao-Huan Zhang, Ning-Bao Huang, Jun-Ling Li, Yu-Ping Pang, Xin-Yu Zhang, Zhi-Qiang Yu, and Zhi-Guang Xu. 2012. "Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls in Freshwater Fish from Taihu Lake, China: Their Levels and the Factors That Influence Biomagnification." *Environmental Toxicology and Chemistry* 31 (3): 542–549. https://doi.org/10.1002/etc.1722.

Yun, Xiaoyan, Yuyi Yang, Minxia Liu, and Jun Wang. 2015. "Concentrations and Risk Assessment of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Surface Sediments from the East Lake, China." *Ecotoxicology* 24 (1): 172–180. https://doi.org/10.1007/s10646-014-1370-z.

Zanaroli, Giulio, Annalisa Balloi, Andrea Negroni, Luigimaria Borruso, Daniele Daffonchio, and Fabio Fava. 2012. "A Chloroflexi Bacterium Dechlorinates Polychlorinated Biphenyls in Marine Sediments under in Situ-like Biogeochemical Conditions." *Journal of Hazardous Materials* 209–210 (3): 449–457. https://doi.org/10.1016/j.jhazmat.2012.01.042.

Zhang, Zhen, Lin H. Chen, Min L. Tao, Dan D. Zhou, Yuan Zhang, Jun Yao, Qing Na Kong, and Bin B. Guo. 2023. "Occurrence and Distribution Characteristics of PCBs and PBDEs in Farmland Soils Adjacent to Electronic Circuit Board Dismantling Ruins." *Frontiers in Environmental Science* 10 (1): 1048345-1048360. https://doi.org/10.3389/fenvs.2022.1048345.

Zhao, Siyan, Matthew J. Rogers, Lifeng Cao, Chang Ding, and Jianzhong He. 2021. "Identification of Reductive Dehalogenases That Mediate Complete Debromination of Penta- and Tetrabrominated Diphenyl Ethers in Dehalococcoides Spp." *Applied and Environmental Microbiology* 87 (17): 1–14. https://doi.org/10.1128/aem.00602-21.

APPENDIX A

Table A. The ingredients of the media related to enrichment and microcosm studies.

Solution	Components	Amount (g or mL)	Volume used in ASM-1 (mL in 1 L)	Volume used in ASM-2 (mL in 1 L)
Stock Solution 1 (S1) (2000 mL in DI)	NaCl	287.9268 g	140	140
	Na_2SO_4	48.2371 g		
	KCl	8.1392 g		
	$NaHCO_3$	2.3642 g		
	KBr	1.1726 g		
	Boric acid (H_3BO_3	0.3126 g		
	Sodium fluoride (NaF)	0.0380 g		
Stock Solution 2 (S2) (2000 mL in DI)	Magnesium chloride tetrahydrate ($MgCl_2.6H_2O$)	364.937 g	50	50
	Calcium chloride dihydrate ($CaCl_2.2H_2O$)	51.1347 g		
	Strontium chloride hexahydrate ($SrCl_2.6H_2O$)	0.829 g		
Stock Solution 3 (S3) (2000 mL in DI)	Sodium nitrate ($NaNO_3$)	90.426 g	10	-
	Sodium phosphate monobasic ($NaH_2PO_4.H_2O$)	6.668 g		
Stock Solution 5 (S5) (2000 mL in DI)	Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$)	0.212 g	50	-
	EDTA disodium salt dihydrate ($C_{10}H_{14}O_8N_2Na_2.2H_2O$)	0.308 g		
	Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) Superstock (0.01 g/L)	94.86 mL		
	Cobalt (II) sulfate heptahydrate ($CoSO_4.H_2O$) Superstock (0.01 g/L)	138.8 mL		
	Sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$) Superstock (0.01 g/L)	17.36 mL		
	Copper sulfate pentahydrate ($CuSO_4.5H_2O$) Superstock (0.01 g/L)	22.62 mL		
	Sodium selenite (Na_2SeO_3) Superstock (0.005 g/L)	0.658 mL		
	Nickel chloride hexahydrate ($NiCl_2.6H_2O$) Superstock (0.01 g/L)	2.84 mL		
Stock Solution 6 (S6) (1000 mL in DI)	Thiamine HCl ($C_{12}H_{18}N_4OSCl$) Superstock (0.01 g/L)	8.616 mL	1	-
	Biotin ($C_{10}H_{16}N_2O_3S$) Superstock (0.005 g/L)	0.0059 mL		
	Vitamin B_{12} ($C_{63}H_{88}N_{14}CoO_{14}P$) Superstock (0.01 g/L)	0.1285 mL		
Deionized Water (DI)			750	810