SUSTAINABLE REMEDIATION OF AQUATIC SEDIMENTS CONTAMINATED WITH POLYBROMINATED DIPHENYL ETHERS AND HEXABROMOCYCLODODECANE

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submitted by **HALE DEMİRTEPE** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Environmental Engineering Department, Middle East Technical University** by,

Prof. Dr. Gülbin Dural Ünver
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Kahraman Ünlü
Head of Department, **Environmental Engineering** _____

Prof. Dr. İpek İmamoğlu
Supervisor, **Environmental Engineering Dept., METU** _____

Examining Committee Members:

Prof. Dr. F. Dilek Sanin
Environmental Engineering Dept., METU _____

Prof. Dr. İpek İmamoğlu
Environmental Engineering Dept., METU _____

Prof. Dr. Ayşegül Aksoy
Environmental Engineering Dept., METU _____

Assoc. Prof. Dr. Perihan Binnur Kurt Karakuş
Environmental Engineering Dept., Bursa Technical University _____

Assoc. Prof. Dr. Mehmet İşleyen
Environmental Engineering Dept., Bursa Technical University _____

Date: 17.05.2017

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name : Hale Demirtepe

Signature :

ABSTRACT

SUSTAINABLE REMEDIATION OF AQUATIC SEDIMENTS CONTAMINATED WITH POLYBROMINATED DIPHENYL ETHERS AND HEXABROMOCYCLODODECANE

Demirtepe, Hale
Ph.D., Department of Environmental Engineering
Supervisor: Prof. Dr. İpek İmamoğlu

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The most widely used brominated flame retardants (BFRs), namely, polybrominated diphenyl ethers (PBDEs), and hexabromocyclododecane (HBCDD) are persistent organic pollutants that pose great risk to human health and the environment. This study aims to investigate degradation of PBDEs and HBCDD contaminated aquatic sediments via biotic and abiotic remediation strategies. In order to obtain an indication of use of these BFRs in Turkey, urban and industrial wastewater treatment plant sludges were sampled, yielding Σ_{22}PBDE concentrations between 66.93-2.46x10^7 ng/g dw, and total-HBCDD ranging from 13.1-616.2 ng/g dw. Results indicated clear evidence of use of BFRs in Turkey.

Sediment microcosms were operated for degradation of BFRs to simulate biostimulation (via addition of carbon and electron source), bioaugmentation (via addition of dechlorinating culture) and natural attenuation (no extraneous substance). Both BDE-209 and gamma-HBCDD microcosms showed the highest degradation rate during biostimulation with pseudo-first-order rate constants of 0.0049 d^{-1}, and 0.0542 d^{-1}, respectively. BDE-209 bioaugmentation microcosm yielded the greatest extent of debromination, with tri-BDEs detected at the end of incubation. Identification of 20 debromination pathways indicate preferential Br loss from *ortho* and *meta* positions. On the other hand, HBCDD natural attenuation and

bioaugmentation microcosms showed no statistically significant difference. Abiotic degradation of BFRs was investigated via catalyzed hydrogen peroxide propagations. The most successful application was through fill-and-draw treatments yielding up to 31.8%, 83% and 93.9% removal of BDE-209, BDE-99, and HBCDD, respectively. BDE-209 and BDE-99 biostimulation mesocosms, with reduced amount of biostimulating agents, showed on average 0.0012 d^{-1} and 0.0020 d^{-1}degradation rates, respectively. Also, changes in debromination pathways compared to microcosms were observed, revealing the impact of amount of biostimulators supplied in sediments. Finally, all four remediation strategies studied under laboratory conditions were evaluated from the perspective of sustainable remediation principles.

Keywords: Brominated flame retardants, biotic transformation, abiotic degradation, sustainable remediation.

ÖZ

POLİBROMLU DİFENİL ETERLER VE HEKZABROMOSİKLODODEKAN İLE KİRLENMİŞ SUCUL SEDİMANLARIN SÜRDÜRÜLEBİLİR REMEDİASYONU

Demirtepe, Hale

Doktora, Çevre Mühendisliği Bölümü
Tez Yöneticisi: Prof. Dr. İpek İmamoğlu

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En çok kullanılan bromlu alev geciktiriciler (BAG) olan polibromlu difenil eterler (PBDE) ve hekzabromosiklododekan (HBCDD), insan sağlığına ve çevreye büyük risk oluşturan kalıcı organik kirleticilerdir. Bu çalışma, PBDEler ve HBCDD ile kirlenmiş sucul sedimanlarda biyotik ve abiyotik iyileştirme stratejileri ile bu kimyasalların bozunmalarının araştırılmasını amaçlamaktadır. Bu BAGların Türkiye'deki kullanımına yönelik bulgular elde etmek amacıyla, kentsel ve endüstriyel atıksu arıtma tesislerinden arıtma çamuru örnekleri alınmıştır. Bu örneklerde, Σ_{22}PBDE derişimleri 66.93-2.46x10^7 ng/g ka aralığında, toplam HBCDD derişimleri 13.1-616.2 ng/g ka aralığında bulunmuştur. Sonuçlar, Türkiye'de BAG kullanımını açıkça kanıtlamaktadır.

BAGların bozunmalarına yönelik sediman mikrokozmları, biyostimulasyon (karbon ve elektron kaynağı eklenerek), biyoogmentasyon (mikroorganizma kültürü eklenerek), ve doğal giderim (hiç bir madde eklemeksizin) yöntemlerini taklit edecek şekilde işletilmiştir. Hem BDE-209, hem de γ-HBCDD mikrokozmları, en yüksek bozunma hızının biyostimulasyon yöntemi ile elde edildiğini, birinci-derece hız sabitlerinin sırasıyla 0.0049 gün^{-1} ve 0.0542 gün^{-1} olduğunu göstermiştir. BDE-209 biyoogmentasyon mikrokozmları, inkübasyon sonunda tri-BDEleri üreterek, en geniş kapsamlı debrominasyonu elde etmiştir. Belirlenen 20 adet debrominasyon

reaksiyonu, brom kaybının tercihen orto ve meta pozisyonlarından gerçekleştiğini göstermiştir. Diğer taraftan, HBCDD doğal giderim ve biyoogmentasyon mikrokozmları arasındaki fark istatistiki olarak anlamlı bulunmamıştır. BAGların abiyotik bozunması katalizörlü hidrojen peroksit çoğalma reaksiyonları ile incelenmiştir. Yöntemin en başarılı uygulaması olan doldur-boşalt işlemi ile BDE-209, BDE-99 ve HBCDD için sırası ile %31.8, %83, ve %93.9 giderim elde edilmiştir. BDE-209 ve BDE-99 biyostimulasyon mezokozmları, kısıtlı miktarda biyostimulant eklenmesi ile, sırasıyla ortalama 0.0012 gün^{-1} ve 0.0020 gün^{-1} bozunma hızları göstermiştir. Ayrıca, debrominasyon reaksiyonlarında mikrokozmlara nazaran değişiklikler görülmüş, bu durum sedimana eklenen biyostimulant miktarının etkisini ortaya koymuştur. Son olarak, laboratuvar koşullarında çalışılan dört iyileştirme stratejisi sürdürülebilir iyileştirme prensipleri açısından değerlendirilmiştir.

Anahtar Kelimeler: Bromlu alev geciktiriciler, biyotik bozunma, abiyotik bozunma, sürdürülebilir iyileştirme.

To my family

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ABBREVIATIONS

ASTM: American Society for Testing and Materials

BA: Bioaugmentation

BDE: Brominated diphenyl ether

BFRs: Brominated flame retardants

Br/dp: bromine per diphenyl ether

BS: Biostimulation

CDT: Cyclododeca-1,5,9-triene

CHP: Catalyzed hydrogen peroxide propagations

CLRTAP: Convention on Long Range Transboundary Pollution

COD: chemical oxygen demand

CRM: certified reference material

CSCCS: Regulation on Control of Soil Contamination and Contaminated Sites due to Point Sources

EI: electron impact

FR: Flame retardants

FRTR: Federal Remediation Technologies Roundtable

F&D: Fill-and-draw

GC-μECD: Gas chromatography coupled with micro-cell electron capture detector

GC-MS: Gas chromatography coupled with mass spectrometry

HBCDD: Hexabromocyclododecane

HPLC: High performance liquid chromatography

IUPAC: International union of pure and applied chemistry

KD: Kuderna-Danish

Koc: Organic carbon-water partitioning coefficient

Kow: Octanol-water partitioning coefficient

LC-MS/MS: Liquid chromatography tandem mass spectrometry

LCS: laboratory control sample

LOQ: limit of quantitation

MDL: Method detection limit

MONET: global passive air monitoring network

MS/MSD: matrix spike/matrix spike duplicate

NA: Natural attenuation

PAH: Polycyclic aromatic hydrocarbons

PBDEs: Polybrominated diphenyl ethers

PBDFs: Polybrominated dibenzofurans

PCBs: Polychlorinated biphenyls

POPs: Persistent organic pollutants

QA/QC: Quality assurance/quality control

RE: rotary evaporator

SIM: selected ion monitoring

UNECE: United Nations Economic Commission for Europe

US EPA: United States Environmental Protection Agency

WFD: Water Framework Directive

WWTP: wastewater treatment plant

ZVI: Zerovalent iron

CHAPTER 1

INTRODUCTION

Urban and industrial development has brought about various synthetic chemicals, produced to improve the industrial quality of commercial goods. These chemicals generate excessive amounts of domestic and industrial wastes, leading to the contamination of natural resources. One such group of chemicals is flame retardants (FRs). They are used to delay ignition and/or protect from fire in commercial and residential products; such as, textiles, plastics, building materials and electronic equipments (Alaee and Wenning, 2002). The most widely used FRs are polybrominated diphenyl ethers (PBDEs), and hexabromocyclododecane (HBCDD) (Covaci et al., 2003). They are classified as additive FRs, i.e. applied to products by mixing, hence they can easily leach from the products (Segev et al., 2009).

The physico-chemical properties of PBDEs and HBCDD reveal their hydrophobic character. When they are released into an aquatic environment, they prefer to accumulate in sediments and biota. Hence, their behavior in environment, especially in aquatic sediments, is of great importance. Previous studies on degradation of PBDEs and HBCDD were relatively scarce, and needs further investigation. Furthermore, toxic effects of PBDEs and HBCDD have been reported by many studies (ATSDR, 2004; Stockholm Convention, 2010). These studies revealed that their presence in the environment pose significant risk to the environment and human health.

Since the start of their manufacture, total global production of PBDEs reached 1.5 million tones in 2005 (UNEP, 2010). Usage of PBDEs started to be phased-out in

Europe and United States starting from 2003. Commercial PBDE mixtures, penta- and octa-BDEs, were regulated by the Stockholm Convention since 2009, including them among the persistent organic pollutants (POPs), while deca-BDE is still under review (Stockholm Convention, 2017). The consumption rate of HBCDD was 12500 tons annually in 2013 in Europe (VECAP, 2014). HBCDD was identified as a POP in 2014 (Stockholm Convention, 2017). Both PBDEs and HBCDD were identified as priority hazardous substances by the Water Framework Directive (European Commission, 2013). As a country which is a party in Stockholm Convention and is trying to adapt the Water Framework Directive, Turkey should identify the current situation on the inventory of and contamination caused by these chemicals.

PBDEs and HBCDD have been ubiquitously measured in environmental matrices; such as soil, sewage sludge, sediments, and atmosphere, as well as aquatic and terrestrial species and humans (Covaci et al., 2006; Hites, 2004). While worldwide PBDE levels in various media have been reported, including Turkey (Cetin and Odabasi, 2007a; Kurt-Karakus et al., 2014; Odabasi et al., 2010), studies on HBCDD levels are recent and rather limited, none yet reported for Turkey. Furthermore, levels in treatment plant sludge were never reported for Turkey for PBDEs or HBCDD. Identification of their levels in treatment plant sludge is important since this is believed to reflect the usage of PBDEs and HBCDD in cities via use of commercial products as well as in industry. This information would also be valuable for Turkey's EU accession studies regarding adaptation to the Water Framework Directive.

Contaminated site is defined as any site that was confirmed by various measurements and/or evaluations to contain hazardous pollutants due to human activity, hence pose significant risk to human health and environment. When the present and future land uses are taken into consideration, the contaminated sites require actions to be taken to reduce or eliminate the risk on human health and environment. Practices for removal, reduction and transformation of contaminants, and systematic application of such actions are called remediation (Mulligan et al., 2010). Formerly, remediation practices included containment or excavation, which are not feasible or sustainable (Ellis and Hadley, 2009). In the last decade, remediation studies were directed

towards application of more sustainable techniques (Bedard, 2003; Sowers and May, 2013).

There are limited studies on degradation of PBDEs in environmental media. These studies investigate the degradation kinetics in soil, sediment and sludge under various conditions, e.g. in the presence of various primers, carbon sources and/or electron donors (Gerecke et al., 2005; Huang et al., 2014; Qiu et al., 2012; Tokarz III et al., 2008). Anaerobic debromination of PBDEs was also studied in dehalogenating culture media in the absence of a solid phase (He et al., 2006; Robrock et al., 2008). All of the previous studies indicated the importance of revealing PBDE degradation mechanisms to assess remediation of contaminated sites. Although addition of carbon source and electron donor which simulate biostimulation as a bioremediation alternative was applied many times for PBDEs, addition of microorganism species which simulate bioaugmentation has never been applied for PBDEs. Furthermore, a comparative evaluation of these bioremediation alternatives through concurrent lab scale microcosms was never attempted. This would provide information on how the results could change regarding the degradation kinetics and debromination pathways of PBDEs when various strategies are applied. Therefore, there appears a knowledge gap for detailed identification of degradation efficiencies, pathways and rates of PBDE debromination when biostimulation, bioaugmentation and natural attenuation strategies are applied.

For HBCDD degradation in environmental media, the studies were much more scarce compared to PBDEs. Biodegradation in soil, sediment, and sludge was investigated in the presence of various primers, and appropriate nutrients (Davis et al., 2006, 2005; Gerecke et al., 2006; Stiborova et al., 2015b). These studies demonstrated that HBCDD has a half-life ranging between 0.66 and 115.5 days under anaerobic conditions. This was believed to be a wide range with three orders of magnitude difference between minimum and maximum half-life values. Since the studies on biodegradation of HBCDD, which was recently included among POPs, started in the last decade, there remains much to be investigated regarding their fate

in the environment. As an example, bioaugmentation has never been tested as a bioremediation alternative for HBCDD degradation in sediments.

For abiotic degradation of PBDEs and HBCDD in the environment, several studies were conducted on photodegradation and catalytic debromination (Ahn et al., 2006; Keum and Li, 2005; Zhou et al., 2014). Also, Fenton's reagent was tested for degradation of PBDEs (J. Li et al., 2016; Wu et al., 2011). However, application of Fenton's reagent in subsurface environments may be infeasible for contaminant removal due to the fast consumption of hydroxyl radical with natural organic matter in soils or sediments. Hence, catalyzed hydrogen peroxide propagation (CHP) reactions were proposed to be used with modifications on Fenton's reactions (Watts and Teel, 2005). Usage of high concentration of hydrogen peroxide and various iron catalysts in CHP reactions yielded high removal percentages for several contaminants; such as PCBs (Ahmad et al., 2011; Manzano et al., 2004) and PAHs (Venny et al., 2012a). However, CHP has never been tested for degradation of PBDEs and HBCDD, hence such a study would provide valuable information to propose new remediation strategies for removal of PBDEs and HBCDD from contaminated sediments.

In the light of the literature studies and knowledge gaps put forth in the area, this study was constructed to contribute to the determination of BFR contamination in Turkey, and elucidation of degradation mechanisms for PBDEs and HBCDD in sediments. More specifically, the aim of the proposed study was to investigate the fate of PBDEs and HBCDD in sediments under conditions representing various biotic and abiotic remediation strategies. An itemized explanation of particular aims of the study is given below by explicitly indicating the novel features of each task. The study concludes with a preliminary sustainability assessment of remediation strategies for aquatic sediments contaminated with these chemicals.

The specific objectives of this study were;

1) To identify BFR levels in four different wastewater treatment plant sludges in Turkey for the first time, and while doing this to validate an analytical

method, to be used in determination of PBDEs and HBCDD in solid matrices, which has low time and materials consumption and high efficiency,

2) To examine the biotic degradation of PBDEs and HBCDD under various bioremediation scenarios in small scale laboratory reactors (microcosms) simulating aquatic sediments, and elucidate debromination pathways and rates,
3) To test an abiotic degradation mechanism, catalyzed hydrogen peroxide propagations, which was not previously attempted for the remediation of PBDE and HBCDD contaminated sediments,
4) To investigate the applicability of the bioremediation scenario that demonstrated the highest degradation efficiency in microcosms, for biodegradation of PBDEs in larger scale laboratory reactors (mesocosms) with the aim of better mimicking environmental conditions, and
5) To evaluate the remediation options investigated throughout the study from the perspective of sustainable remediation.

This dissertation is organized as a collection of manuscripts of which portions have been submitted for publication, or are prepared to be submitted for publication. Apart from these, dissertation also includes literature review and methodology chapters presenting the methods used for PBDE and HBCDD analysis, and details of experimental work conducted in this study. This is the reason why some repetition may appear in the introduction and methodology sections of relevant chapters.

CHAPTER 2

LITERATURE REVIEW

2.1. Pollutants in the Environment

Synthetic chemicals and naturally occurring compounds are being released into the environment, leading to the pollution of natural resources. Especially, since industrial evolution, chemicals manufactured due to human and industrial needs became waste streams, and discharged into freshwaters. Contaminants of emerging concern are then defined as the pollutants existing in the environment for a long time, but not monitored and regulated until recently, while emerging pollutants are defined as the newly identified chemicals in the environment (Sauvé and Desrosiers, 2014; USEPA, 2008a). These pollutants included pesticides, biocides, flame retardants, heavy metals among others (Schwarzenbach et al., 2006). Therefore, in the last decade, great attention was drawn to regulate the production and use of these chemicals, and to asses and remediate the sites contaminated with these pollutants. The countries and/or commissions started acting to examine the production, use, and discharge of these hazardous substances, and in that scope several conventions and legislations have come into force.

First convention to mention is the Stockholm Convention. Persistent organic pollutants (POPs) are defined by Stockholm Convention as the organic chemicals which;

- Persist in the environment for long periods of time;
- Are widely distributed throughout the environmental compartments, water, soil and air and over regions in the world;

- Accumulate in the lipid of living organisms, and increase in their concentrations as gone up in the food chain; and
- Toxic to humans and wildlife (Stockholm Convention, 2017).

Initially, Stockholm Convention identified 12 chemicals as POPs, and aimed for the parties involved to take measures to eliminate (Annex A), restrict (Annex B) the production and use, and/or reduce the unintentional release (Annex C) of these chemicals (Stockholm Convention, 2017). In 2009, the Convention decided to add 9 new chemicals to the POPs list. The review committee under the Convention continues to evaluate chemicals proposed by the parties involved. After screening the chemicals and evaluation of their risk profiles, the decision on the listing of new chemicals as POPs are agreed upon by parties. Since 2009, five new chemicals were included into the POPs list. The chemicals currently under review are decabromodiphenyl ether, dicofol, short chain chlorinated paraffins, and pentadecafluorooctanoic acid, its salts and PFOA-related compounds (Stockholm Convention, 2017). Furthermore, all POPs were categorized according to their usage and production purposes. Table 2-1 presents the POPs under Stockholm Convention with their categories and annexes that they were included in.

Another convention on POPs is the Convention on Long Range Transboundary Pollution (CLRTAP) implemented by United Nations Economic Commission for Europe (UNECE) and its POPs Protocol (Aarhus Protocol on POPs) (UNECE, 2016). European Commission also identifies hazardous and priority hazardous substances, and their environmental quality standards in Water Framework Directive (WFD) (European Commission, 2013). The chemicals listed under the Aarhus Protocol and WFD are marked in Table 2-1. All of these conventions are concerning similar types of chemicals; such as pesticides, industrial chemicals and by-products. One of the groups involved in these conventions is the brominated flame retardants. Hence, their occurrence and fate in the environment became important issues which the countries should engage in.

Table 2-1. List of persistent organic pollutants, their categories and annexes they are involved in Stockholm Convention, placements in Aarhus Protocol and Water Framework Directive (WFD) priority hazardous substances list.

Chemical name	**Category for usage**	**Annex[a]**	**Aarhus Protocol**	**WFD**
Aldrin	Pesticide	A	✓	
Chlordane	Pesticide	A	✓	
DDT	Pesticide	B	✓	
Dieldrin	Pesticide	A	✓	
Endrin	Pesticide	A	✓	
Heptachlor	Pesticide	A	✓	✓
Hexachlorobenzene	Pesticide, Industrial chemical, By-product	A, C	✓	✓
Mirex	Pesticide	A	✓	
Toxaphene	Pesticide	A	✓	
Polychlorinated biphenyls (PCBs)	Industrial chemical, By-product	A, C	✓	✓
Polychlorinated dibenzo-p-dioxins (PCDD)	By-product	C		✓
Polychlorinated dibenzofurans (PCDF)	By-product	C		✓
Chlordecone	Pesticide	A	✓	
α-Hexachlorocyclohexane	Pesticide, By-product	A	✓	✓
β- Hexachlorocyclohexane	Pesticide, By-product	A	✓	✓
Lindane (γ-Hexachlorocyclohexane)	Pesticide	A	✓	✓
Pentachlorobenzene	Pesticide, Industrial chemical, By-product	A, C	✓	✓
Hexabromobiphenyl	Industrial chemical	A	✓	
Hexabromodiphenyl ether and heptabromodiphenyl ether	Industrial chemical	A	✓	✓
Tetrabromodiphenyl ether and pentabromodiphenyl ether	Industrial chemical	A	✓	✓
Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride	Industrial chemical	B	✓	✓
Endosulfan (Technical endosulfan and its related isomers)	Pesticide	A		✓
Hexabromocyclododecane	Industrial chemical	A		✓
Hexachlorobutadiene	Industrial chemical	A	✓	✓
Pentachlorophenol and its salts and esters	Pesticide	A		
Polychlorinated naphthalenes	Industrial chemical and By-product	A, C	✓	

[a] Annex A: elimination of production and use, Annex B: restriction of production and use, Annex C: reduction of unintentional release.

2.2. Chemicals of Concern for This Study – Flame Retardants

Flame retardants (FR) were defined by the American Society for Testing and Materials (ASTM) as "a chemical which, when added to a combustible material, delays ignition and reduced flame spread of the resulting material when exposed to flame impingement" (Weil and Levchik, 2009). These type of chemicals are widely applied to some goods that are in residential and commercial use; such as, textiles, plastics, building materials and electronic equipments (Alaee and Wenning, 2002). Today, more than 175 different FR chemicals are being produced and used for different applications, which are classified into four: inorganic, organophosphorus, nitrogen based and halogenated organic compounds (Alaee and Wenning, 2002). Another way of classification relies on the method of incorporation into the material applied: reactive and additive (Alaee and Wenning, 2002; Segev et al., 2009). Reactive FRs form chemical bonds with the materials, e.g. organophosphorus compounds. Additive FRs, on the other hand, are mixed with the materials, hence they can easily leach from the products. Additive FRs are more frequently used, examples of which are hexabromocyclododecane, aluminium trihydrate and phosphate esters (Segev et al., 2009).

FRs may be released into the environment via several ways: as wastes/wastewaters from FR producing industrial facilities and manufacturing facilities using FRs in their products, leaching and volatilization from manufacturing, use, and disposal of FR products, leaching from landfills, and combustion and recycling of waste products (Segev et al., 2009). When FRs enter into the environment, they can adsorb onto a particle, and transport in water, sediment, or travel long distances with the attached particle in air. Therefore, FRs can be found at regions away from the emission site in terrestrial, freshwater and marine ecosystems (Segev et al., 2009).

2.2.1. Polybrominated Diphenyl Ethers (PBDE)

Polybrominated diphenyl ethers (PBDEs) are aromatic compounds which have two phenyl rings connected with an oxygen atom. A variety of bromine and hydrogen substitutions to carbon atoms results in 209 theoretically possible congeners.

Congeners are numbered according to the International Union of Pure and Applied Chemistry (IUPAC) numbering for polychlorinated biphenyls (PCBs) whose structure is very similar to PBDEs (Hites, 2004). The IUPAC numbering of PBDEs is given in Appendix A. The general molecular structure is shown in Figure 2-1.

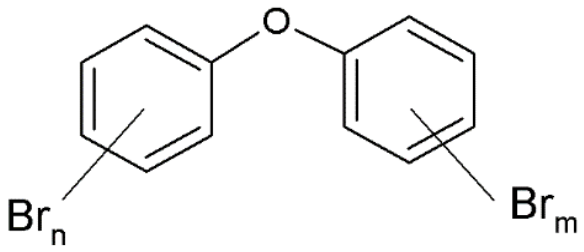


Figure 2-1. The molecular structure of Polybrominated Diphenyl Ethers.

PBDEs are widely used as additive brominated flame retardants (BFRs) in upholstered furniture, electrical and electronic equipments, plastics, insulation materials, and textiles (Alaee et al., 2003; Rahman et al., 2001). PBDEs are produced via bromination of diphenyl ether in the presence of $AlCl_3$ catalyst (Friedel Craft catalyst) in a solvent such as dibromomethane (Alaee et al., 2003). During bromination, Br attachments occur initially at para positions, then ortho positions (Rahman et al., 2001). Commercial production is typically at three different levels of bromination: penta-, octa-, and deca- (Alaee et al., 2003). They have different congener distributions and bromine content (Kim et al., 2017; La Guardia et al., 2006). The distribution of homolog groups in the commercial mixtures is presented in Figure 2-2, and detailed homolog concentrations are given in Appendix B. Deca-BDE, having 83 % Br by weight, is mainly composed of BDE 209 and minute amounts of nona-BDEs. This mixture is the most widely applied flame retardant, hence BDE 209 is the major congener detected in sediments, sludge. Octa-BDE contains BDE 183 with a Br content of 79%. The production amount of this mixture is very low. Penta-BDE is a viscous liquid and has a Br content of 70%. Its composition is 41-42% tetra-BDE (BDE 47), 44-45% penta-BDEs (BDE 99 and minor BDE 100) and 6-7 % hexa-BDEs (BDE 153 & 154). This mixture is generally

used in polyurethane foam and textiles. The congeners BDE 47, 99 and 100 are predominantly observed in biological matrices including the human tissue (Alaee et al., 2003).

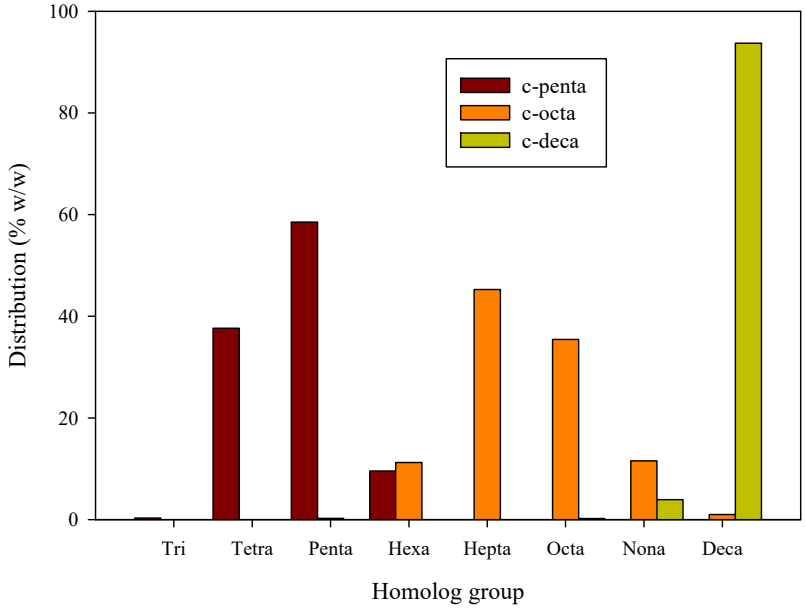


Figure 2-2. Homolog distribution of commercial penta, octa and deca PBDE mixtures (Kim et al., 2017; La Guardia et al., 2006).

Manufacturing of commercial PBDE mixtures worldwide dated back to 1970s. During 1970 – 2005 period, total global production of penta-, octa-, and deca-BDEs were approximately 100000 tonnes, 110000 tonnes and 1.1 to 1.25 million tones, respectively (UNEP, 2010). After the adverse effects have been recognized, European Union banned the use of products containing penta- and octa-BDEs in 2003, and then use of deca-BDE in 2008 (UNEP, 2010). Concurrently, United States manufacturers voluntarily eliminated the production of penta- and octa-BDEs in 2004, and deca-BDE after 2013 (Abbasi et al., 2015). Before the start of these phase-out actions, the worldwide annual market demand of all PBDE mixtures had reached to nearly 70000 tons in 2001 (Hites, 2004).

Since PBDEs were widely used throughout the world, they have been observed in the environment and biota frequently. In marine mammals, fish and birds (mostly birds'eggs), and in river, lake, estuary sediments, sewage sludges, air and water samples, PBDEs were detected (Hites, 2004). PBDE congeners were also found in human blood, milk and adipose tissue (Hites, 2004).

The major exposure routes of PBDEs are via consumption of contaminated food and inhalation. The chronic exposure to PBDEs has adverse effects on liver, kidney and thyroid gland. PBDEs are also developmental neurotoxicants, and endocrine distrupters due to their interaction as antagonists or agonists with androgen, progesterone and estrogen receptors (Costa et al., 2008).

The physical and chemical properties of PBDEs should be well-known to assess their behavior in the environment. There have been several research to identify their physicochemical properties, which are summarized in Table 2-2 and Table 2-3 for the PBDE congeners that are relevant for the debromination studies within the scope of the present study. As can be seen from the tables, aqueous solubility of PBDEs is very low, and octanol-water partitioning coefficients (log Kow) are relatively high, which make them accumulate in the organic matrices in the environment and bioaccumulate in the lipids of organisms. According to organic carbon-water partitioning coefficients (log Koc) estimated using softwares (e.g. EPI Suite), PBDEs can be deemed as very stable in organic carbon and tend to accumulate in solid matrices such as soil. These properties, therefore, allow them to partition in aquatic sediments, when they enter into an aquatic environment. When the properties of individual congeners are examined, it can be observed that the lower bromine atoms on diphenyl ether, the higher the water solubility, vapor pressure and Henry's Law constant, and the lower the log Kow and log Koc. This trend demonstrates that lower brominated congeners have a higher tendency to move in subsurface environment compared to higher brominated ones.

Table 2-2. Physicochemical properties of PBDE commercial mixtures.

	DecaBDE	OctaBDE	PentaBDE
Molecular weight (g/mole)	959.22	801.47	564.69
Density (g/mL @ 25 °C)	3; 3.25 [a]	2.76 [a] 2.8 [b]	2.28 [a] 2.25-2.28 [b]
Melting point (°C)	290 to 306 [b]	85 to 89 [b]	-7 to -3 [b]
Boiling point (°C)	>320 [a]	>330 [b]	>300 [a]
Aqueous solubility (mg/L @ 25 °C)	<0.0001 [c]	<0.001 [b] 0.00198 (hepta-BDE component) [b]	0.0133 [b] 0.0024 (penta-BDE component) [b] 0.0109 (tetra-BDE component) [b]
Log K_{ow}	6.27 [b] 6.265 [b]	6.29 [b]	6.64 – 6.97 [b] 6.57 [b]
Henry's Law constant (atm-m^3/mol @ 25 °C)	$1.62*10^{-6}$ – $4.40*10^{-8}$ [b]	$7.5*10^{-8}$ – $2.6*10^{-7}$ [b]	$3.5*10^{-6}$ – $1.2*10^{-5}$ [b]
Solid state vapor pressure P_s (Pa @ 25 °C)	$9.28*10^{-9}$ [d]	$1.26*10^{-7}$ [d]	$8.60*10^{-7}$ [d]
Log Koc	5.44 – 7.68 [e] 6.80 [f]	4.99 – 5.80 [e] 5.92 – 6.22 [f]	4.34 – 4.76 [e] 4.89 – 5.10 [f]

[a] (WHO, 1994), [b] (ATSDR, 2004), [c] Stenzel and Markley, 1997 cited in (Hardy, 2002), [d] EU 2001, 2002, & 2003 cited in (USEPA, 2010) [e] EPI Suite estimation, [f] EPIWIN estimation in (EPA, 2014).

Table 2-3. Physicochemical properties of individual PBDE congeners.

PBDE congener	Water solubility (mg/L)	Henry's Law constant ($atm.m^3/mol$)	Solid state vapor pressure (Pa @25°C) [e]	Log Kow	Log Koa
BDE 17	0.026 [a]	-	-	5.74 [f]	9.30 [g]
				5.52-5.88 [a]	
BDE 28	0.07 [b]	$5.03*10^{-5}$ [c]	$6.51*10^{-4}$	5.94 [f]	9.50 [g]
		$7.80*10^{-5}$ [d]	$9.07*10^{-4}$	5.98 [e]	
BDE 47	0.001 – 0.015 [a, b]	$1.48*10^{-5}$ [c]	$5.52*10^{-5}$	6.81 [f]	10.53 [g]
		$8.39*10^{-6}$ [d]	$7.42*10^{-5}$	6.01-6.77 [a]	
BDE 77	0.006 [b]	$1.18*10^{-5}$ [c]	$1.40*10^{-5}$	6.73 [e]	10.87 [g]
			$3.21*10^{-5}$		10.70 [e]
BDE 99	$9*10^{-7}$ – $2.4*10^{-3}$ [a]	$2.27*10^{-6}$ [c]	$3.85*10^{-6}$	7.32 [f]	11.31 [g]
	0.009 [b]	$5.92*10^{-6}$ [d]	$1.49*10^{-5}$	6.53-7.66 [a]	11.28 [e]
BDE 100	0.040 [b]	$6.81*10^{-7}$ [c]	$5.50*10^{-6}$	7.24 [e]	11.13 [g]
		$2.37*10^{-6}$ [d]	$7.07*10^{-6}$	6.86 [e]	
BDE 153	0.001 [b]	$6.61*10^{-7}$ [c]	$1.63*10^{-7}$	7.90 [f]	11.82 [g]
		$4.34*10^{-6}$ [d]	$5.80*10^{-6}$	7.62 [e]	12.15 [e]
BDE 154	0.001 [b]	$2.37*10^{-6}$ [c]	$2.64*10^{-7}$	7.82 [f]	11.92 [g]
		$7.90*10^{-7}$ [d]		7.39 [e]	
BDE 183	0.002 [b]	$7.3*10^{-8}$ [c]	-	8.27 [f]	11.96 [g]
BDE 209	$1.3*10^{-8}$ [a]	$3.95*10^{-7}$ [d]	$9.28^{*}10^{-9}$	9.97 [i]	13.21 [h]
				11.15 [a]	

[a] Palm et al. 2002, [b] Tittlemier et al. 2002 cited in ATSDR 2004, [c] ATSDR, 2004, [d] Çetin et al., 2005, [e] Wong et al. 2001, Tittlemier et al. 2002, Wania et al. 2002 cited in USEPA 2010, [f] Braerkvelt et al. 2003 cited in ATSDR, 2004, [g] (Harner and Shoeib, 2002), [h] Webster et al., 2006 cited in USEPA 2010, [i] (Stockholm Convention, 2013).

2.2.2. Hexabromocyclododecane (HBCDD)

Hexabromocyclododecane (HBCDD) is a cycloaliphatic compound, which consists of 12 carbon, 18 hydrogen and six bromine atoms. Bromine atoms are bonded to carbon atoms at 1, 2, 5, 6, 9 and 10 positions. The general molecular structure of HBCDD, whose molecular weight is 641.70 g/mole, is given in Figure 2-3.

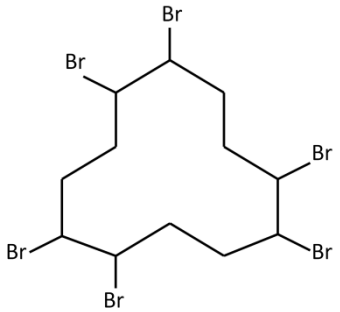


Figure 2-3 The molecular structure of Hexabromocyclododecane.

HBCDD is an additive brominated flame retardant (BFR), which is widely used in extruded, expanded and high impact polystyrene foams for thermal insulation in buildings, and secondarily used in upholstery furniture, automobile interior textiles, car cushions and electrical equipments (Covaci et al., 2006; Marvin et al., 2011). Being produced since 1960s, HBCDD became the mostly used cycloaliphatic BFR (Marvin et al., 2011). The consumption rate of HBCDD was 12500 tons annually in 2013 in Europe (VECAP, 2014), where it is the second highest used BFR (Covaci et al., 2006).

In order to understand the chemical structure of HBCDD, chemistry of isomers is recalled here. Isomers can be divided into two: having different bond pattern (structural isomers) and same bond pattern (stereoisomer). Hence, stereoisomers are defined as molecules having the same order of attachment of the atoms, but different arrangements in space. They have different chemical properties. In this structure, the carbon atom with four different groups attached to it is called stereogenic center. A molecule with a stereogenic center can exist in two stereoisomeric forms, which are

called a pair of enantiomers. Enantiomers are nonsuperimposable mirror images of each other.

Enantiomers differ in the arrangement of the groups attached to the stereogenic center, hence they have opposite configurations. R-S system is used to refer to the specific enantiomer. R-S configuration of enantiomer is identified with the priority order of the four groups attached to the stereogenic center. Priority of the groups is determined according to atomic numbers. The stereogenic center is observed from the side opposite the lowest priority group. If the remaining three groups form a clockwise array, the configuration is R, otherwise it is S. The enantiomers are also distinguished with their optical rotation, if the substance is optically active. A polarimeter is used to analyze optical activity by measuring plane-polarized light. If the analyzer prism is rotated to right, the substance is dextrorotary (+), otherwise, the substance is levorotary (-).

Enantiomers have identical achiral properties, such as, boiling point and density, but have different chiral properties, e.g. optical rotation direction. They generally have different biological activity and biodegradation patterns.

Stereomers that are not mirror images of each other are called diastereomers. They differ in both chiral and achiral properties. An achiral diastereomer of a compound with stereogenic centers is called meso compound. Due to being achiral, meso compounds are optically inactive. Reaction of a chiral reagent with an achiral reagent forms diastereomeric products at different rates and in unequal amounts, when the reaction creates a new stereogenic center.

The manufacturing of technical grade HBCDD mixtures are via bromination of cyclododeca-1,5,9-triene (CDT) isomer, which leads to the formation of six stereogenic centers at positions 1, 2, 5, 6, 9 and 10. By this way, theoretically, 16 stereoisomers (6 pairs of enantiomers and 4 meso forms) are obtained (Heeb et al., 2005). CDT itself also has different isomers depending on the *cis*- and *trans*-positioning. Therefore, while producing HBCDD mixtures, the isomeric composition

of CDT mixture, together with the reaction conditions, will determine the composition of technical grade HBCDD (Heeb et al., 2005). There are five HBCDD diastereomers: alpha (α-), beta (β-), gamma (γ-), delta (δ-) and epsilon (ε-). Mainly, the commercial mixtures contain 75 – 89 % γ-HBCDD, 10 – 13 % α-HBCDD, 1 – 12 % β-HBCDD and minor amounts of δ- and ε-HBCDD (Covaci et al., 2006). The optical rotation measurements also demonstrated the presence of three pairs of enantiomers, (+) and (-) α-, β-, and γ-HBCDDs, while revealing no optical rotations for δ- and ε-HBCDD (Heeb et al., 2005). The schematic representations of all diastereomers are given in Figure 2-4.

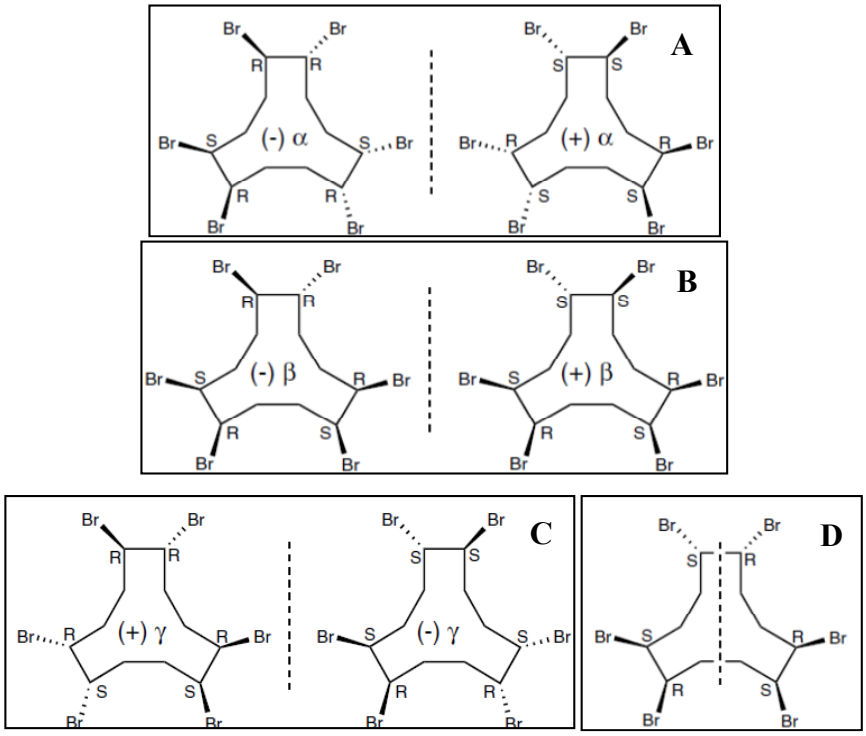


Figure 2-4. Schematic representation of diastereomers **A.** (-) & (+) α-HBCDD **B.** (-) & (+) β-HBCDD **C.** (-) & (+) γ-HBCDD **D.** An example representation for δ- and ε-HBCDD (Heeb et al., 2007).

The first observation of HBCDD in the environment was with fish and sediment samples in River Viskan, Sweden (Sellström et al., 1998). It was revealed that the

river was receiving the discharges from from textile industries and that one industry was known to replace decabrominated diphenyl ether with HBCDD in early 90s. The authors also speculated about the high sediment concentrations showing the recent releases of HBCDD in the river at that time (Sellström et al., 1998). HBCDD were later found in soils, sediment and sewage sludge, which were mostly near the regions of high industrialization and urbanization (Covaci et al., 2006). Moreover, HBCDDs could be found in remote regions from the emission sources due to the long-range transport of the chemical with particulates (Covaci et al., 2006).

Toxicity tests of HBCDD on rats have showed disruption in thyroid hormone system, developmental neurotoxic effects and alteration in uptake of neurotransmitters in brain (Covaci et al., 2006). Exposure of humans may be via ingestion of contaminated food, inhalation of vapor and particles and dermal pathways. HBCDD was found in human adipose tissue and blood (Marvin et al., 2011).

The physical and chemical properties of HBCDD diastereomers have been studied by researchers and summarized by Marvin and colleagues (Marvin et al., 2011) and in European Commission Risk Assessment Report (European Commission, 2008). The properties are presented in Table 2-4. HBCDD is found as white odorless solid, having a density of 2.24 g/cm^3 (European Commission, 2008). Some of the values given in the table are estimates of models; such as COSMOtherm or EPI Suite. While deriving the values for both estimates and measurements, authors' comments on reliability of the data were taken into consideration.

As can be seen from Table 2-4, γ-HBCDD has one order of magnitude lower water solubility than that of other stereoisomers. This may be one of the reasons that γ-HBCDD is predominant in soil, sediments, and sludge, while α-HBCDD is the abundant isomer in biota (Covaci et al., 2006). α-HBCDD also has one order of magnitude higher vapor pressure compared to others according to the model estimates. Lastly, there appears no notable difference in octanol-water partition coefficients between the isomers.

Table 2-4. Physico-chemical properties of HBCDD diastereomers (European Commission, 2008; Marvin et al., 2011).

	technical-HBCDD	α-HBCDD	β-HBCDD	γ-HBCDD
Melting Point (°C) [a]	172-184 to 201-205	179 – 181	170 – 172	207 – 209
Boiling point (°C) [b]	Decomposes at >190°C			
Water Solubility at 20°C (mg/L)	0.0656 [c]	0.0488 [c]	0.0147 [c]	0.0021 [c] 0.0034 [d] (@25°C)
Solid state vapor pressure (Pa)	$6.3 * 10^{-5}$ @ 21°C [e] $7.23 * 10^{-7}$ [f]	$2.72 * 10^{-10}$ [g]	$3.58 * 10^{-11}$ [g]	$1.86 * 10^{-11}$ [g]
Liquid state vapor pressure (Pa)	-	$2.93*10^{-4}$ [g]	$3.86*10^{-5}$ [g]	$2.00*10^{-5}$ [g]
Henry's Law Constant ($atm.m^3/mol$)	$7.4*10^{-6}$ [h] $1.72*10^{-6}$ [i]			
Log Kow	5.62 [j]	5.07 [j]	5.12 [j]	5.47 [j]
	6.73 [k]	5.59 [g]	5.44 [g]	5.53 [g]
Log Koa	10.46 [l]	9.96 [l]	10.47 [l]	10.40 [l]

[a] Smith et al. 2005 cited in EC 2008, [b] Peled et al. 1995 cited in EC 2008, [c] MacGregor and Nixon 2004 cited in EC 2008, [d] Stenzel and Markley 1997 cited in EC 2008, [e] Stenzel and Nixon 1997 cited in EC 2008, [f] Wania et al 2002 cited in Marvin et al., 2011, [g] COSMOtherm estimates given in Marvin et al., 2011, [h] EC 2008, [i] EPI Suite estimate (bond method) given in Marvin et al. 2011, [j] MacGregor and Nixon 1997, Hayward et al. 2006 cited in EC 2008, [k] Average of model estimates given in Marvin et al. 2011., [l] thermodynamically consistent estimates given in Marvin et al. 2011.

2.2.3. Levels of PBDEs and HBCDD in the environment

There are a number of studies revealing environmental levels of PBDEs and HBCDD. Some of the recently published studies were summarized in Table 2-5. The table does not present a complete literature review of environmental levels of PBDEs and HBCDD, rather it introduces an overall understanding of their occurrence.

Concentrations mainly display levels in urban and industrial sites. As can be observed from Table 2-5, PBDE levels ranged from not detect to 10^5 ng/g of dry weight, while total HBCDD levels were in the range of not detect to 10^3 ng/g of dry weight in different parts of the world. The highest observed PBDE concentration was taken from an e-waste recycling site, especially at the area where shredding operation was conducted (Labunska et al., 2013). On the other hand, highest HBCDD level was from a region near manufacturing facilities (Li et al., 2012).

Table 2-5. Recently published environmental monitoring studies for PBDEs and HBCDD.

Environmental matrix	Contaminant	Concentration (ng/g dw)[a]	Location	Ref.
Soil	$\sum_7$PBDE	0.70 – 203	Turkey	(Cetin, 2014)
Sediment	$\sum_{19}$PBDE	0.18 – 13.95	Korea	(Lee et al., 2014a)
Sediment	$\sum_7$PBDE	ND – 44.3	Spain	(Barón et al., 2014)
Sediment	$\sum_{14}$PBDE	236.7 – 1373.4	China	(W. He et al., 2013)
Suspended particulate matter	$\sum_{14}$PBDE	20.9 – 1220.0		
Sediment	BDE-209	0.62 – 477	Czech Republic	(Hloušková et al., 2014)
	∑HBCDD	1.101 – 44.25		
Sediment	$\sum_{14}$PBDE	0.40 – 110	Korea	(Ramu et al., 2010)
	∑HBCDD	0.39 – 59		
Sediment	∑HBCDD	4290	USA (at WWTP outfall)	(La Guardia et al., 2012)
	$\sum_{20}$PBDE	3622		
Sediment	BDE-209	96 – 120000	China	(Labunska et al., 2013)
Sediment	∑HBCDD	0.1 to 2	USA	(Klosterhaus et al., 2012)
Sediment	∑HBCDD	0.5 – 53.1	China	(M.-J. He et al., 2013)
Sediment	∑HBCDD	ND – 2660	Spain	(Guerra et al., 2008)
Soil	∑HBCDD	0.88 – 6901	China	(Li et al., 2012)
Sediment	∑HBCDD	2.93 – 1029		
Soil	∑HBCDD	0.17 – 34.5	China	(Wang et al., 2013)

[a] ND: not detected during analysis.

2.3.Degradation of Brominated Flame Retardants

The degradation of PBDEs and HBCDD in the environment occurs via biotic and abiotic degradation mechanisms. The transformation mechanisms result in the formation of lower brominated congeners/isomers or hydroxylated derivatives depending on the mechanism. Since PBDEs and HBCDD are ubiquitously found in organic media, i.e. solid matrices in the environment, and the present study focuses degradation in sediments, the literature review on degradation mechanisms was directed towards degradation in solid environmental matrices, i.e. soil, sludge and sediments.

2.3.1. Biotic Degradation

PBDEs. Biodegradation of PBDEs is achieved under two conditions: aerobic and anaerobic. Aerobic transformation of PBDEs was initially examined via bacterial cultures to produce lower brominated and hydroxylated derivatives of mono- to hexa-BDEs (Robrock et al., 2009). Following studies showed various microbial consortia could achieve aerobic degradation of PBDEs with differing bromine content under various substrate conditions (Deng et al., 2011; Shi et al., 2013; Zhang et al., 2013). The bacterial species found to be capable of PBDE degradation aerobically are given in Table 2-6. Additional studies have been conducted to demonstrate aerobic biodegradation in environmental media. Degradation of a mono-BDE in aerobic sludge in the presence of carbon sources revealed that the main mechanism under aerobic conditions is debromination (Chen et al., 2010). A total of 62 – 78% reduction in PBDE levels was achieved in aerobic sludge incubations for 11 months, and authors suggested the formation of both lower brominated congeners and hydroxylated derivatives at the end of incubation (Stiborova et al., 2015a). Nyholm and colleagues (2010) studied degradation in aerobic soil amended with sludge containing deca-BDE and BDE 28, and found long half-life for BDE 28 (210 – 260 days) while deca-BDE showed no degradation at all (Nyholm et al., 2010). The degradation of BDE 15 was also observed in aerobic sediment microcosms and bioreactors, with a higher rate of degradation, compared to previous studies, occurring in weeks (Huang et al., 2012).

Table 2-6. The bacterial species having the ability to degrade PBDEs aerobically.

Name of bacterial specie	**Reference**
Rhodococcus jostii RHA1 *Burkholderia xenovarans* LB400 *Rhodococcus sp.* RR1 *Pseudonocardia dioxanivorans* CB1190	(Robrock et al., 2009)
Clostridiales anaerobe	(Chen et al., 2010)
Pseudomonas aeruginosa	(Shi et al., 2013)
Pseudomonas sp. and *Bacillus sp.*	(Huang et al., 2012)
Pseudomonas stutzeri	(Zhang et al., 2013)
Lysinibacillus fusiformis strain DB-1	(Deng et al., 2011)

Anaerobic degradation of PBDEs was first shown in a fixed film plug flow reactor, which was believed to represent the microbial activity occurring in sediments, via the debromination of BDE 15 to BDE 3 in eight weeks (Rayne et al., 2003). Succeeding studies aimed at identifying the mechanisms, pathways, products and rate of reductive debromination, and means to enhance this mechanism in the environment. These studies were summarized in Table 2-7. As can be seen from the table, general approach was to supply the contaminated media with additional carbon sources, electron donors, vitamin and mineral solutions. Some of the studies investigated the efficiency of using various supplements in degradation (Huang et al., 2014; Qiu et al., 2012). For instance, BDE 209 debromination did not improve at the end of 90 days of incubation with the addition of electron donors (methanol, ethanol, acetate, lactate, and pyruvate), yet highest rate was observed in first 30 days for methanol and ethanol (Qiu et al., 2012).

Table 2-7. PBDE anaerobic degradation studies in solid media.

Medium	Initial PBDE concentration	Conditions	Amendments	% reduction	Incubation time (days)	Reference
Digested sludge	Deca-BDE: 537.2 ng/mL	37°C	Primers (2,6-dibromobiphenyl, 4-bromobenzoic acid, and decabromobiphenyl, 9-11 nmol each) and nutrients (starch-20 mg and yeast-50 mg)	30%	238	(Gerecke et al., 2006, 2005)
Two different WWTP sludges	BDE-3: 5 mg/L	27°C rotary shaker 120 rpm	Mineral medium only Medium with glucose as carbon source (5 g/L)	31/77% 74/79%	16	(Shih et al., 2012a)
Municipal digested sludge	BDE-47: 455 ng/g dw BDE-99: 500 ng/g dw BDE-209: 200 ng/g dw	37°C	Basal medium	36% 35% 39.8%	238	(Shin et al., 2010)
Industrial sewage sludges	$\sum_{11}$PBDEs: 1291 ng/g $\sum_{11}$PBDEs: 1608 ng/g	28°C	Medium with starch (20 mg) and yeast (50 mg)	47.4% 68.7%	458	(Stiborova et al., 2015b)
e-wastes contaminated soil	BDE-209: 138 ng/g dw BDE-47: 55 ng/g dw	25°C	buffer solution using lactate (10 mM) as an electron donor	39.7% 29.5%	90	(Song et al., 2015)
Soil mixed with BDE-spiked activated sludge	BDE-28, BDE-209: 40 – 70 ng/g dw	20°C	Distilled water	3.2% 24.7%	160	(Nyholm et al., 2010)
Sediment microcosms	BDE-47: 5000 ng/g BDE-99: 5000 ng/g BDE-209: 5000 ng/g	22°C	phosphate buffer, methanol (50 µL) and dextrose (25 mg)	30% 3% 3.8%	240 240 300	(Tokarz III et al., 2008)

Table 2-7. Cont'd.

Medium	Initial PBDE concentration	Conditions	Amendments	% reduction	Incubation time (days)	Reference
Soil and sediment samples	octa-BDE: 1500 ng/g in TCE 3000 n/g in nonane	30°C	Vitamin-mineral salts medium and pyruvate/lactate/acetate (10 mM each)	Data not shown	60	(Lee and He, 2010)
Sediment microcosms	BDE-209: 480 µg/g ww	30°C	Vitamin-mineral salts medium and yeast (0.2 g/L) with electron donors (methanol/ethanol/acetate/ pyruvate/lactate-10mM)	5 – 12% with varying e-donors	90	(Qiu et al., 2012)
Sediment slurry	BDE-209: 50 µg/g	30°C	Various surfactants/ Vitamin B12(0.025 mg/L)/ ZVI(1 g/L)/ acetate(30 mM)/lactate(20 mM)/ pyruvate(20 mM)/ bicarbonate(30 mM)/ sulfate(20 mM)/nitrate(20 mM)/ inhibitors	98.1 – 99.9%	180	(Huang et al., 2014)
Mangrove/ fresh water/ marine sediment microcosms	BDE-47: 1.03 mg/kg dw BDE-153: 1.08 mg/kg dw BDE-209: 0.92 mg/kg dw	28°C horizontal shaker 150 rpm	Mineral salts medium	62 – 82.4% 31.4 – 98.8% ~0%	90	(Zhu et al., 2014)
Sediment microcosms	BDE-209: 4 µg/g dw	Within an oligotrophic lake (epilimnion & hypolimnion) Dark/light	Distilled water	3.5% & 2.3% ~0%	30 12	(Orihel et al., 2016)
Lake sediment mesocosms	BDE-209: 2523 ng/g (DE83-R)	Within the oligotrophic lake	-	~0%	2 years	(Orihel et al., 2016)

Apart from the studies that incubated the microcosms under laboratory controlled conditions, Orihel and colleagues (2016) preferred to place the microcosms within an oligotrophic lake in Canada. By this way, they aimed to simulate environmental conditions, and monitor degradation in microcosms. At the end of 30 days, although sediments showed slight debromination of BDE-209, researchers concluded to observe debromination products up to di-BDEs (Orihel et al., 2016). They also tested the effect of oxic conditions in sediments, and found that dissolved oxygen concentration had a positive effect on degradation in 12 days. Their consecutive test was the application of a mesocosm within the lake. After 2 years of monitoring, deca-BDE showed little change in sediments, in spite of the observation of product congeners (Orihel et al., 2016).

The debromination PBDE congeners were also studied with various dehalogenating cultures in the absence of a solid phase (He et al., 2006; Robrock et al., 2008; Yen et al., 2009). The bacterial species found to be able to debrominate PBDEs anaerobically are given in Table 2-8.

Table 2-8. The bacterial species having the ability to degrade PBDEs anaerobically.

Name of bacterial specie	**Reference**
Sulfurospirillum multivorans (deca-BDE)	(He et al., 2006)
Dehalococcoides sp.	(He et al., 2006; Lee and He, 2010; Robrock et al., 2008)
Dehalobacter restrictus PER-K23	(Robrock et al., 2008)
Desulfitobacterium hafniense PCP-1	
Pseudomonas sp. SCSWA09	(Qiu et al., 2012)
Clostridium sp.	(Shih et al., 2012a)

HBCDD. There is a limited number of researches focusing on HBCDD as compared to PBDEs. All available studies concerning transformation of HBCDD were examined and mentioned below.

Biodegradation of HBCDD in aerobic and anaerobic soils and sediments, and in activated sludge and digested sludge were investigated in microcosms (Davis et al., 2006, 2005). Anaerobic degradation of HBCDD in sewage sludge was also studied in the presence of primers (2,6-dibromobiphenyl, 4-bromobenzoic acid, and decabromobiphenyl) and nutrients (starch and yeast) (Gerecke et al., 2006). A recent study investigated the aerobic and anaerobic degradation of HBCDD in rhizosphere and non-rhizosphere soil in the presence and absence of additional humic acid and glucose (Le et al., 2017). All of these studies observed the loss of HBCDD in time with the half-lives given in Table 2-9. Davis and colleagues (2006) revealed that the biodegradation rates of HBCDD isomers differed in sludge and sediments. In digester sludge, rates were on the order of β- > α- > γ-HBCDD and that of aquatic sediments were β - > γ- > α -HBCDD (Davis et al., 2006). Different from the study of Davis et al. (2006), Gerecke and colleagues (2006) found that the degradation rates for HBCDD isomers in anaerobic sludge were on the order of γ- ≈ β- > α-HBCDD. Peng et al. (2015) also demonstrated biodegradation of HBCDD isomers in mixed liquor with pure cultures isolated from their previous anaerobic reactors for degradation of tetrabromobisphenol A (Peng et al., 2012), and found degradation rates with an order of α- > β- > γ-HBCDD (Peng et al., 2015). In soils, on the other hand, relative percentage of α-HBCDD was found to be increased with anaerobic degradation (Le et al., 2017). Hence, these studies revealed that biodegradation rate of isomers change according to the environmental media and conditions, as well as capabilities of microbial consortia present in the media.

Table 2-9 Half-lives of HBCDD in different media under aerobic/anaerobic conditions.

Media/Condition	**Half-life**	**Reference**
Soil/aerobic	63 d*	Davis et al., 2005
Sediment/aerobic	11 d*	Davis et al., 2005
Sediment/aerobic	32 d*	Davis et al., 2005
Soil/anaerobic	6.9 d*	Davis et al., 2005
Sediment/anaerobic	1.5 d*	Davis et al., 2005
Sediment/anaerobic	1.1 d*	Davis et al., 2005
Sludge/anaerobic	15 d	Davis et al., 2006
Sludge/anaerobic	0.66 d*	Gerecke et al., 2006
Mixed liquor/ anaerobic	5.4 d (α), 8.2 d (β), 8.8 d (γ)	Peng et al., 2015

* Pseudo first order reaction

Although these studies showed the loss of HBCDD in different environmental media and conditions, only Davis and colleagues (2006) and Peng and colleagues (2015) demonstrated the degradation products. After operating the sludge reactors for 28 days and sediment microcosms for 113 days (Davis et al., 2006), and anaerobic reactors of mixed liquor with inoculums for 8 days (Peng et al., 2015); three products of HBCDD degradation were observed: tetrabromocyclododecene, 1,2-dibromocyclododecadiene, and 1,5,9-cyclododecatriene. These were formed via the removal of two moles of bromine in each reaction, i.e., via dihaloelimination, which is said to be favored in environments having scarce H_2 concentrations (Davis et al., 2006). A novel product was also observed in mixed liquor reactor. 2-dodecene was formed by cycloaliphatic ring cleavage, and it was the last product identified in LC-MS/MS analysis (Peng et al., 2015).

Different than the previous studies that involved the spike of HBCDD onto media under laboratory conditions, Stiborova and colleagues (2015) used an HBCDD-

contaminated sewage sludge for degradation studies. Two industrial sludge samples, whose contamination levels were known, were collected, and incubated in anaerobic reactors with nutrients necessary for indigenous microorganisms growth (Stiborova et al., 2015b). At the end of three months, HBCDD in sludge reactors were below detection limit (Stiborova et al., 2015b). Since there were no sampling time between day zero and 90 days, kinetics of degradation cannot be studied.

Identification of bacterial species responsible for HBCDD biodegradation was also investigated. Among 13 bacterial strains tested for aerobic γ-HBCDD degradation, *Pseudomonas* sp., isolated from a contaminated soil, was found to degrade 70% of γ-HBCDD in 5 days (Yamada et al., 2009). During aerobic degradation of HBCDD in soil, population of gram positive bacteria (*Brassia rhizosphere* and *Sphingomonas* sp.) have reported to be increased (Le et al., 2017). Another bacteria specie isolated from an anaerobic sludge reactor was *Achromobacter* sp. (Peng et al., 2015).

2.3.2. Abiotic degradation

PBDE. Abiotic degradation mechanisms acting on PBDEs are photochemical and catalytic debromination. Photochemical debromination of PBDEs was studied under natural sunlight and in photoreactors in soils, sediments and alike solid phases (Ahn et al., 2006; Hua et al., 2003; Söderstrom et al., 2004). All of these studies focused on debromination of deca-BDE. Hua and colleagues investigated photodegradation in humic acid coated sand particles under sunlight, and observed 10.9% reduction of BDE-209 in 96 hours (Hua et al., 2003). The study that examined degradation of BDE-209 adsorbed onto sand, soil and sediment under artificial UV light and natural sunlight found longer half-lives for natural sunlight conditions (12 h vs 37 h for sand; 40-60 h vs 80 h for sediment) (Söderstrom et al., 2004). This finding also supported by another study, but with much longer half-lives, such as 150 d under artificial UV light vs 990 d under natural sunlight for sediment (Ahn et al., 2006). Hence, it can be concluded that the photochemical degradation rate was highly dependent on the irradiation conditions, as well as solid phase properties such as composition and porosity of the media (Pan et al., 2016). For example, the higher the organic content

of media, the lower photolysis rate was observed (Hua et al., 2003; Söderstrom et al., 2004). The main mechanism for photolysis was found to be the formation of lower brominated congeners via stepwise reductive debromination, while some studies observed the formation of polybrominated dibenzofurans (PBDFs) via intramolecular elimination of HBr (Pan et al., 2016; Söderstrom et al., 2004). Even though PBDFs were photochemicaly unstable, their occurrence should be considered important due to PBDFs' toxicity to human and wildlife (van den Berg et al., 2013).

The catalytic debromination of PBDEs with zerovalent iron (ZVI) was shown mostly in aqueous systems (Keum and Li, 2005; Zhuang et al., 2012), while there were few studies (Huang et al., 2014; Xie et al., 2016, 2014) to investigate the effectiveness of ZVI in soil and sediment remediation of PBDEs. Beyond their biodegradation studies, Huang and colleagues (2014) evaluated the use of ZVI in sediments, and observed the highest BDE-209 debromination with ZVI added sediment (half life of 16.9 d). Consecutive studies examined the modifications on the use of ZVI and factors affecting their performance. The need for modifications on ZVI resulted from their tendency to aggregate due to van der Waals forces which reduces their reactivity (Xie et al., 2014). Therefore, to prevent aggregation and to provide mobility of ZVI in subsurface systems, either formation of bimetallic species or the use of a carrier for its distribution was adopted. The use of Ni/Fe bimetallic nanoparticles in contaminated soil was found to decrease BDE-209 by 72% in 70 h under optimized pH and Ni/Fe dosage (Xie et al., 2014). The same research group tested the immobilization of nano-ZVI in mesoporous silica microspheres to be used in contaminated soil, and showed a reduction of 78% in BDE-209 in 120 h (Xie et al., 2016). Another study used biochar as a support for Ni/Fe bimetallic nanoparticles to remove BDE-209 in soil, and observed enhanced removal percentage compared to only biochar and only Ni/Fe systems (87.7% reduction in 72 h) (Wu et al., 2016). The mechanism for catalytic degradation in soil by ZVI was found to be sequential debromination up to mono-BDE (Wu et al., 2016; Xie et al., 2014).

HBCDD. Possible abiotic transformation mechanisms for HBCDD were proposed to be photodegradation, elimination catalyzed by Lewis base, and nucleophilic

substitution, all of which were deemed to be not considerable in the environment compared to reducing subsurface environment (European Commission, 2008). Abiotic degradation of HBCDD in soil and/or sediment systems was investigated with very few studies; hence previous research conducted in all kind of media was summarized here. Abiotic degradation mechanisms included thermal degradation, photolysis, debromination via reduced sulfur species and zero valent iron. Thermal degradation of HBCDD was studied by Barontini and colleagues (2001a; 2001b) in a reactor within a furnace at 500 °C followed by condensers and absorbers. The main mechanism for thermal degradation was reported to be the dehydrobromination reactions until formation of three unsaturated bonds in the ring. The following steps of degradation were proposed to form several non-, mono-, di-, tri-, tetra-, and penta-brominated compounds (Barontini et al., 2001a, 2001b).

Photodegradation of HBCDD was first studied in an aqueous solution by UV-C irradiation, and yielded 29% - 35.6% reduction in 4 h of irradiation under acidic or alkali pH, together with the identification of hydroxylated products (Zhou et al., 2012). Another study investigated the photodegradation by Fe(III) complexes of oxalate and citrate under artificial light (Zhou et al., 2014). At corresponding optimum pH values, both iron complexes demonstrated increased degradation with increased carboxylate concentrations, while addition of H_2O_2 enhanced degradation of HBCDD further for citrate complexes (Zhou et al., 2014). Yu and colleagues (2015) showed photodegradation of HBCDD with UV-C lamps active on acetonitrile-water mixture by direct and indirect photolysis, and as a result dihalodebromination products were formed (Yu et al., 2015a). As mentioned in previous parts, technical HBCDD mixture includes predominantly γ- isomer. However, HBCDD-treated products may involve α-HBCDD in greater proportions, close to γ- (Heeb et al., 2010; Kajiwara et al., 2013). It has been observed that γ-HBCDD can be transformed to form α-HBCDD at temperatures above 140 °C during manufacturing of HBCDD-treated products (Heeb et al., 2010). However, any composition change, i.e. isomeric conversion, was not reported in HBCDD-treated products via photodegradation (Kajiwara et al., 2013).

Ultrasound assisted Fenton reaction was tested in hydrogen peroxide solution, and it was revealed that α-HBCDD had the highest rate of degradation, followed by β- and γ- isomers (Ye et al., 2014). Debromination of HBCDD was also demonstrated with nZVI particles in aqueous solution, yielding less brominated derivatives formation (Tso and Shih, 2014). The researchers also revealed the increase in removal rate with increased temperature and ZVI dosage, and decrease in rate with presence of anions that are possibly found in subsurface systems (Tso and Shih, 2014).

There were also studies investigating the reactions between HBCDD and the reactive species that can be present in sediment/subsurface systems. Lo and colleagues (2012) tested the reactivity of HBCDD with reduced sulfur species, i.e. polysulfide and bisulfide, under anoxic conditions. They found that γ-HBCDD had greater reaction rate constant than the other isomers for both sulfur species, but they also concluded that when the compounds are sorbed onto sediment particles, they would be less reactive with dissolved reduced sulfur species (Lo et al., 2012). Another study to examine the reactivity of iron monosulfide (FeS), which is abundant specie in anoxic sediments, showed that HBCDD had a half-life of about 8 h, and that over 90% reduction could be achieved in 24 h (D. Li et al., 2016). In this study also, γ- and β-isomers exhibited higher transformation rates over α-HBCDD, and the formation of dibromoelimination products were presented (D. Li et al., 2016).

The only transformation mechanism studied on soil so far was the mechanochemical degradation of HBCDD (K. Zhang et al., 2014). Iron-quartz, calcium oxide, and quartz sand were used in two different soil samples as the co-milling agents individually. The results revealed that the efficiency of agents differed with the composition of soil, and that usage of co-milling agents achieved 99% HBCDD removal, while zero usage of co-milling agents also yielded up to 75% reduction in 2 h (K. Zhang et al., 2014).

Catalyzed Hydrogen Peroxide Propagations (CHP)

The abiotic degradation mechanism investigated throughout the present study is the catalyzed hydrogen peroxide propagations process. For effective remediation of soils

and groundwater, in situ chemical oxidation processes have been investigated and applied for several years. One of these processes is the use of Fenton's reagent in which hydroxyl radical generated decomposes the recalcitrant contaminants (Watts and Teel, 2005). Going back to 1894, Fenton's reaction using ferrous ion was successful to oxidize tartaric acid by hydrogen peroxide (Fenton, 1894). From then on, this solution of ferrous ion and hydrogen peroxide, known as "Fenton's reagent", was effectively used to oxidize variety of organic compounds (Walling, 1975). Being a weak acid (pK_a=11.65), H_2O_2 can itself oxidize organic compounds, whereas this oxidation process may not be feasible for high contaminant concentrations (Venny et al., 2012b). Hence, a catalyst, such as Fe^{2+}, is needed to produce reactive radicals via decomposition of H_2O_2 so that contaminants can be oxidized. Fenton reaction mechanism is as follows:

$$H_2O_2 + Fe^{2+} \xrightarrow{k} OH\bullet + \mathrm{OH}^- + Fe^{3+} \qquad k = 76 M^{-1}s^{-1} \tag{1}$$

The hydroxyl radical produced with reaction (1) is an electrophile, and can commonly participate into (i) electrophilic substitution reactions in aromatic compounds, (ii) hydrogen abstraction reactions from saturated compounds (Watts and Teel, 2005), and (iii) addition to double bonds. Also, additional radicals are formed by subsequent reactions with hydrogen peroxide, and proceed in Fenton mechanism as given in the following reactions (W. Barb et al., 1951):

$$Fe^{2+} + OH\bullet \rightarrow \mathrm{OH}^- + Fe^{3+} \tag{2}$$

$$OH\bullet + H_2O_2 \xrightarrow{k} H_2O + OOH\bullet \qquad k = 2.7 \times 10^7 M^{-1}s^{-1} \tag{3}$$

$$OOH\bullet + Fe^{2+} \rightarrow {}^{-}OOH + Fe^{3+} \qquad k = 1.2 \times 10^6 M^{-1}s^{-1} \tag{4}$$

$$OOH\bullet + Fe^{3+} \rightarrow Fe^{2+} + H^+ + O_2 \tag{5}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\bullet + \mathrm{H}^+ \tag{6}$$

Application of Fenton oxidation to halogenated and/or aromatic compounds in subsurface systems has been studied by many researchers, and reviewed by Venny and colleagues (2012) and Cheng et al. (2016). For instance, polycyclic aromatic hydrocarbons (PAH) removal was investigated in sludge, soil and sediment matrices (Flotron et al., 2005), and PCB mixture (Aroclor 1016) degradation was monitored in

soil column experiment (Viisimaa et al., 2012) with Fenton reaction. There were also combined chemical (Fenton) and biological treatment methods for the removal of PCBs from soil/sediment systems (Aronstein and Rice, 1995).

There were only two studies that examine the applicability of Fenton's reagent for PBDE removal. The first study used Fenton oxidation as a unit in treatment scheme for landfill leachate, and achieved 72.1% removal of BDE-3 in the individual Fenton unit (Wu et al., 2011). The other study on PBDE removal in soil via hydrogen peroxide used tourmaline as a catalyst, and microorganisms in combination (J. Li et al., 2016). As a result of H_2O_2 application with tourmaline, having approximately 20% Fe_2O_3 content, to the contaminated soil, nearly 42% and more than 50% of total PBDEs were removed in 70 days with and without the addition of microorganisms, respectively (J. Li et al., 2016). The study also proposed possible pathways for BDE-47 and -153, by the attack of hydroxyl radical at *ortho* and *para* positions, attaining complete mineralization (J. Li et al., 2016).

Application of Fenton's reagent for contaminant removal in subsurface systems may become infeasible due to scavenging of hydroxyl radical with the constituents in soil, such as natural organic matter (Watts and Teel, 2005). Therefore, modifications on Fenton's reagent have attracted attention for the transformation of organic contaminants. The catalyzed hydrogen peroxide propagations (CHP) is a modified Fenton's reagent by the use of high hydrogen peroxide concentrations (2 – 20%) and various catalysts e.g. Iron (III), iron chelates, or iron minerals (Watts and Teel, 2005). While iron (II) is the most effective catalyst at dilute peroxide concentrations, iron (III) is effective at high peroxide concentrations (Watts and Teel, 2005). Thus, CHP is initiated with equation 6 (W. G. Barb et al., 1951):

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\bullet + \mathrm{H}^+ \qquad k = 2 \times 10^{-3} s^{-1} \ (\mathrm{pH} = 3) \qquad (6)$$

Iron (II) produced with this reaction is then used to initiate classical Fenton's reactions as given in equation 1. The hydroxyl radical generated in equation 1 reacts with hydrogen peroxide and the following propagation reactions occur (Watts and Teel, 2005):

$$OH\bullet + H_2O_2 \xrightarrow{k} H_2O + OOH\bullet \qquad k = 2.7 \times 10^7 M^{-1}s^{-1} \qquad (3)$$

$$OOH\bullet \leftrightarrow O_2\bullet^- + H^+ \qquad pK_a = 4.8 \qquad (7)$$

$$R\bullet + H_2O_2 \xrightarrow{k} ROH + OH\bullet \qquad k = 10^6 - 10^8 M^{-1}s^{-1} \qquad (8)$$

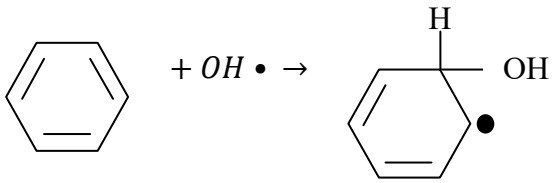
$$OOH\bullet + Fe^{2+} \rightarrow {}^-OOH + Fe^{3+} \qquad k = 1.2 \times 10^6 M^{-1}s^{-1} \qquad (4)$$

As noted earlier, the most reactive radical, OH•, can abstract hydrogen from organic compounds as given in reactions (Tripathi, 1998):

$$RH + OH\bullet \rightarrow R\bullet + H_2O \qquad (9)$$

$$R\bullet + OH\bullet \rightarrow ROH \qquad (10)$$

In equation 9, the organic compound R become reactive, and reacts with hydroxyl radical again to form alcohol functional group in equation 10. Another, but the most important, reaction mechanism of hydroxyl radical for aromatic compounds is hydroxyl addition to double bonds, as given in equation 11 (Watts and Teel, 2005). This is the mechanism expected to occur with the target contaminants of this study.



$+ OH\bullet \rightarrow$ (11)

The formation of nonhydroxyl radicals in the presence of high H_2O_2 concentrations leads to an increased range of reactivity. Perhydroxyl radical (HO_2•) is a relatively weak oxidant, superoxide radical anion (O_2•$^-$) is a weak reductant and nucleophile in aqueous systems, and hydroperoxide anion (HO_2^-) is a strong nucleophile (Watts and Teel, 2005). This mixture of radicals is believed to degrade most of the organic chemicals.

Previous studies on contaminant degradation showed CHP to be useful in soil systems. These studies are summarized in Table 2-10. Furthermore, there were numerous studies investigating the effectiveness of CHP on various organic compounds in aqueous solutions (Mitchell et al., 2014; Smith et al., 2009; Teel and Watts, 2002). CHP has never been applied for the degradation of PBDEs or HBCDD in soils and sediments.

Table 2-10. Literature studies testing catalyzed hydrogen peroxide propagations in contaminated soil systems

Contaminant	Fe^{3+} concentration	H_2O_2 concentration	pH	T(°C)	Time	% removal	Ref.
PCB (Aroclor 1242)	100 ppm	5%	2.75	15	72 h	98%	(Manzano et al., 2004)
PCB	-	4 – 30%	-	<40	8 treatments[a]	2 – 94%[b]	(Ahmad et al., 2011)
PCB (di- to hexa-CBs)	100 ppm	5%	2.75	30	72 h	96 – 99.7%	(Quiroga et al., 2009)
PAH	Fe^{3+}/soil= 0.025 wt	15%	-	28	3 h	61 – 72.5%	(Venny et al., 2012a)
Diesel	Magnetite/ Goethite = 5wt%	15%	3	20	200 h	50%	(Kong et al., 1998)

[a] 8 treatments indicated the withdrawal and refill of peroxide solution 8 times.
[b] The range was given as minimum and maximum values obtained for two different soil types.

The effectiveness of CHP reactions depends on system parameters, such as concentrations of reagents, pH and temperature. Previous studies demonstrated the necessity to determine the optimum conditions of parameters for the specific test system. For example, varying concentrations of iron and hydrogen peroxide were tested, and very low (e.g. usage of 1% H_2O_2) and very high concentrations (e.g. usage of 1500 ppm Fe^{3+}) were found to decrease the contaminant removal efficiency (Manzano et al., 2004). On the other hand, Ahmad and co-workers (2011) used no additional iron since the soil had adequate amounts of iron oxide. They also observed high demand for hydrogen peroxide (up to 50%) due to the high organic content of soil when testing ex-situ application of CHP (Ahmad et al., 2011). The effect of pH has also been reported in many studies. At low pH, iron is maintained in soluble form and this provides effective catalysis of CHP reactions (Watts and Teel, 2005). However, due to the strong buffering capacity of subsurface environment, adjustment of pH becomes crucial in order to prevent iron precipitates. Moreover, maintaining pH below 3 – 4 allows the carbonic acid dominate in subsurface system so that hydroxyl radical will not be scavenged by bicarbonate and carbonate ions (Watts and Teel, 2005).

The last parameter to be taken into consideration is temperature. Although the literature shows no consensus on the optimum temperature for CHP reactions, researchers used varying temperatures between 15 and 50 °C (Ahmad et al., 2011; Quiroga et al., 2009), and commented on the effects of temperature adjustment. High temperatures can generally increase the rate of reactions; on the other hand it can also cause hydrogen peroxide decomposition. Therefore, adjusting the temperature is not deemed as a requirement, but it should be monitored for system performance.

2.4.Remediation of Contaminated Sites

Contaminated site is defined by “Regulation on Control of Soil Contamination and Contaminated Sites due to Point Sources” (CSCCS) of Turkey as any site that has confirmed by various measurements and/or evaluations to have hazardous pollutants due to human activity and pose significant risk to human health and environment, and decided to be treated when the present and future land use are taken into

consideration (Ministry of Environment and Urbanization, 2010a). The major sources of contamination are direct discharge of wastes, uncontrolled waste runoff, spills from industrial facilities, improper storage and illicit dumping of hazardous substances. Although some measures can be implemented to avoid possible future disposal of hazardous wastes at the discharge points when identified, past contamination still remains requiring intense treatment, i.e. remediation. Remediation is defined by CSCCS of Turkey as the actions taken to control or reduce the risk on human health and environment posed by the contaminated sites (Ministry of Environment and Urbanization, 2010a). These actions involve strategies to remove, reduce or transform the contaminants in a systematic approach.

In the USA, the issue of contaminated sites started with the Superfund program (The Comprehensive Environmental Response, Compensation, and Liability Act – CERCLA) in 1980 (USEPA, 2017a). With this Act, the threats associated with the release of hazardous substances have been taken under control, and corresponding contaminated sites were identified. Additionally, USA governmental agencies constructed the Federal Remediation Technologies Roundtable (FRTR) in 1990 to work collaboratively on management of contaminated site cleanup activities (FRTR, n.d.). Therefore, it can be said that remediation efforts began almost 40 years ago in the USA. However, in Turkey, there were few attempts for remediation of contaminated sites. Past cleanup efforts included petroleum hydrocarbon spill at Devegeçidi Dam and Midyat Aquifer (Mardin) in 1996 and Batman in 2005, PCB contamination due to wild dumping in İncirlik (Adana) in 1997, and acrylonitrile spill due to earthquake in Yalova in 1999. A database of potentially contaminated sites is recently being compiled by the Ministry of Environment and Urbanization. Furthermore, with CSCCS, contaminated site management has started to be regulated in Turkey (Ministry of Environment and Urbanization, 2010a).

Remediation strategies are mainly divided into two: in situ and ex situ. Both methods include biological, chemical and physical treatment strategies, while the technologies applied may differ. Applicable in situ and ex situ remediation technologies were presented by many studies, e.g. FRTR treatment technologies screening matrix

(FRTR, n.d.), and Sustainable Remediation Forum – United Kingdom (SuRF-UK) (Cl:Aire, 2010). Remediation strategies should take into account the characteristics of the site (soil/sediment/groundwater), properties of contaminants, their distribution in the site and future use of the site (Mulligan et al., 2010). Hence, applicable remediation technologies differ depending on the contaminants of concern and site properties. Here, the remediation technologies evaluated within the scope of the present study were introduced.

Natural attenuation. Natural attenuation relies on natural physical, biological and chemical processes to achieve reduction of mass, toxicity or concentration of contaminants in a reasonable time period (Fuchsman et al., 2014). The reduction in concentration/mass is the result of assimilative capacity of the site, either soil or sediments. The assimilative capacity is dependent on contaminant-soil interactions, which include physical, biological and chemical reactions and their combinations occurring in the environment (Yong and Mulligan, 2005). These naturally occurring processes involve biotic and abiotic reactions, i.e. transformation and degradation, partitioning via sorption, volatilization and accumulation, dispersion and dilution (Yong and Mulligan, 2005).

Biostimulation. This remediation strategy is the addition of extraneous substrates, carbon sources or electron acceptors to enhance the activity of indigenous microorganisms when they show little or no degradation activity (Reible, 2014). In this technique, the important point is to determine the most appropriate substrate for the field in terms of the suitability to conditions and cost-effectiveness (Bedard, 2003).

Bioaugmentation. Bioaugmentation is introducing degrading microorganisms enriched from the same or another site, if the microbial activity is insufficient for degradation at the site (Reible, 2014). For bioremediation to be effective, the augmented strain should maintain adequate viable cell count (Singh et al., 2011). Some environmental parameters, such as pH and redox, presence of other toxic pollutants, and bioavailability of target contaminant, can constrain the

bioaugmentation performance (Perelo, 2010). Furthermore, the selection of appropriate microorganism specie is of significance for a successful bioaugmentation application.

Chemical oxidation. For remediation of organic pollutants, chemical oxidation by using permanganate, hydrogen peroxide, ozone, and persulfate is widely applied in situ and ex situ. The stability of the oxidant agent in subsurface environment is one of the most important concerns. When applied in situ, the distribution of oxidant agent should be taken into account (Brown, 2010). Furthermore, usage of environmentally benign agents should be promoted, together with their cost-effectiveness.

The feasibility of in situ and ex situ biotic and abiotic processes depends on the characteristics of environmental media, type and properties of the contaminant, as well as economic and social factors. When economic and social aspects are taken into consideration, sustainability concept becomes important. Hence, sustainable remediation aims to protect human health and the biota via removing contaminants from environment with minimum negative impact, and use of limited resources in an economic way (Fortuna et al., 2011). The remediation approaches should;

- Minimize energy and natural resources consumption, use renewable energy and reuse materials, if possible,
- Reduce emissions to the environment
- Simulate a natural process occurring in the environment,
- Result in removal of contaminants at maximum efficiency, or permenant destruction, if possible (Ellis and Hadley, 2009).

Within this context, sustainability assessment of remediation options prior to implementation on a site became crucial in the last decade. While assessing sustainable remediation strategies, performance of various scenarios should be evaluated based on sustainability indicators. These environmental, social and economic indicators are listed in Table 2-11.

Table 2-11. Sustainability indicators identified by Sustainable Remediation Forum (SuRF-UK, 2010).

Environment
• Emissions to air • Soil and ground conditions • Groundwater and surface water • Ecology • Natural resources and waste
Economic
• Direct economic costs and benefits • Indirect economic costs and benefits • Employment and employment capital • Induced economic costs and benefits • Project lifespan and flexibility
Social
• Human health and safety • Ethics and equity • Neighbourhoods and locality • Communities and community involvement • Uncertainty and evidence

The sustainability assessment should proceed with i) setting clear remediation objectives, ii) defining comprehensible boundaries of the system, iii) identifying scope of relevant sustainability indicators, and iv) selection of methodology for comparison of remediation alternatives (Bardos, 2014).

CHAPTER 3

MATERIALS AND METHODS

This chapter includes the methodologies followed thoughout this study. All materials and equipments used, methods followed, and laboratory setups are explained in detail for:

- The analysis of PBDEs (a total of 22 congeners) and HBCDD (total, and 3 individual isomers) in solid matrices (i.e., sludge and spiked sediments),
- Sampling of wastewater treatment plant sludges,
- Biotic degradation studies,
- Abiotic degradation studies.

Initially, extraction, cleanup and instrumental analysis methods for PBDE and HBCDD analysis in solid matrices are presented. Quality assurance/quality control protocols are then summarized. Subculturing of DF-1 strain is described. Experimental setups for biotic and abiotic degradation studies are then explained in detail. Lastly, the information on sediment samples used in degradation studies, and dewatered sludge samples collected is presented.

3.1.Reagents and standards

All solvents (n-hexane (HEX), dichloromethane (DCM), acetone(ACE)) used for analysis, anhydrous sodium sulfate (granular), copper fine powder (<63 μm), and aluminum oxide (0.063-0.200 mm) were purchased from Merck KGaA (Darmstadt, Germany). Individual standards of PBDE-209 (2,2',3,3',4,4',5,5',6,6'-BDE), surrogate standard PCB-141 (2,2',3,4,5,5'-CB), internal standard PCB-209

(2,2’,3,3’,4,4’,5,5’,6,6’-CB), PCB-61 (2,3,4,5-CB) and PCB-23 (2,4,5-CB) were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Individual HBCDD isomers (α-, β-, γ-) and PBDE predominant congeners mixture (BDE-CSM) were purchased from AccuStandard (New Haven, USA). A standard mixture of octa-, nona-, and deca-BDEs (BDE-OND), surrogate standard BDE-77 (3,3’,4,4’-BDE), BDE-17 (2,2’,4-BDE), BDE-49(2,2’,4,5’-BDE), BDE-66 (2,3’,4,4’-BDE) were purchased from Wellington Laboratories (Canada). For PBDE microcosm studies, BDE-209 supplied from Dr. Ehrenstorfer GmbH was used as the spiking standard, while for mesocosm studies, deca-BDE and BDE-99 (2,2’,4,4’,5-BDE) purchased from CPA (Bulgaria) were used.

3.2.Extraction methods

The methods used in the analysis of PBDEs and HBCDD in solid matrices are presented in Table 3-1. All of the methods are published by United States Environmental Protection Agency (US EPA). The extraction and cleanup procedures used throughout this study utilized the guidelines given in the relevant methods, however they do not completely follow the methods of US EPA.

PBDEs. PBDE extraction from solid matrices was studied by comparing efficiencies of BDE-209 extraction with three methods, i.e. Soxhlet, ultrasonic and vial extraction. The details of these methods are presented in Table 3-2. The efficiency of methods were determined according to the acceptable analyte recovery criteria proposed in method 8000D as 70 – 130% (USEPA, 2014a). Method validation experiments were based on quantitative comparison of recoveries via analyzing laboratory control samples spiked with a known amount of BDE-209.

Table 3-1. Methods used in extraction and analysis of PBDEs and HBCDD.

Method No – Name	Purpose	Reference
8000D – Determinative chromatographic separations	Analytical chromatographic methods, QA/QC requirements	(USEPA, 2014a)
3540C – Soxhlet extraction	Extraction of nonvolatile and semivolatile organic compound from solid matrices	(USEPA, 1996a)
3550C – Ultrasonic extraction	Extraction of nonvolatile and semivolatile organic compound from solid matrices	(USEPA, 2007a)
3665A – Sulfuric acid/ permanganate cleanup	Cleanup of sample extracts	(USEPA, 1996b)
3660B – Sulfur cleanup	Cleanup of elemental sulfur	(USEPA, 1996c)
3630C – Silica gel cleanup	Column cleanup of sample extracts	(USEPA, 1996d)
3610B – Alumina cleanup	Column cleanup of sample extracts	(USEPA, 1996e)
1614 – Brominated diphenyl ethers in water, soil, sediment and tissue by HRGC/HRMS	Determination of BDE congeners in environmental matrices and other sample matrices	(USEPA, 2007b)
Chapter 4 – Organic analytes	Sampling, storage, and sample preparation methods	(USEPA, 2014b)
Definition and procedure for the determination of the method detection limit	Estimation of detection limit for physical and chemical methods	(USEPA, 2016)

Table 3-2. Methods used in extraction of PBDEs from solid matrices.

Method	Details of method *	Ref
Soxhlet extraction	Ten grams of sample mixed with ten grams of anhydrous sodium sulfate was placed into Soxhlet thimble and 300 mL of solvent mix (HEX:DCM:ACE, 7:7:1 v/v) was used for extraction. Sample was extracted with Soxhlet apparatus for 17 h	(USEPA, 1996a)
Ultrasonic extraction	Five grams sample was placed into 40 mL vials and mixed with five grams of anhydrous sodium sulfate. Sample was kept overnight in 30 mL solvent mix (HEX:DCM:ACE, 7:7:1 v/v) and extracted in ultrasonic bath for 30 min twice.	(USEPA, 2007a)
Vial extraction	0.5 g of samples were extracted by vigorous shaking on a horizontal shaker at 350 rpm for 16 h with 10 mL solvent mix (HEX:DCM:ACE, 4.5:4.5:1 v/v) in 15 mL amber vials with Teflon-lined caps.	(Kaya et al., 2015a)

* HEX: n-hexane, DCM: dichloromethane, ACE: acetone.

During extraction of solid material, sulfur removal was achieved with the addition of copper powder into the extraction solvents at the beginning of extraction (USEPA, 1996c). The extract from extraction step was concentrated to 2 – 5 mL via rotary evaporator (Heidolph, Hei-Vap Advantage HL/G1). To remove the possible interfering organic compounds, the colored extract after concentration step was treated with 1:1 concentrated sulfuric acid (U.S. EPA Method 3665A). The top clear extract was purified with 0.5 to 3 g of alumina (deactivated to 6%) or silica (deactivated to 4.5%) topped with anhydrous sodium sulfate, and eluted with 2 mL of n-hexane. The collected extract was concentrated to 2 mL by high purity nitrogen stream.

HBCDD. HBCDD extraction method validation studies were based on verified PBDE extraction method with a few modifications. Main differences between HBCDD extraction and PBDE extractions are i) deactivation percentage of alumina, ii) the use of elution solvent type and volume from alumina column, and iii) use of rotary evaporator at final concentration step. During method validation studies for HBCDD extraction, use of rotary evaporator vs nitrogen evaporation was tested. Then, elution solvents during clean-up step were altered to find the optimum solvent type and volume. Finally, deactivation percentage of alumina was optimized.

Extraction of HBCDD from solid matrices was based on US EPA method 3550C (USEPA, 2007a). One gram of sample, mixed with one gram of anhydrous sodium sulfate in 40 mL vials, were extracted ultrasonically in an ultrasonic bath with 30 mL mL hexane:dichloromethane:acetone mixture (7:7:1 v/v) for 30 minutes twice, after being soaked into the solvent mixture overnight. Sulfur removal was achieved with the addition of copper powder into the extraction solvents at the beginning of extraction (USEPA, 1996c). The two extracts were combined and concentrated to 2-5 mL via rotary evaporator. To remove the possible interfering organic compounds, the colored extract after concentration step was treated with concentrated sulfuric acid (U.S. EPA Method 3665A). The top clear extract was purified with 0.5 g of alumina (deactivated to 3%) topped with anhydrous sodium sulfate, and eluted with 5 mL of n-hexane, followed by 2 mL of n-hexane:dichloromethane mixture (1:1). The collected extract was concentrated to 2 mL via rotary evaporator.

3.3.Instrumental analysis

PBDEs. One mL extract was spiked with internal standard (PCB-209) and analyzed with gas chromatography coupled with micro-cell electron capture detector (Agilent 6890N GC-μECD) with DB-5 MS capillary column (15 m x 0.25 mm ID x 0.10 μm). Instrumental conditions were as follows: Helium was used as the carrier gas with 1.8 mL/min flowrate using a constant flow mode. The make-up gas for the detector was nitrogen with a flowrate of 30 mL/min. The injector and detector temperatures were 250°C and 350°C, respectively. The sample injection was carried out at 1 μL with

splitless injection mode. Oven temperature program started at 90 °C, raised at 20°C/min to 310 °C, and hold there for 6 min. PBDE congeners 17, 28, 47, 49, 66, 99, 100, 153, 154, 183, 194,195, 196, 201, 202, 198/199/200/203 (co-eluting peak), 197/204, 205, 206, 207, 208, and 209 were identified according to the retention times given with certificates of standards and previous studies.

Analysis of BDE-209 standard (CAS No:1163-19-5) in GC-μECD revealed nona-BDE peaks, increasing linearly with the concentration of standard analyzed (Figure 3-1). Hence, analysis of 100 ppb, 200 ppb, and 400 ppb BDE-209 standard solutions five times consecutively resulted in the derivation of ratios for each nona-BDE in the standard (Table 3-3). Average ratios were found to be 0.0097, 0.0191, and 0.0214 for BDE-208, -207, and -206, respectively, all of which has RSDs lower than 15%. These ratios were similar to commercial deca-BDE content, as was also mentioned by Alaee et al. (2003) and Tokarz et al. (2008). Hence, a correction in nona-BDE quantitation was performed by subtracting the value found by multiplying the corresponding ratio with BDE-209 concentration from the chromatogram result of nona-BDE during degradation studies. Calibration of congeners in GC was conducted without considering these ratios.

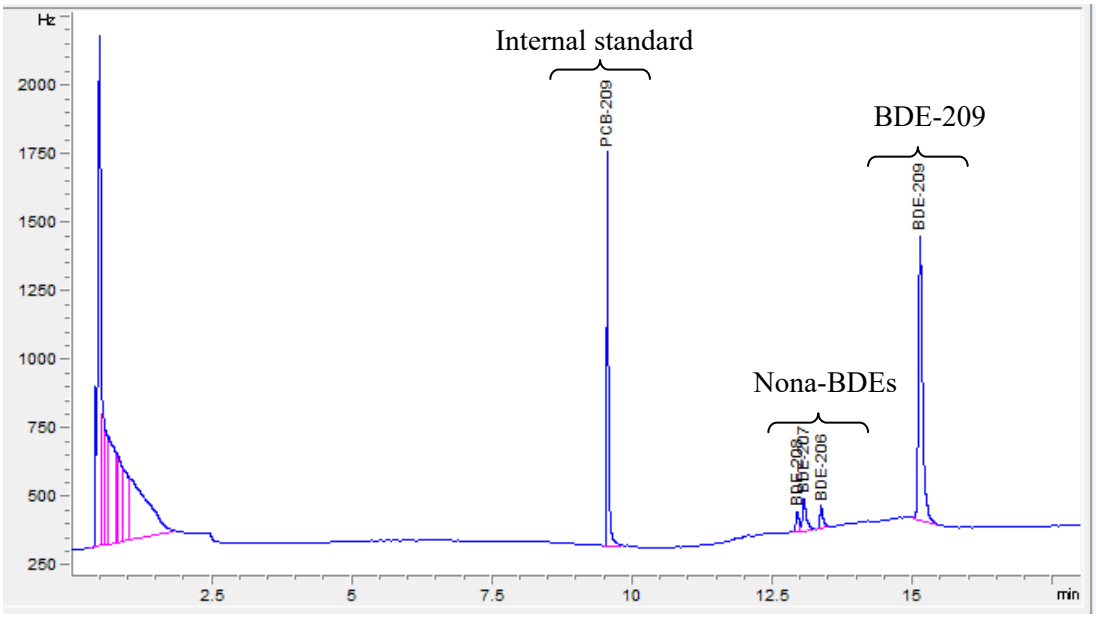


Figure 3-1. Occurrence of BDEs-208/207/206 during GC analysis of BDE-209 standard (CAS No:1163-19-5).

Table 3-3. Occurrence ratios of BDE-209 to BDE-208, -207, and -206 during GC analysis.

	BDE-208	BDE-207	BDE-206
100ppb-1	0.0104	0.0233	0.0194
100ppb-2	0.0093	0.0205	0.0212
100ppb-3	0.0090	0.0201	0.0211
100ppb-4	0.0087	0.0192	0.0179
100ppb-5	0.0090	0.0200	0.0211
200ppb-1	0.0107	0.0228	0.0238
200ppb-2	0.0106	0.0215	0.0236
200ppb-3	0.0083	0.0180	0.0199
200ppb-4	0.0082	0.0176	0.0201
200ppb-5	0.0075	0.0158	0.0177
400ppb-1	0.0105	0.0171	0.0219
400ppb-2	0.0120	0.0187	0.0257
400ppb-3	0.0117	0.0191	0.0229
400ppb-4	0.0080	0.0152	0.0206
400ppb-5	0.0114	0.0179	0.0244
Average	0.0097	0.0191	0.0214
Std.dev.	0.0014	0.0023	0.0023
RSD	14.78	12.24	10.79
Min	0.0075	0.0152	0.0177
Max	0.0120	0.0233	0.0257

Analysis of PBDEs was done primarily with GC-ECD. However, since identification of compounds in ECD was only based on retention times, a confirmation was deemed necessary for sludge samples, which were found to have very high BDE-209 concentration, with GC mass spectrometry (GC-MS). For this purpose, Agilent 7890A GC 5975C inert mass spectrometry (GC-MSD) in EI mode with DB5-MS column (15 m x 0.25 mm ID x 0.10 μm) was used to confirm BDE-209 concentrations in the sludge samples analyzed to have very high level. Injection temperature was 320°C, ion source temperature was 300°C and quadrupole temperature was 150°C. Injection was performed with pulsed splitless injection with injection pulse pressure of 15.8 psi until 1.8 min, and purge flow to split vent of 50 mL/min at 2 min. Helium was used as the carrier gas at a constant rate of 1.8 mL/min. Oven program was as follows: 90°C for 1 min, raised to 340°C at

20°C/min, and held for 2 min. Analysis in scan mode revealed that primary ion (m/z) used for confirmation was 799.

HBCDD. Agilent 7890A GC 5975C inert mass spectrometry (GC-MSD) in EI mode with DB5-MS column (15 m x 0.25 mm ID x 0.10 μm) was used for the instrumental analysis of HBCDD. α-, β- and γ-HBCDD isomers could not be separated in GC analysis when all isomers were present in the solution, while they can be identified at very similar retention times individually. Hence, in GC analysis, sum of three isomers can be determined.

PCB-209 was used as the internal standard, and as surrogate standard, BDE-208 was used for biotic microcosm studies, while BDE-99 was used for abiotic degradation studies. Injection temperature was 200°C, ion source temperature was 230°C and quadrupole temperature was 150°C. Helium was used as the carrier gas at a constant rate of 1.5 mL/min. Oven program was as follows: 60°C for 1 min, raised to 200°C at 15 °C/min, to 310°C at 10°C/min and held there for 5 min. Analysis in scan mode revealed that primary/secondary ions (m/z) used for confirmation are 79/159.1 for HBCDD, 497.8/427.8 for PCB-209, 721.6 for BDE-208, and 403.8/563.6 for BDE-99. These ions were then used to analyze samples in SIM mode.
To identify individual isomers, LC-MS/MS analysis was initiated in Central Laboratory of METU, at the same time with GC analysis in our department. HBCDD isomers were identified by Agilent 6460 triple quadrupole (ESI ionization) coupled with Agilent 1200 HPLC. Zorbax SB-C18 (2.1 x 50 mm x 1.8 μm) column was used. Injection volume was 2 μL. Mobile phase gradient was (A) water/acetonitrile (95:5) and (B) methanol/acetonitrile (95:5). Column temperature was 40°C. The flowrate was 0.4 mL/min. The elution program started at an initial composition of 50:50 A/B (v/v), and was ramped to 60% B in 1 min, 95% B in 5 min, 90% B in 1 min and 60% B in 1 min. Then, it was returned to starting conditions in 2 min.

During HBCDD analysis in GC-MS, degradation of HBCDD to form intermediates was observed. HBCDD injection led to two small peaks in the chromatogram due to the low melting point of HBCDD as 275 °C (EPI-Suite). These were believed to be

the degradation products, ie. pentabromocyclododecane and tetrabromocyclododecane as was previously mentioned in the literature (Zeng et al., 2014; K. Zhang et al., 2014). Zhang et al. (2014) observed small peaks in the chromatogram before HBCDD peak during GC-MS analysis of standard sample, and attributed this to dehydrobromination (K. Zhang et al., 2014), and tried to eliminate these peaks by reducing injection temperature from 280°C to 220°C (Zeng et al., 2014). In the current study, injection temperature was 200°C, hence no more reduction was deemed necessary in the analysis. Since the scope of this study does not cover the quantification of HBCDD degradation products, decomposition ratios was not computed. Similar to PBDE case, calibration of HBCDD in GC was conducted regardless of this decomposition amount.

3.4.QA/QC Protocols

Quality assurance/control protocols (QA/QC) include cleaning of glassware, establishing standardized instrumental analysis (calibration curves and identification of detection limits), analysis of blanks, laboratory control samples, matrix spikes/matrix spike duplicates (MS/MSD), and certified reference material along with the test samples.

Laboratory equipment cleaning was performed with care according to the procedure by Organic Analytes Chapter 4 of US EPA (USEPA, 2014b). Glassware and syringe cleaning was also supported by ultrasonic bath when these equipments were used in highly contaminated samples; such as wastewater treatment plant sludges. Cleaned glassware was rinsed with n-hexane just prior to use.

Six-point internal calibration was performed for 22 PBDE congeners/congener groups in GC-ECD. For BDE-99 and BDE-209, high concentration calibration was also performed. PCB-141 and BDE-77 were used as surrogate standards during analysis of PBDEs. Calibrations yielded RSDs lower than 20% and R^2 greater than 0.99 for all congeners and surrogate standards (see Appendix C). For BDE-209 concentration confirmation in GC-MS, three-point external calibration was performed for 1000, 1500, and 2000 ppb concentrations.

Method detection limit (MDL) and limit of quantitation (LOQ) were determined according to US EPA method (USEPA, 2016). MDL is found by multiplying the appropriate one-sided 99% t-statistic (2.82) by the standard deviation obtained from ten analyses of the lowest calibration standard. Then, LOQ is calculated by multiplying MDL with 3.18 (Muir and Sverko, 2006). The results for all PBDEs analyzed are presented in Table 3-4.

Table 3-4. Method detection limit and limit of quantitation values for PBDEs.

PBDE congener	Method Detection Limit (ppb)	Limit of Quantitation (ppb)	PBDE congener	Method Detection Limit (ppb)	Limit of Quantitation (ppb)
BDE 17	0.09	0.27	BDE 202	0.32	1.02
BDE 28	0.15	0.48	BDE 201	0.21	0.67
PCB 141 (SS)	0.20	0.63	BDE 204/197	0.43	1.36
BDE 49	0.10	0.33	BDE 198/199/200/203	0.92	2.93
BDE 47	0.06	0.19	BDE196	0.41	1.30
BDE 66	0.13	0.41	BDE205	0.81	2.59
BDE 77 (SS)	0.16	0.50	BDE194	1.29	4.09
BDE 100	0.12	0.38	BDE195	1.96	6.24
BDE 99	0.10	0.30	BDE208	1.82	5.77
BDE 154	0.16	0.52	BDE 207	1.03	3.27
BDE 153	0.17	0.53	BDE 206	1.43	4.54
BDE 183	0.23	0.74	BDE 209	3.94	12.53

Five-point internal calibration of GC-MS was performed for γ-HBCDD in the range of 250 ppb to 1500 ppb and BDE-208 in the range of 120 ppb to 600 ppb, yielding RSDs lower than 20% and R^2 greater than 0.99 for all (see Appendix C). MDLs were

34.6 ppb and 80.3 ppb, and LOQs were 110.1 ppb and 255.5 ppb, for γ-HBCDD and BDE-208, respectively. The same calibration can be used for sum of isomers with appropriate verification standards. For HBCDD abiotic degradation studies, BDE-99 was the surrogate standard, and its MDL and LOQ values were 8.70 ppb and 27.7 ppb, respectively. For LC-MS/MS analysis, five point external calibration was conducted for γ-HBCDD in the range of 50 to 1000 ppb, and seven point external calibration was conducted for each isomer (α-, β-, and γ-HBCDD) in the range of 25 to 1000 ppb. Detection limit for each isomer was found as 10 ppb.

Within the scope of method validation study, several laboratory control samples (LCS) were analyzed together with sludge samples and MS/MSD samples. Method blanks were analyzed in every batch of 10 – 15 samples, or in each batch of sludge samples collected from different treatment plants. There were no peaks observed for the analytes in blanks of sediment microcosms. However, BDE-209 peak was detected in the blanks analyzed after one of the treatment plant sludge sample. Hence, glassware cleaning was conducted with special care, i.e. repeating acid cleaning and hexane rinsing several times, after the extraction of that specific sludge sample. Overall, blank correction was not performed in any of the samples analyzed.

LCSs are the samples prepared by spiking a known amount of analyte or analyte mixture to a clean matrix. The matrix used in this study was soil and sediment samples, cleaned via Soxhlet extraction with hexane:dichloromethane:acetone for 17 hours. Spike of analytes to clean matrix is at a concentration that is around the middle of the calibration range of analytes (USEPA, 2014a). MS/MSD sample spike is also suggested to be at 20 times the LOQ of analytes, or at the middle of the calibration range similarly with LCS (USEPA, 2014a). Hence, 300 ppb, 30 ppb each, and 400 ppb of analytes were spiked for MS/MSD analysis for BDE-209, other PBDE congeners, and γ-HBCDD, respectively. Lastly for QA/QC requirements, certified reference material analysis has been conducted with both ultrasonic and Soxhlet extraction methods for PBDEs. There is no certified reference material for HBCDD, currently. Results of all QA/QC samples will be presented in Chapter 4.

Performance of analytical methods is checked with the analyte and surrogate recoveries for LCS and MS/MSD samples. The following formulas are used for performance evaluation for accuracy of the results:

$$Surrogate\ recovery(\%) = \frac{Concentration\ found}{Concentration\ added} \times 100 \quad (1)$$

$$Analyte\ recovery\ (\%) = \frac{C_s - C_u}{C_n} \times 100 \quad (2)$$

where C_s is the measured concentration of the spiked sample, C_u is the concentration of unspiked sample (=0 for LCS), and C_n is the theoretical concentration added to spiked sample.

The acceptable range for analyte and surrogate recovery is 70 – 130% (USEPA, 2014a). However, throughout this study, a range of 80 – 120% was utilized to check the performance of analysis.

Precision can be estimated via calculation of relative percent difference between spiked samples, and calculated from the formula:

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \times 100 \quad (3)$$

where C_1 is the concentration of analyte for sample 1, and C_2 is the concentration of analyte for sample 2.

Relative standard deviation (coefficient of variation) can also be used for precision of analysis, and calculated as:

$$RSD\ (CV) = \frac{S}{\bar{X}} \times 100 \quad (4)$$

Where S is the square root of variance of measurements, and $\bar{X}$ is the arithmetic mean of measurements.

3.5.Subculturing of *Dehalobium chlorocoercia* strain DF-1

Dehalobium chlorocoercia strain DF-1 was originally enriched from Charleston Harbor sediments (Wu et al., 2000), and was routinely grown in estuarine mineral medium (ECl) in Dr. Kevin Sowers' Lab of Institute of Marine & Environmental

Technology, University of Maryland, Baltimore, MD, USA. DF-1 culture grown in 50 mL medium in a serum bottle was transported to our laboratory and stored at room temperature in the dark. This culture was subcultured in our laboratory to grow DF-1 as follows: Fresh ECl medium (Table 3-5) is prepared according to the specifications described by previous studies (Berkaw et al., 1996; Fagervold et al., 2011; Payne et al., 2011). Subculturing was done by transferring 1:10 of original culture into fresh ECl medium. As an electron donor, 10 mM formate was added, together with 10 ppm of PCB 61 (2,3,4,5-CB) to the fresh medium before subculturing. Subculturing was done anaerobically under N_2:CO_2 in an anaerobic glovebox (PlasLabs 818GB/Exp). Culture was incubated statically in the dark at 30°C.

Table 3-5. ECl medium ingredients (for a total volume of 500 mL).

Ingredient	Amount	Ingredient	Amount
H_2O	498 ml	Trace mineral solution (1000x)	0.5 ml
NaCl	4.21 g		
$MgSO_4 * 7H_2O$	2.4 g	Vitamin solution (1000x)	0.5 ml
KCl	0.14 g	HCl	0.25 ml
$CaCl_2 * 2H_2O$	0.025 g	$Na_2HPO_4 * 7H_2O$	0.56 g
NH_4Cl	0.25 g	cysteine	0.125 g
Resazurin (1000x)	0.5 ml	Na_2CO_3	1.5 g

Growth of DF-1 was monitored by dechlorination of PCB 61 to PCB 23 (2,3,5-CB) (Payne et al., 2011). Hence, PCB extraction from culture media was done to observe whether dechlorination was achieved. The extraction method is as follows (Lombard et al., 2014): 1 mL of sample is taken to a glass tube and 5 mL of hexane is added. The sample is shaken vigorously by hand for 10 s and let the tube rest for a few seconds for phase separation. A purification column is prepared in Pasteur pipette with alumina and copper (4:1), and rinsed with 5 mL hexane. Top part of the extracted sample (solvent part) is eluted from the column, with additional 1 mL hexane. The collected extract is ready for GC analysis (Lombard et al., 2014). For the instrumental analysis of PCB 61 and 23, Agilent 6890N GC-μECD with DB-5 MS capillary column (15 m x 0.25 mm ID x 0.10 μm) was used. Instrumental

conditions were as follows: Helium was used as the carrier gas and the make-up gas for the detector was nitrogen. The injector and detector temperatures were 250°C and 350°C, respectively. The sample injection was at 1 μL with splitless injection mode. Oven temperature program started at 100°C, raised at 20°C/min to 160°C, held there for 2 min, raised to 200°C at 3°C/min, then to 240°C at 8°C/min, held there for 5 min, and finally raised at 30°C/min to 290°C (3 min). PCB-209 was used as the internal standard and no surrogate was used since only mass ratio of congeners was monitored. A six-point internal calibration was performed for PCB-61 and -23 in the range 1 ppb to 500 ppb, yielding RSDs lower than 20% and R^2 0.995 for both.

3.6.Experimental setup

Degradation of PBDEs and HBCDD was investigated via two distinct mechanisms: biotic and abiotic degradation. The experimental setup of each system is explained in detail in this section.

3.6.1. Biotic degradation studies

Biodegradation of PBDEs in aquatic sediments was investigated in two laboratory scales, namely microcosms and mesocosms. Microcosm sets were established to observe biodegradation of PBDEs under varying conditions so that the efficiency of each treatment would be identified. Mesocosm sets were then prepared to examine the applicability of biodegradation mechanism, which is determined to degrade PBDEs most efficiently in microcosm experiments, in larger scale. Biodegradation of HBCDD, on the other hand, was investigated in only sediment microcosms under varying conditions, similarly with PBDEs.

PBDE microcosms. Sediment microcosm reactors were put into operation under various conditions to evaluate the enhancement of degradation rate. The PBDE congener that was examined in the microcosm study was BDE-209. Some part of sediments was air-dried overnight. Air-dried sediments were spiked with BDE-209 standard in acetone. Spiked dry sediments were mixed completely. Then, wet sediments were added onto spiked dry sediments and a homogeneous mixture was

obtained, as described in Tokarz et al. (2008). BDE-209 contaminated wet sediments were distributed equally among the microcosm bottles to get approximately 3 g wet sediments in each. Similarly, non-spiked control set was established by spiking dry sediment with high grade acetone and mixed with wet sediments. These contamination control microcosm sediments were found to have no PBDE contamination throughout the incubation period. The samples were also confirmed with GC-MS analysis, and hence it can be said that all congeners observed in spiked sediment microcosms were due to degradation of the spiked BDE-209. Similar microcosm studies had approximate concentrations such as, 5 ppm of octa-BDE in sediments (Lee and He, 2010), 5 ppm of BDE-209 in sediments (Tokarz III et al., 2008), and 1000 ng/g dw of PBDEs in sediment (Zhu et al., 2014) were tested in degradation studies. Hence, the target concentration was selected as 1000 ng/g dw.

The details of microcosm sets are given in Table 3-6. Microcosms were prepared as sacrificial reactors and operated for 6 months. For each set and each sampling time, duplicate 20 mL reactors were established. All sets were topped with distilled water while the biostimulation set was topped only with an organic medium. In all reactors sediment-to-liquid ratio was kept constant as 3 g:3.5 mL. In bioaugmentation set, microorganism culture was added in a liquid medium, and volume of added water was adjusted accordingly.

Table 3-6. Details of PBDE sediment microcosm sets.

Reactor Type	Reactor Name	BDE-209 spike	Sediment	Topping Liquid Ingredients (volume)
Test microcosms	Natural attenuation	+	+	DI water (3.5 mL)
	Biostimulation	+	+	e- donor & C source rich organic medium (3.5 mL)[a]
	Bioaugmentation	+	+	DF-1 culture (0.5 mL)[b] + DI water (3.0 mL)
Control microcosms	Negative control	+	+	Spent growth medium (0.5 mL)[c] + DI water (3.0 mL)
	Contamination control	-	+	DI water (3.5 mL)
	Sterile	+	+ (Autoclaved)	DI water (3.5 mL)

[a] Prepared as given in Berkaw et al. 1996 and supplied with sodium formate and ethanol.
[b] *Dehalobium chlorocoercia* strain DF-1 culture.
[c] DF-1 medium with no DF-1 cells, obtained by passing the culture medium through 0.22 μm filter.

Organic medium of biostimulation set was prepared by dissolving several vitamins and minerals in water together with various salts under N_2:CO_2 atmosphere, and pH was adjusted to 6.8 (Berkaw et al., 1996). Medium also contained 10 mM of sodium formate and ethanol as the carbon source and electron donor, respectively. *Dehalobium chlorocoercia* strain DF-1 was used in bioaugmentation set. This strain was successful in reductive dechlorination of PCBs (Payne et al., 2011). For natural attenuation set, no amendment was made with extraneous substances to sediments.

There were three types of control sets: i) A negative control set was established to serve as a control for bioaugmentation set so that the effects of adding a culture medium without DF-1 cells can be observed. Hence, a spent growth medium was formed by passing DF-1 culture through 0.22 μm filter so that no cells will remain in the medium. ii) A sterile control set was established where microcosms were

autoclaved at 120 °C at 1.1 atm pressure for 20 min on three consecutive days to hinder any microbial activity in sediments. iii) A contamination control set was established without any spike of BDE-209 to check for any contamination resulting from incubation conditions in unspiked sediments. All sets were purged with high purity nitrogen stream after closed with Teflon lined septa crimp caps. They were incubated in the dark at 25°C. During sampling, duplicate reactors were opened and all sediments were analyzed. An example photograph showing sediment microcosms is presented in Figure 3-2.

Sampling from microcosms was performed on days 0, 20, 40, 60, 90, 120, 150, and 180. Analysis of sediments was conducted to observe any change in BDE-209 concentrations, and formation of product congeners, tri-BDEs (BDE-17, 28), tetra-BDEs (BDE-47, -49, -66), penta-BDEs (BDE-99, -100), hexa-BDEs (BDE-153, 154), hepta-BDE (BDE-183), octa-BDEs (BDE-194, -195, -196, -201, -202, -198/199/200/203 (co-eluting peak), -197/204, -205), and nona-BDEs (BDE-206, -207, -208).

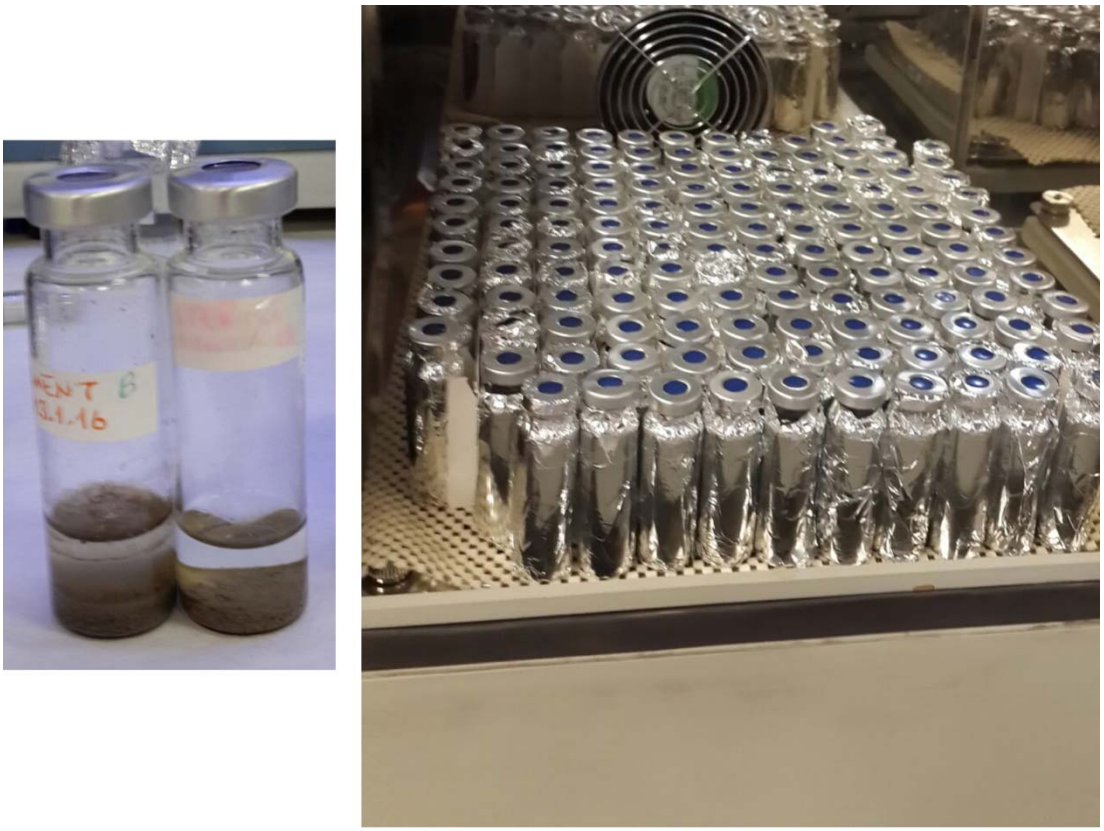


Figure 3-2. An example demonstration for sediment microcosms. On the left, sediment and liquid contents of microcosm, and on the right, placement of microcosms in incubator are shown.

HBCDD microcosms. Similarly, biodegradation of HBCDD was investigated in sediment microcosms under various conditions. The details of HBCDD microcosm sets are presented in Table 3-7. The target concentration of HBCDD in microcosms was 1000 ng/g dw. The sets were prepared similar with PBDE sets. Different than PBDE sets; i) an abiotic control set was established, and ii) sterilization was performed via both mercury poisoning and autoclaving at 120 °C at 1.1 atm pressure for 20 min on three consecutive days.

Table 3-7. Details of HBCDD sediment microcosm sets.

Reactor Type	Reactor Name	HBCDD spike	Sediment	Topping Liquid Ingredients (volume)
Test microcosms	Natural attenuation	+	+	DI water (3.5 mL)
	Biostimulation	+	+	e- donor & C source rich organic medium (3.5 mL)[a]
	Bioaugmentation	+	+	DF-1 culture (0.5 mL)[b] + DI water (3.0 mL)
Control microcosms	Negative control	+	+	Spent growth medium (0.5 mL)[c] + DI water (3.0 mL)
	Contamination control	-	+	DI water (3.5 mL)
	Sterile[d]	+	+	DI water (3.5 mL)
	Abiotic control[e]	+	-	DI water (5 mL)

[a] Prepared as given in Berkaw et al. 1996 and supplied with sodium formate and ethanol.
[b] *Dehalobium chlorocoercia* strain DF-1 culture.
[c] DF-1 medium with no DF-1 cells, obtained by passing the culture medium through 0.22 μm filter.
[d] Sterile set was poisoned with mercury chloride and then autoclaved on three consecutive days.
[e] Abiotic control set included kaolinite instead of sediment as the solid media.

Almost all of the previous studies on HBCDD biodegradation in the literature showed a sterilization problem. Studies of Davis et al. (2005, 2006) and Gerecke et al. (2006) showed a considerable HBCDD concentration decrease (e.g. 48% decrease in 14 days in sediments, reaching not detected values in 61 days) in sterile control sets. The same problem was examined in pre-set microcosms operated before real set microcosms in our laboratory. Pre-set microcosms included only DF-1 added bioaugmentation set and autoclaved sterile set, operated for 26 days. Initial HBCDD concentration in the sets was 705.6 ± 55.0 ng/g dw, while day 26 concentrations were 45.4 ng/g (93.6% reduction) and 79.1 ng/g (88.8% reduction) in bioaugmentation and sterile set, respectively.

Two approaches were implemented in HBCDD microcosms to solve sterilization problem. First one was to treat sterile set sediments with mercury ($HgCl_2$) together with autoclaving. By this way, the enzymatic activities that can remain after autoclaving would be ceased. $HgCl_2$ was applied to sediments with the ratio of 500 mg $HgCl_2$/kg of soil (Trevors, 1996) in each bottle. Second approach was to operate another control set which includes an abiotic solid medium that resembles sediments structurally. For this purpose, kaolinite ($Al_2Si_2O_5(OH)_4$) was used. Previous abiotic degradation studies for POPs used several abiotic media, such as kaolinite, montmorillonite, silica gel, etc., besides soil and sediments (Ahn et al., 2006; Venny et al., 2012b). Among those, silica gel and kaolinite was tested for HBCDD spike and extraction from the media. Kaolinite was selected due to the HBCDD recovery it presented with the validated extraction method (90.6 ± 8.14 %, *n*=4). It was used in microcosms after it was washed with distilled water and dried in oven at 105°C overnight, three times. Similar to sediments, kaolinite was spiked with HBCDD, mixed vigorously, and distributed to microcosm bottles to involve 2 g dry weight kaolinite. On top of kaolinite, 5 mL of distilled water was added to provide similar medium/liquid ratio among all microcosm sets.

At each sampling time, duplicate bottles were taken for each set, and all sediments were extracted for analysis of γ-HBCDD. Sampling from microcosms was performed on days 0, 4, 8, 12, 16, 20, 24, and 36. Analysis of sediments was conducted to observe any change in γ-HBCDD concentrations. Products of HBCDD were not monitored throughout this study.

PBDE mesocosms. Monitoring PBDE biodegradation was also performed in sediment mesocosms. These larger scale reactors were operated under the treatment conditions showing satisfactory biodegradation according to the results of sediment microcosms. Therefore, biostimulation set was established to observe biodegradation of PBDEs since this set has given the highest BDE-209 reduction rate in microcosms. Furthermore, in sediment mesocosms, BDE-99 biodegradation was also monitored in individual reactors.

Mesocosm reactors of approximately 2400 mL total volume (LxWxH: 21 cm x 5.7 cm x 20.4 cm) were used in duplicate for each set. Preparation of mesocosms was performed completely under N_2:CO_2:H_2 environment in an anaerobic glovebox (PlasLabs 818GB/Exp). PBDE spike was done similarly as the microcosms: spike of BDE-209 and BDE-99 onto dried sediments, thorough mixing until solvent evaporation, addition of wet sediments and complete mixing as a whole. Target contamination concentration was 1000 ng/g dw for both BDE-209 and BDE-99. Unspiked control sets were added with the same volume of solvent (isooctane:toluene mix of 9:1) as BDE spikes. After mixing provided under anaerobic atmosphere, sediments were poured into mesocosm reactors. On top of sediments resazurin was added to observe the color change throughout the incubation. The details of sediment mesocosms are presented in Table 3-8.

Table 3-8. Details of PBDE sediment mesocosms.

Reactor	Sediment	Spike	Organic medium
BDE-209 Biotic	+	BDE-209	+
BDE-209 Sterile	+ *	BDE-209	-
BDE-99 Biotic	+	BDE-99	+
BDE-99 Sterile	+ *	BDE-99	-
Contamination Control	+	-	-

* Reactors were autoclaved at 120°C at 1.1 atm pressure for 20 min on three consecutive days.

Each reactor contained 720 g of wet sediments, and total of 550 mL liquid. For biotic sets, 50 mL of organic medium was added onto sediments, mixed gently, and then 500 mL of distilled water, purged with nitrogen, was added. Although in microcosms topping liquid included only the organic medium, a diluted organic medium was provided in mesocosms. The reason for this was to supply an acceptable chemical oxygen demand (COD) in sediments. The organic medium had a COD of 2500 mg/L. According to Water Pollution Control Regulation, receiving water discharge criteria

for many industries has COD values around 200 – 250 mg/L (Ministry of Environment and Urbanization, 2004). Additionally, surface water classification criteria for COD lies between less than 25 mg/L and greater than 70 mg/L (Ministry of Environment and Urbanization, 2004). Therefore, COD load to the sediments was kept as low as possible by diluting the organic medium with a ratio of 1/11. Accordingly, for sterile and control sets 550 mL distilled water was added.

After sediment mesocosms were prepared, they were taken out of glovebox, and put into incubator at 25°C in dark. Sterile sets were autoclaved at 120°C at 1.1 atm pressure for 20 min on three consecutive days before placing into the incubator. Although they were prepared under anaerobic atmosphere, this atmosphere could not be provided during incubation. The reactors were allowed to contact with normal atmosphere to mimic the environmental conditions, hence the surface water was exposed to aerobic conditions. These type of reactor systems resemble the natural conditions in the ways that the contaminants are exposed to volatilization and solubilization, i.e. physicochemical weathering processes. For PBDEs case, since their vapor pressures and solubilities are very low, these processes may not be expected to be effective in mesocosms. The mesocosm reactors have a glass lid on top, only opened when sampling was done. An example photograph showing sediment mesocosms is presented in Figure 3-3.

Triplicate sediment samples were taken from each reactor with minimum disturbance to sediments (i.e. as a core sample with a glass pipette with tip cut-off). Sediment sampling was performed on days 0, 15, 30, 60, 90, and 120 for BDE-209, and on days 0, 15, 30, 45, 60, 75, 90, 120 for BDE-99. Nona- to tri-BDEs were monitored for BDE-209 degradation products, and tetra- and tri-BDEs were monitored for BDE-99 degradation. Every 30 days, COD of supernatant water was measured with COD kit prepared.

Headspace gas monitoring was not conducted during biotic degradation studies. Supernatant water was also not analyzed for PBDEs/HBCDD since these compounds and their degradation products have water solubilities of <1 to 70 μg/L. They also

have vapor pressures of 10^{-5} to 10^{-9} Pa, hence they were expected to neither dissolve in supernatant water nor partition into headspace.

Figure 3-3. A photograph showing sediment mesocosms.

3.6.2. Abiotic degradation studies

For abiotic degradation of PBDEs and HBCDD, catalyzed hydrogen peroxide propagation reactions (CHP) was investigated. Parameters affecting the efficiency of this process are pH, H_2O_2, and Fe(III) concentrations. Preliminary sets were established with single-fill 24-hour experimental setups to observe the response of these chemicals to CHP reactions, and to detect the effects of changing operational parameters; such as hydrogen peroxide concentrations, pH, and contaminant concentration. Initial experiments were conducted with BDE-209, and then HBCDD set was operated with the selected CHP process and parameters. Results of single-fill microcosms revealed the optimum conditions and operation of CHP process as fill-and-draw treatment. Therefore, fill-and-draw experimental setups were established

for BDE-209, BDE-99, and HBCDD. For the abiotic degradation studies, products of any compound were not monitored.

Single-fill microcosms. The details of single-fill sets are presented in Table 3-9. In each reaction set, 40 mL amber bottles were used as the reactors and operated sacrificially for each sampling time. Each reactor contained approximately 3 g wet sediments and a total of 10 mL solution. Controls for single-fill microcosms were i) no H_2O_2: using distilled water instead, ii) no contaminant: to check any contamination/interference caused from CHP reactions, starting from "H_2O_2-repeat" experiment set. Except the first set in which duplicate reactors were operated, all sets included triplicate test reactors and duplicate control reactors. In all sets, 3 g wet sediment to 10 mL total solution volume ratio was kept constant. Sampling was performed at t = 0, 1, 4, and 24 hours. An example CHP setup is demonstrated in Figure 3-4.

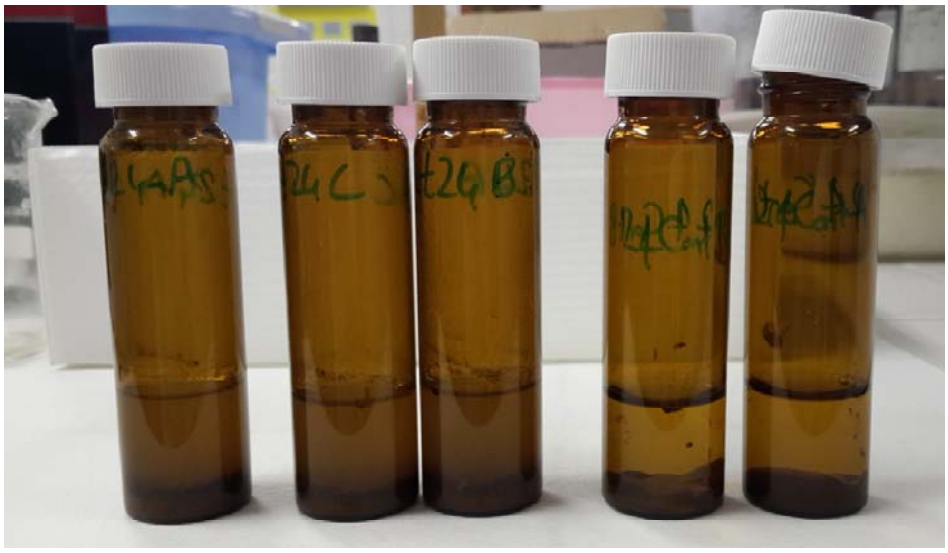


Figure 3-4. An example CHP setup, showing CHP reaction microcosms in first three bottles from left, and no-H_2O_2 controls in the last two.

Table 3-9. Details of single-fill microcosms for abiotic degradation studies.

Experiment name	Contaminant	pH[a]	H_2O_2	Fe(III)	Monitoring of	
					H_2O_2	T
pH unadjusted	BDE-209	-	2 M	100 ppm	-	+
H_2O_2	BDE-209	+	10 M	100 ppm	+	+
H_2O_2-repeat	BDE-209	+	10 M	100 ppm	+	+
BDE-high	BDE-209	+	10 M	100 ppm	+	-
Fenton	BDE-209	-	10 M	Fe(III): - Fe(II):300 ppm	+	+
HBCDD	γ-HBCDD	+	10 M	300 ppm	+	+

[a] pH adjustment (+) was done to pH=2 by addition of sulfuric acid.

H_2O_2 concentration in reaction vessels were monitored with Quantofix H_2O_2 strips. Fe(III)sulfate hydrate and Fe(II)sulfate were used to prepare iron catalyst solutions. For the first three sets (i.e. "pH unadjusted", "H_2O_2", "H_2O_2-repeat"), t=0 samples were taken without the addition of any solution onto sediments. However, in "BDE-high", "Fenton" and "HBCDD" sets, solutions were also added to t=0 sample bottles, and discarded right after peroxide addition.

Fill-and-draw microcosms. Fill-and-draw (F&D) operation means withdrawal of reaction solution at predetermined time intervals and refilling the reactor with fresh solution. The experimental setup of F&D treatments are given in Table 3-10, while operation of control reactors is presented in Table 3-11.

Table 3-10. Details of fill-and-draw (F&D) CHP experimental setup.

Contaminant	Concentration of contaminant (ng/g dw)	H_2O_2 (M)	Fe(III) (ppm)	F&D period	pH	Monitoring of	
						H_2O_2[a]	T[b]
BDE-209	1000	10	300	1 day	2	+	+
BDE-209 & BDE-99	1000 each	10	300	8 days	2	+	+
γ-HBCDD	1000	10	300	8 days	2	+	+

[a] H_2O_2 was monitored only at fill-and-draw treatment times.
[b] Temperature was monitored only at first few hours of reaction.

Table 3-11. Details of F&D CHP control reactor setup.

	H_2O_2	Fe(III)	Contaminant	Sampling time
No H_2O_2 control	-	+	+	t=0, F&D-1, F&D-5
No contaminant control	+	+	-	t=0, F&D-5
Spike control	-	-	+	t=0
LOI control	+	+	-	t=0, F&D-1, F&D-5

Similar to the single-fill microcosms, 40 mL amber bottles consisting of 3 g wet sediments and 10 mL solution were employed, and operated sacrificially. F&D process included 5 treatments. At each F&D treatment, sacrificial bottles were collected for sediment extraction so that withdrawal and refilling was conducted 5 times for the last sample. The test systems and spike control were operated in triplicate, while no H_2O_2 and no contaminant control systems were in duplicate. Time zero samples were taken right after they were amended with solutions. At each sampling, approximately 0.5 mL of ethanol was added onto solution before withdrawal to quench CHP reactions.

No H_2O_2 controls were established using distilled water instead of H_2O_2 to observe contaminant concentration change without CHP reactions. No contaminant controls were operated to check for a contamination resulting from the CHP process. Spike controls were prepared to determine the contaminant concentration in sediments without addition of CHP solutions. The purpose of LOI controls was to observe the change in organic content of sediments during CHP process.

3.7.Sediments used in degradation studies

The sediments that were used in degradation studies were collected from the pond in Çamkoru National Park, Ankara. It is located 110 km northwest of Ankara, and has an altitude of approximately 1350 m (Figure 3-5). The park is remote from residential areas, and it is seasonally used as a recreational area. There are no known pollution sources for the pond, but the Ankara-Bolu highway is on the northeast of the pond and is approximately 2 km away from the nearest point of the pond. The National Park site is one of the air monitoring stations of MONET (global passive air monitoring network) since December 2009. According to atmospheric and soil samples collected four times and once per year, respectively, the station consistently showed very low POPs concentrations, and no previous PBDE contamination have been recorded (Jarkovský et al., 2015). Sediments under 70 cm water depth were collected with a scoop at five different points in the pond and stored in glass jars. Sediments having large particles and stones were wet-sieved (2 mm) in the field as suggested by the guidance document on chemical monitoring under Water Framework Directive (European Commission, 2010). The pond water above the sediments was also collected. Samples were transported to the laboratory in coolers and stored at 4 °C in amber glass bottles until use. Sediments collected were blackish in color. Moisture content and total organic content of sediment was determined to be 36.5±1.53% and 1.43±0.16% (n=3), respectively.

Figure 3-5. Çamkoru Natural Park pond satellite view.

Particle size distribution of wet sediments was analyzed using Master Sizer (Central Laboratory of METU). The results are given in Table 3-12 and Figure 3-6. According to American Society for Testing and Materials specifications for types of sediments based on grain size, Çamkoru sediments can be classified as mostly sand (75-2000 µm) and silt (5-75 µm) (Mulligan et al., 2010). Sediments were also analyzed in terms of elemental content using X-ray florescence spectrometry, after they were dried in 65°C overnight and 105°C for 2 hours. The results are presented in Table 3-13.

Table 3-12. Results of sediment particle size distribution analysis.

Specific surface area	0.653 m^2/g
Size range	0.02 to 2000 µm
Surface weighted mean	9.186 µm
Volume weighted mean	295.933 µm
d(10%)	4.103 µm
d(50%)	28.644 µm
d(90%)	1251.751 µm

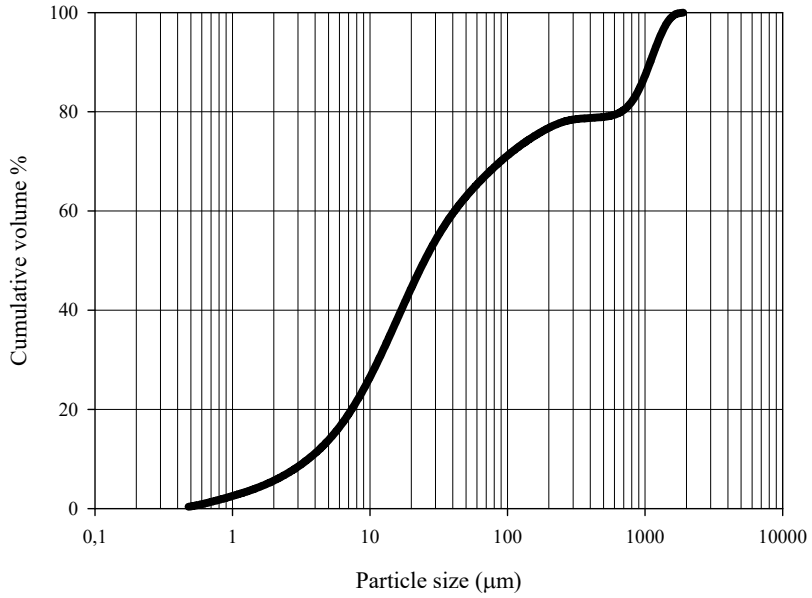


Figure 3-6. Particle size distribution (analyzed by Central Laboratory, METU).

Table 3-13. Results of content analysis of sediments.

Component	Percent by weight (%)	Component	Percent by weight (%)
O	45.3	SiO_2	52.5
Si	28.0	CO_2	17.9
Al	9.54	Al_2O_3	16.3
C	5.48	Na_2O	3.86
K	3.84	K_2O	3.84
Na	3.09	Fe_2O_3	2.78
Fe	2.42	CaO	1.61
Ca	1.40	TiO_2	0.540
Ti	0.397	MgO	0.232
Mg	0.153	SO_3	0.125
Ba	0.0919	P_2O_5	0.124
Sr	0.0751	BaO	0.0836
P	0.0647	SrO	0.0703
S	0.0599	MnO	0.0419
Mn	0.0402	ZrO_2	0.0410
Zr	0.0383	Cl	0.0138
Cl	0.0165	Rb_2O	0.0132
Rb	0.0152	ZnO	0.0081
Zn	0.0082		

3.8.Wastewater Treatment Plant Sludge Samples

Selection of sludge sampling points was based on the types, serving areas and locations of wastewater treatment plants (WWTPs). Dewatered sludge samples were collected from WWTPs located in four different geographical regions of Turkey: two being urban (U-1, U-2) and two being industrial (I-1, I-2). Urban WWTPs are both high capacity plants receiving over 500,000 m^3/d of flow. They can also receive some industrial discharges. Urban WWTPs have biological wastewater treatment (conventional activated sludge), followed by sludge stabilization (anaerobic digester) and dewatering. Industrial WWTPs serve organized industrial districts (OID) that consist mainly of textile factories, and receive a flow of over 20,000 m^3/d. Industrial WWTPs have biological wastewater treatment, while I-1 has also a chemical precipitation unit. Their sludge treatment consists of dewatering units.

Two sampling campaigns have been implemented: cold and warm seasons. Average ambient air temperatures for these cities at the sampling months are presented in Table 3-14. Collected samples were transported to the laboratory in a cooler, and then stored at -18°C until analysis. According to Chapter 4 of U.S. EPA Method (USEPA, 2014b), solid samples for polychlorinated biphenyls/dibenzo-p-dioxins/furans can be stored under 6°C with no specific holding time. Additionally, OSPAR guidelines suggest -20 °C for long-term storage (OSPAR, 2003). Samples were dried in a lyophilizator (Christ Alpha 1–4 model) prior to extraction. Dried samples were immediately ground and sieved through a 2.0-mm sieve and stored in amber-glass bottles just before extraction.

Table 3-14. Monthly average ambient air temperatures at sampling areas (State Meteorological Works, n.d.) and moisture and organic content of the samples.

Sampling area – time	Average temperature °C (min-max)	Moisture content (%)	Organic content (%)
U-1 – November 2014	8 (-2 – 19)	73.7	56.9
U-1 – September 2015	22 (11 – 34)	68.3	57.7
U-2 – September 2014	22.8 (16.1 – 31.1)	65.2	69.8
U-2 – March 2016	8.1 (3 – 14)	68.2	67.6
I-1 – March 2016	10.1 (5.2 – 15.8)	67.2	32.1
I-1 – August 2014	26.9 (19.7 – 34.3)	61.5	26.5
I-2 – November 2014	8 (-1 – 19)	75.8	71.0
I-2 – September 2015	20 (13 – 34)	69.4	77.0

Moisture and organic content of sludge samples were analyzed right after collection. The procedures for analyses were given below, and the values are provided in Table 3-14.

Moisture content analysis. Moisture content of samples was determined by weighing 10 grams of sample in a constant weight crucible and drying it in 105 °C oven overnight. Calculation was based on the formula:

$$Moisture\ content\ (\%) = \frac{sample\ weight - dried\ sample\ weight}{sample\ weight} \times 100$$

Organic content analysis. Organic content analysis of samples was performed by loss-on-ignition procedure via igniting the sample analyzed for moisture content in 550°C furnace for 4 hours (Heiri et al., 2001). Total organic content of samples was then calculated with the equation:

$$Organic\ content\ (\%) = \frac{dry\ sample\ weight - ignited\ sample\ weight}{dry\ sample\ weight} \times 100$$

CHAPTER 4

BROMINATED FLAME RETARDANTS IN TREATMENT PLANT SLUDGES: METHOD VALIDATION AND LEVELS IN TURKEY

4.1.Introduction

Flame retardants (FRs) have been used in residential and commercial goods to delay ignition and/or protect from fire. FRs may be released into the environment via wastes/wastewater streams, leaching, and volatilization from FR producing industrial facilities and manufacturing facilities using FRs in their products, and after disposal of FR products, leaching from landfills, and combustion and recycling of waste products (Segev et al., 2009). Two mostly used brominated FRs are polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD). PBDEs are widely used in upholstered furniture, electrical equipments, plastics, insulation materials, and textiles (Alaee et al., 2003; Rahman et al., 2001). HBCDD is used in extruded, expanded and high impact polystyrene foams for thermal insulation in buildings, and secondarily used in upholstery furniture, automobile interior textiles, car cushions and electrical equipments (Covaci et al., 2006; Marvin et al., 2011).

During 1970 – 2005, total global production of penta-, octa-, and deca-BDEs were approximately 100000 tonnes, 110000 tonnes and 1.1 to 1.25 million tones, respectively (UNEP, 2010). Commercial deca-BDE demand in European countries was 2500-5000 tons per year in 2012 (*The Voluntary Emissions Control Action Programme*, 2012). After the adverse effects have been recognized, European Union banned the use of products containing penta- and octa-BDEs in 2003, and then use of deca-BDE in 2008 (UNEP, 2010). Concurrently, United States manufacturers

voluntarily eliminated the production of penta- and octa-BDEs in 2004, and deca-BDE after 2013 (Abbasi et al., 2015). Tetra- & penta- and hexa- & hepta-BDEs were included among the persistent organic pollutants (POPs), identified by the Stockholm Convention, in 2009, while deca-BDE is still under review (Stockholm Convention, 2017). Being produced since 1960s, HBCDD is the mostly used cycloaliphatic BFR in the present decade (Marvin et al., 2011). The consumption rate of HBCDD was 12500 tons annually in 2013 in Europe (VECAP, 2014), where it is the second highest used BFR (Covaci et al., 2006). HBCDD was identified as a POP in 2014 (Stockholm Convention, 2017). Both PBDEs and HBCDD were identified as priority hazardous substances by the Water Framework Directive (European Commission, 2013).

As a part of National Implementation Plan preparations for Stockholm Convention, PBDE inventory study for Turkey was conducted and the data gathered was based on estimates of use in related sectors (Ministry of Environment and Urbanization, 2014). Furthermore, according to Waste Electric and Electronic Equipments Control Regulation of Turkey, deca-BDE usage in polymeric applications are exempt from the ban of their applications in household goods, electronic equipments, etc (Ministry of Environment and Urbanization, 2012). Additionally, HBCDD was identified to be one of the chemicals that are produced or imported more than one ton annually in Turkey (Ministry of Environment and Urbanization, 2017). Hence, their applications in some industries can still continue.

Due to the wide usage and persistency of PBDEs and HBCDD, studies focusing on these chemicals have increased in the last decade throughout the world. On the other hand, studies conducted on PBDE levels in Turkey have been relatively scarce, yet they indicate presence of use of PBDEs. There are no published studies on the determination of environmental HBCDD levels in Turkey. National studies on PBDEs were summarized in Table 4-1. Studies on PBDEs were conducted generally to determine the atmospheric levels and soil contamination around industrial regions, such as İzmir, Hatay-İskenderun (Cetin and Odabasi, 2007a; Odabasi et al., 2016, 2015b). All showed that these atmospheric and soil concentrations were comparable

or higher than concentrations measured worldwide (Cetin and Odabasi, 2008, 2007a, Odabasi et al., 2015b, 2010). Recent studies revealed indoor PBDE concentrations in dust in İstanbul (Kurt-Karakus et al., 2015), and in Kocaeli (Civan and Kara, 2016). A more comprehensive study on atmospheric levels in 16 cities in Turkey has already revealed its preliminary results (Ugranli et al., 2016). Additional studies have evaluated the levels in human milk (Erdoğrul et al., 2004; Ozcan et al., 2011), fish (Erdogrul et al., 2005), and butter (Uçar et al., 2011) and all found that the levels were similar with literature data from European countries and USA.

Since PBDEs and HBCDD consumption is high in industries and they are globally found in commercial goods, industrial and domestic WWTPs can be sinks for these substances. Therefore, sludge BFR levels were under investigstion worldwide for several years (Barón et al., 2014; Davis et al., 2012; La Guardia et al., 2010; Morris et al., 2004). Currently, there is no information about the level of PBDEs and HBCDD in wastewater treatment plant (WWTP) sludges in Turkey.

Table 4-1. National PBDE levels in different matrices.

Region	Sample	Year	Basis	Level	Ref.
İzmir – urban and industrial	Soil	2004 – 2005	$\sum_7$PBDEs 28,47,100,99, 154,153,209	$5.04*10^2$ – $2.84*10^6$ ng/kg dw	(Cetin and Odabasi, 2007a)
İzmir – industrial and background	Soil, pine needle, bark, branch, stem, litter	2011	$\sum_7$PBDEs 28,47,100,99, 154,153,209	Air: 162±138, 18.6±0.78 pg/m^3, Soil: 15.2±23.6, 0.34±0.21 µg/kg dw	(Odabasi et al., 2015b)
Hatay-İskenderun – industrial	Soil	2008	$\sum_7$PBDEs 28,47,100,99, 154,153,209	37±64 µg/kg dw	(Odabasi et al., 2010)
Hatay-İskenderun – industrial	Soil, pine needle, bark, branch, stem, litter	2010	$\sum_7$PBDEs 28,47,100,99, 154,153,209	Air: 181±196 pg/m^3, Soil: 9.7±13.0, Litter: 16.0±18.8, Bark: 5.0±8.0, Needle: 4.5±2.0, Branch: 2.5±1.3, Stem: 16.3±9.3 µg/kg dw	(Odabasi et al., 2016)
Kocaeli – industrial	Soil	2010	$\sum_7$PBDEs 28,47,100,99, 154,153,209	0.70 – 203 µg/kg dw	(Cetin, 2014)
İzmir – urban and industrial	Atmospheric	2004 – 2005	$\sum_7$PBDEs 28,47,100,99, 154,153,209	11 – 149 pg/m^3 (summer), 6 – 81 pg/m^3 (winter)	(Cetin and Odabasi, 2008)
16 different cities in Turkey	Atmospheric	2014	$\sum_{14}$PBDEs 17,28,71,47, 66,100,99,85, 154,153,138, 183,190,209	6.86 – 641 pg/ m^3	(Ugranli et al., 2016)

Table 4-1. Cont'd.

Region	Sample	Year	Basis	Level	Ref.
İzmir - rural	Near-ground air	2012	$\sum_5$PBDEs 28,47,66,100,99	8.5 pg/m^3	(Lammel et al., 2015)
Bursa – suburban, rural				0.7 pg/m^3	
İstanbul – urban and rural residential	Indoor dust	2012	$\sum_{12}$PBDEs	330 – 32000 ng/g	(Kurt-Karakus et al., 2015)
Kocaeli - residential	Indoor dust	2015	$\sum_{14}$PBDEs 17,28,71,47, 66,100,99,85, 154,153,138, 183,190,209	29.32 – 4790 ng/g	(Civan and Kara, 2016)
İzmir –urban and industrial	Aerosol	2006	$\sum_7$PBDEs 28,47,100,99, 154,153,209	Urban $PM_{2.5}$ = 80±64 pg/m^3 PM_{10} = 132±92 pg/m^3, Industrial $PM_{2.5}$ = 116±85 pg/m^3 PM_{10} = 206±246 pg/m^3	(Odabasi et al., 2015a)
İzmir – urban	Sea water	2005	$\sum_7$PBDEs 28,47,100,99, 154,153,209	212±65 pg/L (summer), 87±57 pg/L (winter)	(Cetin and Odabasi, 2007b)

Table 4-1. Cont'd.

Region	Sample	Year	Basis	Level	Ref.
Kahramanmaraş	Human milk	2003	$\sum_7$PBDEs 28,47,100,99, 154,153,183	0.005 – 0.014 ng/g ww	(Erdoğrul et al., 2004)
Konya	Human milk	2010	$\sum_5$PBDEs 47,100,99, 154,153	ND – 363.68ng/g lipid wt.	(Ozcan et al., 2011)
Kahramanmaraş – Ceyhan Watershed	Fish	2003	$\sum_6$PBDEs 28,47,100,99, 154,153	ND – 6.7 ng/g ww	(Erdogrul et al., 2005)
14 different cities in Turkey	Butter	2007	$\sum_{18}$PBDEs 17,28,47,49,66, 71,75,77,85,99,100, 119,138,153,154, 183,190,209	0.18 – 5.00 ng/g fat	(Uçar et al., 2011)

Several analysis methods have been developed for the determination of these BFRs in different matrices, and summarized in comprehensive review studies (Covaci et al., 2007; Fulara and Czaplicka, 2012; ten Dam et al., 2012). Here, validation of an analysis method, which uses less chemicals, requires small amounts of samples for extraction and provides numerous samples extraction in one batch, was targeted.

The objective of this study is, therefore, two fold: (1) to compare the efficiency of different analytical methods and to optimize the steps involved for the determination of BFRs in solid matrices and (2) use the verified method to investigate the BFR levels of WWTP sludges for the first time in Turkey.

4.2.Materials and methods

4.2.1. Sludge samples

Dewatered sludge samples were collected from WWTPs located in four different geographical regions of Turkey: two being urban (U-1, U-2) and two being industrial (I-1, I-2). The rationale behind the selection of WWTPs to be sampled was to collect dewatered sludge samples that can represent a relatively high population equivalent or relevant industrial facilities for BFR usage, size, and accessibility of the WWTPs. Urban WWTPs are both high capacity plants receiving over 500,000 m^3/d of flow. They can also receive some industrial discharges. Urban WWTPs have biological wastewater treatment (conventional activated sludge), followed by sludge stabilization (anaerobic digester) and dewatering. Industrial WWTPs serve organized industrial districts (OID) that consist mainly of textile factories, and receive a flow of over 20,000 m^3/d. Industrial WWTPs have biological wastewater treatment, while I-1 has also a chemical precipitation unit. Their sludge treatment consists of dewatering units.

Two sampling campaigns have been implemented: cold and warm seasons. Average ambient air temperatures the sampling points at the sampling months were presented in Table 4-2. Collected samples were transported to the laboratory in a cooler, and then stored at -18°C until analysis. Samples were dried in a lyophilizator (Christ

Alpha 1–4 model) prior to extraction. Dried samples were ground and sieved through a 2.0-mm sieve just before extraction and stored in amber-glass bottles. Moisture content of samples was analyzed by drying at 105°C oven overnight, and organic content was analyzed by loss-on-ignition via igniting the sample analyzed for moisture content in 550°C furnace for 4 hours (Heiri et al., 2001). Moisture content and organic content of samples are also provided in Table 4-2.

Table 4-2. Monthly average ambient air temperatures at sampling areas (State Meteorological Works, n.d.) and moisture and organic contents of dewatered sludge samples.

Sampling area – time	**Average temperature °C (min-max)**	**Moisture content (%)**	**Organic content (%)**
U-1 – November 2014	8 (-2 – 19)	73.7	56.9
U-1 – September 2015	22 (11 – 34)	68.3	57.7
U-2 – September 2014	22.8 (16.1 – 31.1)	65.2	69.8
U-2 – March 2016	8.1 (3 – 14)	68.2	67.6
I-1 – March 2016	10.1 (5.2 – 15.8)	67.2	32.1
I-1 – August 2014	26.9 (19.7 – 34.3)	61.5	26.5
I-2 – November 2014	8 (-1 – 19)	75.8	71.0
I-2 – September 2015	20 (13 – 34)	69.4	77.0

4.2.2. Analysis of PBDEs and HBCDD in solid matrices

Extraction procedures. Three different extraction methods were tested in PBDE analysis. These methods are summarized below. Several different solvents were used for these methods in the literature, but solvent types and volumetric ratios were kept similar while testing the methods. After the analysis of each method with laboratory control samples (LCSs), the most efficient method was selected for the analysis of sludge samples. Method validation studies were conducted with BDE-209 due to its

being the most abundant congener in almost all types of environmental matrices, and then LCSs were analyzed for other PBDE congeners with the verified method. Also, the verified method was tested for HBCDD extraction, and then optimized with altering certain extraction parameters.

Soxhlet extraction. For this extraction procedure, U.S. EPA Method 3540C was followed (USEPA, 1996a). Ten grams of sample, mixed with ten grams of anhydrous sodium sulfate, was placed into Soxhlet thimble, and 300 mL of solvent mix (hexane:DCM:acetone, 7:7:1 v/v) was used for extraction. Sample was extracted with Soxhlet apparatus for 17 h after the addition of surrogate standard. The extract was eluted from a column of anhydrous sodium sulfate, and concentrated to 2 – 5 mL with Kuderna-Danish (KD) evaporator with a 3-ball Snyder column or a rotary evaporator (RE) (Heidolph, Hei-Vap Advantage HL/G1).

Ultrasonic extraction. This extraction procedure was based on U.S. EPA Method 3550C (USEPA, 2007a). Five grams sample was placed into 40 mL vials and mixed with five grams of anhydrous sodium sulfate. After addition of surrogate standard, sample was kept overnight in 30 mL solvent mix (hexane:DCM:acetone, 7:7:1 v/v) and extracted in ultrasonic bath for 30 min. Extract was centrifuged at 1800 rpm for 5 min and collected. This procedure was repeated twice and total 60 mL extract was concentrated down to 2 – 5 mL with a RE.

Vial extraction. This method was developed in a previous study for PCB analysis from WWTP sludges (Kaya et al., 2015b). 0.5 g of samples were extracted by vigorous shaking on a horizontal shaker at 350 rpm for 16 h with 10 mL solvent mix (hexane:DCM:acetone, 4.5:4.5:1 v/v) in 15 mL amber vials with Teflon-lined caps. Separation of solvent from the sample was achieved by centrifuging the vials at 2500 rpm for 5 min.

Purification of extracts. During extraction of solid material, sulfur removal was achieved with the addition of copper powder into the extraction solvents at the beginning of extraction (USEPA, 1996c). The colored extract after extraction and

concentration steps was treated with concentrated sulfuric acid, based on U.S. EPA Method 3665A. The top clear extract was purified with 0.5 to 3 g of alumina (deactivated to 6% for PBDEs, 3%, 6% or 12% for HBCDD) or silica (deactivated to 4.5%) topped with anhydrous sodium sulfate. The collected extract was concentrated with KD apparatus or RE, and/or to 2 mL by high purity nitrogen stream.

Instrumental analysis. For PBDE analysis, one mL extract was spiked with internal standard (PCB-209) and analyzed with gas chromatography coupled with micro-cell electron capture detector (Agilent 6890N GC-μECD) with DB-5 MS capillary column (15 m x 0.25 mm ID x 0.10 μm). Instrumental conditions were as follows: Helium was used as the carrier gas with 1.8 mL/min flowrate using a constant flow mode. The make-up gas for the detector was nitrogen with a flowrate of 30 mL/min. The injector and detector temperatures were 250°C and 350°C, respectively. The sample injection was carried out at 1 μL with splitless injection mode. Oven temperature program started at 90 °C, raised at 20°C/min to 310 °C, and hold there for 6 min. Confirmation of BDE-209 concentration was carried out with an Agilent 7890A GC 5975C inert mass spectrometry (GC-MSD) in EI mode with DB5-MS column (15 m x 0.25 mm ID x 0.10 μm). Injection temperature was 320 °C, ion source temperature was 300 °C and quadrupole temperature was 150 °C. Injection was performed with pulsed splitless injection. Helium was used as the carrier gas at a constant rate of 1.8 mL/min. Oven program was as follows: 90 °C for 1 min, raised to 340 °C at 20 °C/min, and held there for 2 min. Analysis in scan mode revealed that primary ion (m/z) used for confirmation was 799.

For total (sum of α-, β-, and γ-) HBCDD analysis, Agilent 7890A GC 5975C inert mass spectrometry (GC-MSD) in EI mode with DB5-MS column (15 m x 0.25 mm ID x 0.10 μm) was used. Injection temperature was 200 °C, ion source temperature was 230 °C and quadrupole temperature was 150 °C. Helium was used as the carrier gas at a constant rate of 1.5 mL/min. Oven program was as follows: 60 °C for 1 min, raised to 200 °C at 15 °C/min, to 310 °C at 10 °C/min and held there for 5 min. Analysis in scan mode revealed that primary/secondary ions (m/z) used for confirmation are 79/159.1 for HBCDD, 497.8/427.8 for PCB-209 (internal standard)

and 721.6 for BDE-208 (surrogate standard). These ions were then used to analyze samples in SIM mode. HBCDD isomers (α-, β- and γ-HBCDD) were identified by Agilent 6460 triple quadrupole (ESI ionization) coupled with Agilent 1200 HPLC. Zorbax SB-C18 (2.1 x 50 mm x 1.8 μm) column was used. Injection volume was 2 μL. Mobile phase gradient was (A) water/acetonitrile (95:5) and (B) methanol/acetonitrile (95:5). Column temperature was 40°C. The flowrate was 0.4 mL/min. The elution program started at an initial composition of 50:50 A/B (v/v), and was ramped to 60% B in 1 min, 95% B in 5 min, 90% B in 1 min and 60% B in 1 min. Then, it was returned to starting conditions in 2 min.

QA/QC. A six-point internal calibration was performed for 22 PBDE congeners/congener groups in GC-ECD, yielding relative standard deviations (RSD) lower than 20% and R^2 greater than 0.99. PCB-141 and BDE-77 was used as surrogate standards during PBDEs analysis. Method detection limit (MDL) and limit of quantitation (LOQ) were determined according to US EPA method (USEPA, 2016). MDLs were in the range of 0.06 ppb (BDE-47) to 3.94 ppb (BDE-209), and LOQ were 0.19 ppb (BDE-47) to 12.53 ppb (BDE-209). For BDE-209 concentration confirmation in GC-MS, three-point external calibration was performed for 1000, 1500, and 2000 ppb concentrations. A five-point internal calibration of GC-MS was performed for γ-HBCDD, yielding RSD lower than 20% and R^2 greater than 0.995. MDLs were 34.6 ppb and 80.3 ppb, and LOQs were 110.1 ppb and 255.5 ppb, for γ-HBCDD and BDE-208, respectively. The same calibration can be used for sum of isomers with appropriate verification standards. For LC-MS/MS analysis, seven point external calibration was conducted for each isomer (α-, β-, and γ-HBCDD). Detection limit for each isomer was found as 10 ppb.

Performance of analytical methods is checked with the analyte and surrogate recoveries for LCS and MS/MSD samples. The acceptable range for analyte and surrogate recovery is suggested by US EPA as 70 – 130% (USEPA, 2014a). However, throughout this study, a range of 80 – 120% was utilized to check the performance of analysis. Precision can be estimated via calculation of relative

percent difference between spiked samples, and relative standard deviation (RSD) can also be used for precision of analysis.

4.3.Results and Discussion

4.3.1. PBDE method validation

Soxhlet, ultrasonic and vial extraction methods were tested in this study with laboratory control samples (LCS). Together with the LCS, solvent blank were also analyzed. The criteria for selection among methods were based on five parameters: i) high accuracy and precision achieved in LCSs, ii) amount of solvent and chemicals used during extraction, iii) number of samples that can be analyzed in one batch, iv) time requirements, and v) amount of solid sample extracted.

The results of the method validation study for BDE-209 (Table 4-3) demonstrated that use of alumina in Soxhlet extraction gave higher precision (lower RSD) than use of silica gel, while the recoveries were comparable with both adsorbents in ultrasonic extraction. Especially, the surrogate recoveries in LCS extracted with Soxhlet and purified with silica exceeded the acceptable range provided by U.S. EPA as 70 – 130% (USEPA, 2014a). Although vial extraction method yielded acceptable surrogate recoveries with alumina, this method showed low accuracy for analyte recovery. On the other hand, the recoveries of both surrogate and analyte were similar and within acceptable range for Soxhlet and ultrasonic extractions with alumina cleanup with high precision and accuracy. With respect to the solvent and chemical usage per sample during extraction, vial and ultrasonic extraction methods demonstrated better performance over Soxhlet extraction. The number of samples that can be extracted in one batch can reach up to 6 to 10 samples for vial and ultrasonic extraction methods, while that for Soxhlet extraction is limited to two. Time requirements for a complete procedure per single sample are similar for all methods; nevertheless when the number of samples that can be analyzed in one batch is taken into consideration, Soxhlet extraction performed worse than others. Lastly, amount of solid sample needed for extraction is much less in vial and ultrasonic extraction methods than Soxhlet extraction. When all these parameters are taken into

consideration, ultrasonic extraction with alumina cleanup was selected to be used for detection of PBDEs in sludge samples.

Table 4-3. Surrogate and BDE-209 recoveries for three extraction methods and two purification adsorbents.

Sample	Extraction method	Clean up[a]	*n*	Surrogate Recovery (%)		BDE-209 Recovery (%)	
				Average ± SD (range)	RSD	Average ± SD (range)	RSD
Solvent Blank	Soxhlet	S	4	113.9±9.11 (106-127)	8.00		
	Soxhlet	A	2	98.2±5.44 (94.3-102)	5.55		
	Ultrasonic	S	2	84.5±3.46 (82-86.9)	4.10		
	Ultrasonic	A	4	89.4±4.68 (84.6-94.1)	5.23		
	Vial	S	4	89.5±1.65 (88.2-91.8)	1.85		
	Vial	A	4	88.4±3.22 (84.3-91.8)	3.65		
Laboratory Control Sample	Soxhlet	S	4	131.1±36.9 (81.9-171.5)	28.2	108.5±24.9 (73-130.5)	22.9
	Soxhlet	A	4	101.9±2.21 (99.6-104.8)	2.17	86.3±7.02 (78.3-95.4)	8.13
	Ultrasonic	S	2	95.7±2.69 (93.8-97.6)	2.81	96.2±7.42 (90.9-101.4)	7.72
	Ultrasonic	A	4	88.4±1.77 (86.4-90.6)	2.00	96.8±8.35 (86-105.7)	8.63
	Vial	S	4	74.6±3.96 (69.2-77.8)	5.30	52.9±11.2 (43.6-67.7)	21.2
	Vial	A	6	90.3±3.72 (83.9-95.3)	4.12	78.0±13.6 (62.3-96.1)	17.5

[a] S: silica gel, A: alumina cleanup

n: number of samples.

During preliminary validation studies, PCB-141 was used as the surrogate standard due its structural resemblance to PBDE molecules and its absence in the environment. Then, one more surrogate standard was decided to be used, which is a PBDE congener. BDE-77 was used as the surrogate standard in previous studies (Cetin and Odabasi, 2007a). Also, it was found as not-detected or at very low levels (maximum observed concentration was 1.94 ng/g dw only once) in previous sludge sampling studies (De la Torre et al., 2011; Hwang et al., 2012). Therefore, BDE-77 was also used as the surrogate standard in the consecutive analyses.

In order to test whether freeze-drying operation leads to any loss of analytes in the samples, dry soil samples were spiked with a known amount of BDE-209 and then

wetted with distilled water. The samples were then freeze-dried and extracted with the verified method. The results showed BDE-209 recoveries of 88.2 – 103.8% for three samples analyzed. Hence, it can be concluded that freeze-drying operation did not cause any analyte loss during the extraction procedure.

The verified extraction method was then tested with LCS analysis for other PBDE congeners (BDE-17, 28, 47, 49, 66, 99, 100, 153, 154, 183, 194, 195, 196, 201, 202, 198/199/200/203, 204/197, 205, 206, 207, 208). Figure 4-1 demonstrates the range of recovery percentages of individual congeners, appear as in the order of retention times in GC. Extraction of seven samples revealed that recoveries were within acceptable range provided by US EPA (USEPA, 2014a). Furthermore, Soxhlet extraction was also tested for these PBDE congeners to compare the recoveries with verified ultrasonic method. The results are presented as red diamonds in Figure 4-1. The recoveries obtained in both methods yielded compatible results, with some congeners showing lower recoveries in Soxhlet (e.g. BDE-28, 153, 154, 194) and some higher (e.g. BDE-66, 99, 206). Nevertheless, both methods resulted in acceptable recoveries.

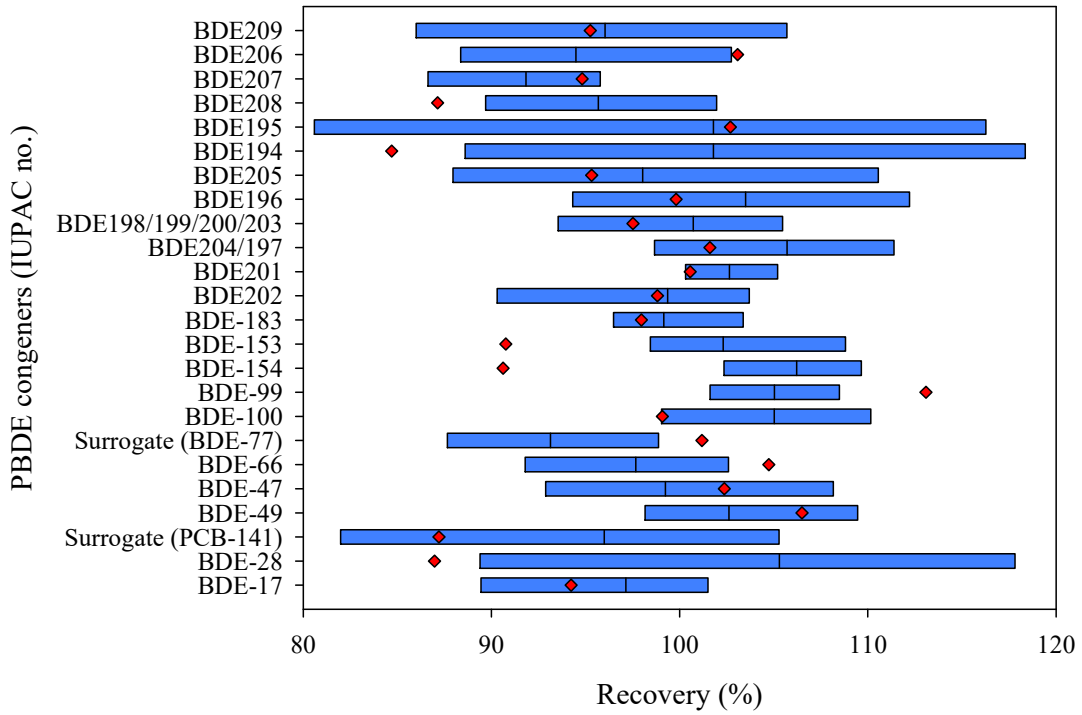


Figure 4-1. Recovery percentages of 22 PBDE congeners/groups and two surrogates analyzed in laboratory control sample (LCS) of ultrasonic extraction method. Bars show minimum and maximum values, and line within the bar shows the average of seven samples. Red diamonds shows LCS for Soxhlet extraction (average of two samples).

One of the WWTP sludge samples was also analyzed with Soxhlet extraction to observe the variation between methods. The relative percent difference between ultrasonic and Soxhlet extraction methods was 1.7%, which indicates the precision between methods in the analysis of sludge samples.

Certified reference material analysis was conducted to test the extraction efficiency of the validated method. PCB and PBDE contaminated sandy loam sediment (RTC CNS329) samples were analyzed and the results were presented in Table 4-4. For PBDE congeners 47, 99, 100, 153, 154, and 183, a recovery range of 84.1 – 101.3% was obtained with ultrasonic extraction method, and 83.0 – 97.3% with Soxhlet

extraction method. Both methods achieved recoveries within the acceptable range given by US EPA. In the reference material, BDE-209 concentration is given as “not certified”, hence concentration of this congener was not reported in this study. Results obtained from ultrasonic extraction are all within confidence or prediction intervals reported in the certificate. The percent recoveries as calculated from certified concentrations are within the acceptable range of US EPA (USEPA, 2014a). CRM was also extracted using Soxhlet extraction for confirmation purposes. Similar to ultrasonic extraction, all results were within confidence or prediction intervals specified in the certificate.

Table 4-4. Results of certified reference material analysis[a].

PBDE congener	Certified concentration (µg/kg)	Confidence Interval	Prediction Interval	Concentration (µg/kg) – Ultrasonic (*n*=4)	Percent Recovery– Ultrasonic	Concentration (µg/kg) – Soxhlet (*n*=2)	Percent Recovery – Soxhlet
BDE-47	**149±33.4**	130-167	75.5-222	150.9±8.71	101.3	144.9	97.3
BDE-100	**108±20.0**	94.9-121	63.5-152	90.8±1.65	84.1	89.7	83.0
BDE-99	**192±48.5**	164-219	85.4-298	169.6±4.48	88.5	162.9	84.8
BDE-154	**108±29.1**	91.4-125	44.1-172	96.0±2.99	88.9	92.3	85.4
BDE-153	**160±14.5**	146-174	126-194	140.3±3.01	87.7	134.2	83.9
BDE-183	**52.6±3.93**	50.2-55	43.8-61.4	53.3±2.72	101.3	49.1	93.3

[a] percent recoveries are calculated based on certified concentration.

MS/MSD analysis was conducted using one of the WWTP sludge samples by spiking PBDE mixture containing the congeners present in the CRM. The results are given in Table 4-5, yielding an average recovery of 94.4% for all congeners. The MS/MSD analysis was important to show the recovery of BDE-209 since it could not be confirmed with CRM analysis. The only congener having relatively low recovery was BDE-100, which was one of the congeners having high variation in this particular sludge sample.

Table 4-5. Results of matrix spike/matrix spike duplicate analysis (*n*=3).

PBDE congener	Matrix concentration (ng/g dw)	Spike concentration (ng/g dw)	Target MS concentration (ng/g dw)	Achieved concentration (ng/g dw)	Recovery (%)
BDE-28	3.51	30	33.51	33.17±4.50	99.0
BDE-47	2.95	30	32.95	35.29±5.97	107.1
BDE-100	2.50	30	32.50	25.46±1.86	78.4
BDE-99	6.00	30	36.0	30.36±2.54	84.4
BDE-154	1.40	30	31.40	27.35±1.03	87.1
BDE-153	2.12	30	32.12	35.54±2.11	110.7
BDE-183	0.83	30	30.83	28.20±3.83	91.5
BDE-209	213.13	300	513.13	500.35±128.01	97.5

4.3.2. HBCDD method validation

Extraction method validation studies for HBCDD were based on the results of PBDE method validation. Therefore, ultrasonic extraction method, having the highest recovery for PBDEs, is used also for HBCDD extraction similar to the previous studies using this technique (García-Valcárcel and Tadeo, 2009; Zhang et al., 2012, Zhang et al., 2014). Silica gel clean-up was generally used for purification of extracts (Feng et al., 2012; Zeng et al., 2014), however in this study only alumina cleanup was tested with changing deactivation ratios (3%, 6%, 12%). Preliminary analysis

was conducted for γ-HBCDD extraction. During method validation, same extraction procedure for PBDEs was applied initially. Observation of very low recoveries with this procedure (Table 4-6) led to investigation of recoveries in each and every step of extraction procedure. Last volumetric concentration step was examined for nitrogen blowdown vs. rotary evaporator (RE). Nitrogen blowdown yielded lower recoveries than RE, hence RE was applied in the succeeding analysis. Purification of extracts was tested initially by eluting 6% deactivated alumina column with 2 mL of hexane, but this yielded zero recovery of γ-HBDD (data not shown). Therefore, elution solvent and volume was altered to 5 mL hexane, followed by 2 mL of hexane:DCM (1:1). This time, different deactivation percentages were tested as given above. Highest recovery was achieved with 3% deactivation. Lastly, efficiency of ultrasonic extraction was examined and found that recoveries were within the acceptable range of US EPA (USEPA, 2014a). Hence, analysis of method blank and LCS were conducted together with matrix blank and solvent LCS. Blank samples did not give any peaks for HBCDD, and solvent LCS yielded 88% γ-HBCDD recovery for one sample analyzed. After method was verified, LCS spiked with three isomers (α-, β-, and γ-) was also extracted, yielding satisfactory total HBCDD recovery as analyzed in GC-MS.

Table 4-6. Surrogate and HBCDD recoveries for complete extraction procedure and single steps in the procedure.

Extraction*	Purification**	Concentration	*n*	Surrogate recovery (%)		HBCDD recovery(%)	
				Avg.±SD.	RSD	Avg.±SD.	RSD
Ultrasonic	6% alumina, 2 mL HEX	Nitrogen blowdown	6	84.5±14.7	17.4%	40.1±19.9	49.7%
-	-	Nitrogen blowdown	4	87.3±6.76	7.74%	78.6±3.51	4.47%
-	-	Rotary evaporation	3	89.9±4.76	5.30%	83.2±2.26	2.72%
-	3% alumina, 5 mL HEX + 2 mL DCM:HEX	Rotary evaporation	3	99.7±12.4	12.5%	91.9±5.25	5.71%
-	6% alumina, 5 mL HEX + 2 mL DCM:HEX	Rotary evaporation	3	88.0±8.61	9.78%	87.8±1.32	1.50%
-	12% alumina, 5 mL HEX + 2 mL DCM:HEX	Rotary evaporation	3	93.1±18.0	19.3%	91.1±0.55	0.61%
Ultrasonic	-	-	3	92.5±3.21	3.47%	87.4±3.95	4.52%
Ultrasonic	3% alumina, 5 mL HEX + 2 mL DCM:HEX	Rotary evaporation	3	106.2±12.3	11.6%	89.3±7.92	8.87%
Ultrasonic	**3% alumina, 5 mL HEX + 2 mL DCM:HEX**	**Rotary evaporation**	**3**	**94.3±5.82**	**6.17%**	**92.2±0.92**	**1.00%**

* Row given in bold was the LCS including three isomers; α-, β- and γ-HBCDD. The result showed total HBCDD recovery as analyzed by GC-MS.

** HEX: n-hexane, DCM: dichloromethane.

HBCDD extraction was also tested with Soxhlet extraction procedure, which gave satisfactory recoveries in PBDE analysis. The Soxhlet extraction procedure was same as PBDEs. However, the column elution method was adjusted for this procedure as follows: alumina (3%) column elution was done first with 125 mL hexane and then 50 mL of hexane:DCM (1:1). Also, final concentration was performed with only RE. The results revealed that γ-HBCDD recovery was 89.4%, and surrogate recovery was 98% as average of two samples. Therefore, both ultrasonic and Soxhlet extraction methods used were consistent and valid for HBCDD analysis in solid matrices.

HBCDD quantification with GC-MS was also confirmed with concurrent LC-MS/MS analysis. The method validation samples were prepared for LC-MS/MS analysis by evaporating the extracts until dryness under high-purity nitrogen, and redissolving in one mL LC-grade methanol. GC-MS can only quantify total HBCDD, whereas LC-MS/MS can quantify all isomers. The comparison of GC and LC analyses were, therefore, based on total HBCDD, and they were presented in Table 4-7. LC-MS/MS analyses yielded acceptable recoveries for HBCDD, in the range 75.0 – 107.1%, with method validation samples. Furthermore, relative percent difference between two instruments was less than 25%. Therefore, it can be evaluated that both analyses were consistent with each other, and LC-MS/MS provided satisfactory recoveries for the purposes of this study.

Table 4-7. Comparison of GC-MS and LC-MS/MS quantification of method validation samples.

Samples	Target concentration (ppm)	Quantification in GC-MS (ppm)	Quantification in LC-MS/MS (ppm)				Percent Recovery (based on total HBCDD)		Relative percent difference
		Total HBCD	α-HBCD	β-HBCD	γ-HBCD	Total HBCD	GC-MS	LC-MS/MS	
Blank		<0.035[a]			<0.025[b]				
Soil blank		<0.035[a]			<0.025[b]				
Solvent LCS	0.5	0.4415			0.4713	0.4713	88.3	94.3	6.5
LCS #1	0.5	0.4862			0.3819	0.3819	97.2	76.4	24.0
LCS #2	0.5	0.4461			0.5354	0.5354	89.2	107.1	18.2
LCS #3	0.6	0.5469	0.1496	0.1450	0.1553	0.4499	91.2	75.0	19.5
LCS #4	0.6	0.5539	0.1663	0.1679	0.1550	0.4892	92.3	81.5	12.4

[a] Method detection limit for GC-MS, [b] Method detection limit for LC-MS/MS.

During the analysis of sludge samples, GC-MS response for HBCDD was so low that other peaks dominated the chromatogram and quantification of HBCDD could not be achieved. Therefore, only for sludge analysis, a fractionation method was employed during alumina column elution in order to eliminate the abundant peaks in the chromatogram. After extract elution, 5 mL of hexane and 2 mL of hexane:DCM (1:1) was collected in separate vials and analyzed separately. As a result of solvent LCS, first fraction showed no HBCDD and 88.6% surrogate recovery, while second fraction yielded no surrogate and 90.7% HBCDD recovery. Hence, this technique was adopted for sludge analysis and MS/MSD analysis in GC-MS. One of the sludge samples was extracted with fractionation method employed during alumina cleanup and analyzed with GC-MS. The results yielded total HBCDD of 593.06±59.97 ng/g dw (*n*=3) in this particular sludge sample, revealing 3.83% RPD between LC-MS/MS and GC-MS analyses.

MS/MSD analysis was conducted with one of the WWTP sludge samples by spiking γ-HBCDD to achieve a target concentration of approximately 1390 ng/g dw (1390.64 ng/g for average of three samples). As a result, an average of 1359.72±166.7 ng/g dw (*n*=3) γ-HBCDD was obtained by yielding 97.8% recovery and 2.25% RPD.

4.3.3. BFR levels in sludge samples

Within the scope of this study, BFR levels in treatment plant sludge samples were identified for the first time in Turkey. 22 PBDE congeners were analyzed in sludge samples, and individual congener concentrations and their sum in cold and warm seasons' samples are presented in Table 4-8 for urban WWTPs and in Table 4-9 for industrial WWTPs.

When BDE-209 levels in urban WWTPs are compared, U-1 exhibited lower BDE-209 concentrations than U-2. Both plants serve more than one million population equivalents. The higher levels found in U-2 sample are attributed to the industrial inputs to this WWTP, which was known to receive some discharges from chemical, food and textile industries. The sludge samples of I-1 WWTP showed the lowest levels of contamination among other samples in this study. This WWTP serves the

textile sector, mainly manufacturing towels and bed sheets. I-2 WWTP, on the other hand, had much greater BDE-209 levels. The level observed with GC-μECD was also verified with GC-MS analysis. BDE-209 concentrations were quantified at same order of magnitude with both seasons' samples by GC-MS. This huge amount of BDE-209 accumulated in sludge is attributable to the products manufactured in the industries that the WWTP serves for. The main production for this district is based on automotive industry, its accessories, upholstery for car cushions and insulation materials. These are among the major usage areas of PBDEs. Although the information on the inventory of PBDEs in Turkey is based on estimates for sectors, it was revealed that 200 tons of PBDEs can be expected annually due to transport sector (Ministry of Environment and Urbanization, 2014). The critically high level of BDE-209 found in this WWTP sludge is crucial since it demonstrates the direct usage of PBDEs in Turkey. The differences observed between urban and industrial WWTP sludge samples revealed that urban WWTPs reflect the BFR-applied commercial products usage in domestic and residential areas, while industrial WWTPs showed the usage of PBDEs in the production of specific goods in Turkey.

Seasonal variations in PBDE congeners showed similar behavior in all sludge samples: cold season samples showed higher total PBDE concentrations than warm season samples. The reason for this observation might be the PBDE load contribution of urban runoff to the treatment plants due to relatively higher amount of precipitation in cold seasons. Additionally, wet deposition of atmospheric particles onto which PBDEs can sorb might be another effect of precipitation. For industrial plants, another reason of seasonal concentration change could be seasonal production of facilities within the OID. However, when the monthly consumption amounts of electricity, water and natural gas of the OIDs were examined, no net trends were observed, indicating continuous manufacturing and hence wastewater generation throughout the year.

Table 4-8. PBDE levels in ng/g dw (average ± standard deviation, *n*=5) in urban treatment plant sludge samples*.

		U-1 - Cold	U-1 - Warm	U-2 - Cold	U-2 - Warm
Tri-BDEs	**BDE17**	2.24±1.97	2.96±0.41	3.39±1.10	1.44±0.30
	BDE28	3.50±1.92	3.99±1.26	3.18±1.49	2.35±0.65
Tetra-BDEs	**BDE49**	2.12±0.38	1.93±0.53	3.90±0.67	3.11±1.23
	BDE47	2.94±1.46	3.23±2.02	3.41±1.35	3.39±0.82
	BDE66	1.92±1.04	1.77±0.67	5.19±1.81	3.51±0.96
Penta-BDEs	**BDE100**	2.48±1.68	2.70±1.58	2.87±1.16	1.62±0.50
	BDE99	5.98±1.90	7.74±0.93	6.78±1.49	5.00±2.47
Hexa-BDEs	**BDE154**	1.40±1.19	3.84±0.69	1.45±0.47	1.36±0.78
	BDE153	2.12±1.31	2.63±0.97	2.43±1.02	1.61±0.49
Hepta-BDE	**BDE183**	0.83±0.73	1.42±0.83	1.84±0.51	1.29±0.65
Octa-BDEs	**BDE202**	BDL	BDL	3.22±2.70	1.40±0.71
	BDE201	12.95±5.86	20.48±6.58	11.94±5.62	5.14±2.12
	BDE204/197	9.47±2.77	6.98±2.45	5.00±2.14	7.14±1.69
	BDE198/199/ 200/203	BDL	BDL	4.02±1.64	3.92±1.78
	BDE196	1.67±1.52	BDL	1.90±1.11	1.43±0.83
	BDE205	BDL	BDL	3.18±0.62	BDL
	BDE194	13.21±1.81	12.74±5.08	8.53±3.25	5.48±1.89
	BDE195	BDL	BDL	BDL	BDL
Nona-BDEs	**BDE208**	BDL	BDL	BDL	BDL
	BDE207	6.67±1.25	5.59±0.90	8.83±2.92	8.15±1.55
	BDE206	141.12±57.12	34.02±12.10	154.02±32.99	118.67±40.69
Deca-BDE	**BDE-209**	212.7±36.3	197.5±21.2	424.2±90.8	381.8±20.5
Sum of tri-BDEs		5.29±2.05	6.46±0.61	6.03±2.18	3.58±0.87
Sum of tetra-BDEs		6.87±1.55	6.21±1.74	12.11±3.6	10.0±2.39
Sum of penta-BDEs		8.47±2.63	9.90±1.61	9.65±2.12	6.61±2.74
Sum of hexa-BDEs		3.36±1.93	6.15±1.13	3.87±1.16	2.96±1.19
Sum of octa-BDEs		38.94±9.52	42.61±14.90	35.61±12.9	28.20±6.76
Sum of nona-BDEs		144.84±57.44	38.53±12.67	166.78±34.3	131.25±39.6
Sum of tri- to nona-BDEs		205.72±55.00	108.23±26.44	232.88±47.10	185.14±32.48
Sum of all BDEs		423.46±91.42	300.06±46.4	655.37±122.34	575.7±75.97

*BDL: Below detection limit.

Table 4-9. PBDE levels in ng/g dw (average ± standard deviation, *n*=5) in industrial treatment plant sludge samples*.

		I-1 -Cold	I-1 -Warm	I-2 -Cold	I-2 -Warm
Tri-BDEs	**BDE17**	1.29±0.81	0.68±0.17	4.68±1.04	5.08±1.94
	BDE28	0.83±0.45	1.19±0.14	4.67±0.64	7.50±1.16
Tetra-BDEs	**BDE49**	1.50±0.56	1.09±0.52	7.61±1.40	8.21±0.81
	BDE47	1.94±1.21	0.88±0.57	3.84±1.01	4.42±1.14
	BDE66	2.12±0.65	1.22±0.72	BDL	BDL
Penta-BDEs	**BDE100**	1.39±0.89	0.97±0.75	1.95±1.19	3.78±2.30
	BDE99	1.65±0.85	2.21±1.24	35.27±6.51	6.03±2.45
Hexa-BDEs	**BDE154**	1.91±0.97	1.16±0.93	4.05±1.43	4.31±2.25
	BDE153	1.09±0.82	1.69±0.96	4.30±3.74	6.33±3.12
Hepta-BDE	**BDE183**	BDL	0.78±0.45	8.62±4.65	6.99±2.05
Octa-BDEs	**BDE202**	BDL	BDL	3.37±1.05	BDL
	BDE201	0.82±0.27	1.19±0.13	10.08±1.80	17.83±5.47
	BDE204 /197	BDL	BDL	9.69±2.45	11.59±5.92
	BDE198/199/ 200/203	BDL	BDL	33.21±9.37	39.97±10.84
	BDE196	BDL	BDL	37.09±9.44	33.90±8.25
	BDE205	BDL	BDL	BDL	BDL
	BDE194	BDL	BDL	BDL	BDL
	BDE195	BDL	BDL	BDL	BDL
Nona-BDEs	**BDE208**	BDL	BDL	111.0±40.09	160.16±79.59
	BDE207	BDL	BDL	442.0±155.2	537.22±250.18
	BDE206	BDL	BDL	2140.4±777.2	2786.7±1263.0
Deca-BDE	**BDE-209**	129.0±23.5	44.0±5.6	$2.46*10^7$±$0.7*10^7$	$3.66*10^6$±$0.9*10^6$
Sum of tri-BDEs		1.95±0.83	1.68±0.57	9.35±1.40	12.07±2.64
Sum of tetra-BDEs		5.41±1.23	3.04±1.18	11.45±1.90	11.81±3.45
Sum of penta-BDEs		3.03±1.70	3.07±1.09	36.90±7.67	8.86±4.67
Sum of hexa-BDEs		3.00±1.70	2.50±1.08	8.34±4.91	10.21±5.27
Sum of octa-BDEs		0.82±0.27	1.19±0.13	91.51±15.01	101.51±27.9
Sum of nona-BDEs		-	-	2693.5±967.0	3484.0±1589.6
Sum of tri- to nona-BDEs		24.85±5.71	22.0±2.55	2871.0±978.6	3649.1±1609.0
Sum of all BDEs		152.75±25.77	66.93±7.63	$2.46*10^7$±$0.7*10^7$	$3.66*10^6$±$0.9*10^6$

*BDL: Below detection limit.

As can be seen from the tables, urban WWTPs showed very similar levels and I-1 WWTP had the lowest level among others. Also, the cumulative percent distribution of BDE homologs is presented in Figure 4-2, together with that of commercial PBDE mixtures (La Guardia et al., 2006), and Turkey's background PBDE profiles (Jarkovský et al., 2015; Odabasi et al., 2015b). When PBDE congener distribution of sludge samples for cold and warm seasons were examined, it can be observed that percent contribution of congeners were similar for same WWTPs.

A number of deductions may be made based on the similarity of congener distributions of sludges versus commercial mixtures and background profiles. Contribution of deca-mixture is apparent for urban WWTPs since deca-BDE was the most abundant congener in the sludge samples, as in previous studies (Daso et al., 2012; Gorga et al., 2013; Lee et al., 2014b). Presence of lower brominated congeners in the urban sludge samples revealed also the usage of penta-, and octa-, besides deca-mixture-treated household products; such as TV/PC housings, plastics, and furniture textiles (Alaee et al., 2003; USEPA, 2010). Also, I-2 samples obviously showed deca-mixture use during production of upholstery for car cushions, automotive industry accosories, and insulation materials in the OID. Deca-mixture is typically used in polymeric materials for construction and transportation sectors (Alaee et al., 2003), which this particular OID serves for.

I-1 WWTP sludge sample congener profile, on the other hand, resembles that of Turkey's background samples, hence it can be speculated that the appearance of PBDE congeners in this WWTP represents background contamination, indicating that PBDE mixtures may not have been used by the OID facilities. As mentioned before, main products of this OID are towels and bed sheets, in which BFRs are not typically used. Here, it is important to note that nona- and octa-BDEs were not measured in background air and soil samples in the corresponding studies (Jarkovský et al., 2015; Odabasi et al., 2015b). They only measured indicator PBDEs. However, in this study, nona-, and octa-BDEs were measured, where nona-BDEs were below detection limit for I-1 WWTP.

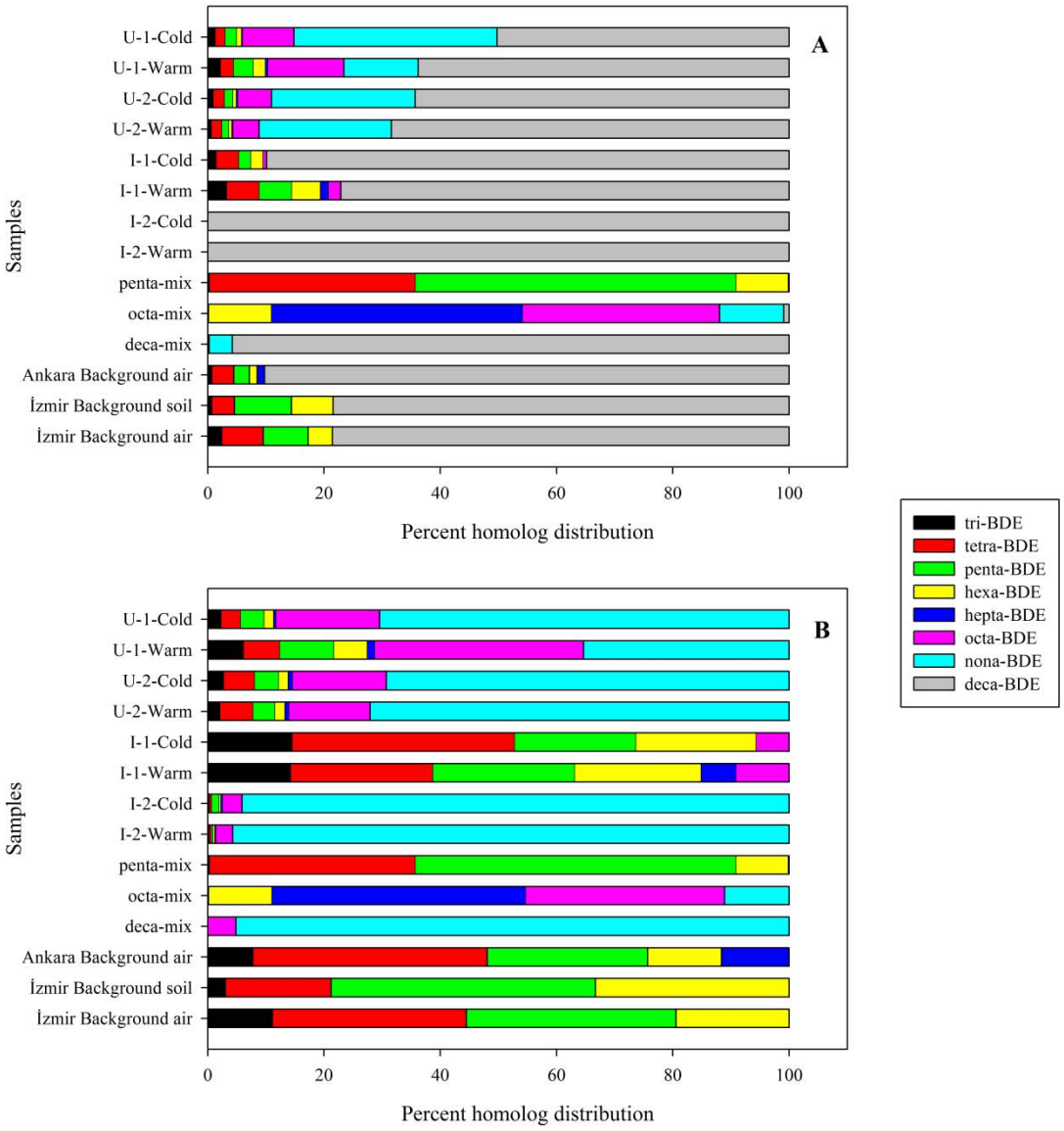


Figure 4-2. PBDE homolog distribution of sludge samples A) for all homologs, and B) excluding deca-BDE. Commercial mixtures penta-, octa-, and deca- are formed from the congener profiles presented in La Guardia et al., 2006, Ankara background air distribution is formed via Genasis website (Jarkovský et al., 2015), İzmir background PBDE profiles are deriven from concentrations presented in Odabaşı et al. 2015.

Mainly, nona-BDEs were abundant in samples after deca-BDE, except I-1. Especially, BDE-206 showed the highest concentration among all congeners in U-1 and U-2 WWTPs. These treatment plants have anaerobic digesters for sludge treatment, and BDE-206 is the main product of anaerobic degradation of BDE-209 (Gerecke et al., 2005). Therefore, abundance of BDE-206 is attributed to the degradation of BDE-209 in anaerobic digesters. Additionally, BDE-194 and -201 were observed to be relatively higher among others. These congeners are also products of anaerobic degradation, and they are not among the frequently monitored congeners. There are few studies monitoring nona- and octa-BDEs in sludge, and a similar trend was also observed (De la Torre et al., 2011; Hwang et al., 2012). For instance, Hwang and colleagues (2012) found BDE-206 as the congener having second highest level following BDE-209 in almost all samples. Additionally, De la Torre et al. (2011) discussed that the level of nona-BDEs were compatible with that of BDE-99. Furthermore, the congener compositions of commercial mixtures identified by La Guardia et al. (2006) revealed the occurrence of BDE-194 and BDE-201 to be at not detected levels or lower compared to other congeners in octa-mixtures. Although BDE-206 was identified to be present in the octa- and deca-BDE mixtures, its weight percent was low (La Guardia et al., 2006). Other than anaerobic treatment processes, aerobic degradation of deca-BDE during biological treatment processes of WWTPs was also demonstrated to produce lower brominated congeners (Stiborova et al., 2015a). However, the solids retention time in aerobic wastewater treatment and/or sludge digestion units might be low for the transformation of BDE-209 via aerobic debromination. Hence, identification of the nona- and octa-BDEs also in this study showed the importance of possible product congeners, treatment schemes in WWTPs potentially causing degradation of higher BDEs, and their possible contribution while evaluating toxicity and/or bioaccumulation in environment. BDE-99 was generally observed to be the predominant congener in environmental samples. Also, in this study, it was found to have a higher level among hepta- and lower brominated congeners.

Levels of HBCDD in sludge samples are shown in Table 4-10. The predominant isomer in sludge samples was interchangeably α- and γ-HBCDD, as in other studies

(García-Valcárcel and Tadeo, 2009b; Xiang et al., 2015). γ-HBCDD is the abundant isomer in technical mixture, and generally found dominantly in environmental samples (Covaci et al., 2006). However, α-HBCDD can be predominantly found in living organisms. This variety in abundance was attributed to different biodegradation rates of isomers (Davis et al., 2006; Gerecke et al., 2006). The biodegradation studies on HBCDD also demonstrated the change in the order of degradation rates of isomers depending on the environmental media and conditions, as well as capabilities of microbial consortia present in the media (Davis et al., 2006; Gerecke et al., 2006; Peng et al., 2015). This may also help to explain the change in the abundance of isomers in cold and warm period sludge samples of the same WWTP (e.g. U-2, and I-2).

The lowest total HBCDD level was observed for I-1 WWTP. This was an expected result since this sample demonstrated the lowest PBDE levels as well. However, highest HBCDD concentration was observed for U-2 WWTP sample, different from the PBDE results. This might imply that HBCDD is not directly applied to the manufacturing products of the OID that I-2 WWTP serves for. Therefore, the levels of HBCDD found in urban and industrial plants of this study most probably represented the usage of HBCDD-applied commercial products; such as, insulation boards, electrical and electronic equipments, upholstery fabric, and bed mattress (European Commission, 2008). Similar to the seasonal PBDE concentration change of sludge samples, cold season samples showed higher HBCDD levels for U-2 and I-2 WWTPs. For two sludge samples, there was a net concentration difference between cold and warm season, cold season being approximately five to six times greater. U-1 WWTP, on the other hand, showed an opposite ratio where warm season sample was approximately three times that of cold sample concentrations. A clear reason for opposite ratios between seasons observed in these WWTPs could not be identified. For I-1 WWTP, both samples had very low levels; therefore, the difference between warm and cold season was deemed not to be noteworthy.

Table 4-10. Levels of HBCDD (ng/g dw) in treatment plant sludge samples. Percentage of each isomer present in the sample is given in paranthesis.

Wastewater Treatment Plant	Sampling Campaign	α-HBCDD	β-HBCDD	γ-HBCDD	Total HBCDD
U-1 WWTP	Cold	36.3 (48)	13.4 (18)	25.7 (34)	75.4
	Warm	130.3 (55)	35.2 (15)	72.2 (30)	237.7
U-2 WWTP	Cold	386.0 (63)	105.7 (17)	124.5 (20)	616.2
	Warm	31.9 (36)	≤10 (-)	56.9 (64)	88.9
I-1 WWTP	Cold	≤10[b] (-)	≤10 (-)	13.1 (100)	13.1
	Warm	≤10 (-)	≤10 (-)	21.0 (100)	21.0
I-2 WWTP	Cold	131.9 (32)	70.2 (17)	213.8 (51)	415.8
	Warm	50.6 (57)	≤10 (-)	37.7 (43)	88.4

4.3.4. Comparative evaluation and implications of findings

The sludge sampling studies from different parts of the world showed comparable values with this study except for I-2 WWTP (Table 4-11). All studies identified concentration ranges for different number and type of WWTPs. Studies did not particularly indicate whether WWTPs were industrial, except the South Korean study (Lee et al., 2014b). Although a very high level was observed in China, I-2 WWTP of this study yielded thousand times greater level. This finding has also an implication in terms of worldwide PBDE levels, clearly indicating that Turkey is one of the hot spots for BDE-209 levels in treatment plant sludges.

It is interesting to note that none of the sludge samples from Turkey yielded not detect for BDE-209. USA is one of the biggest consumers of BFRs and sludge BDE-209 levels indicate this, as the lowest concentration observed were one to two orders of magnitude greater when compared with concentrations from other countries.

Table 4-11. Comparison of BDE-209 levels in dewatered sludge samples of this study with studies worldwide.

Country (Sampling year)	**BDE-209 level (ng/g dw)**	**No. of WWTPs**	**Ref.**
Turkey (2014-2016)	*44.0 – 2.46x10^7*	*4*	*This study*
Spain (2009)	ND – 2303 (Median: 285)	17	(Gorga et al., 2013)
Spain (2010)	ND – 319	6	(Barón et al., 2014)
United States of America (2001)	1420 – 1.4x10^4	94	(Venkatesan and Halden, 2014)
Switzerland (2003–2005)	138 – 617	16	(Kupper et al., 2008)
Sweden (2004–2010)	Median: 513	9	(Olofsson et al., 2012)
United States of America (2006–2010)	636 – 2933	4	(Davis et al., 2012)
South Korea (2006)	5.05 – 9740	8	(Lee et al., 2014b)
South Africa (2010–2011)	ND – 297	1[a]	(Daso et al., 2012)
China	150 – 2.29x10^4	2	(Peng et al., 2009)

[a] The same WWTP was monthly sampled for 6 months.

The level of total PBDEs, except BDE-209, was also compared with levels from various parts of world (Table 4-12). Although total PBDEs given in table reflects various numbers of congeners (since researchers measure a variety of BDE congeners) and analyzed congeners may not overlap, it can be concluded that the sludge samples of this study demonstrated comparable values with other studies.

Table 4-12. Total PBDE levels, excluding BDE-209, observed in this study and other countries.

Country	**No. of WWTPs**	**Total PBDE concentration (ng/g)**	**Ref.**
Turkey	*4*	*Σ_{21} PBDE: 22.0 – 3649.13*	*This study*
Spain	17	Σ_7 PBDE: 20.7 – 2326	(Gorga et al., 2013)
South Africa	1 (6 month)	Σ_7 PBDE: 41.8 – 558	(Daso et al., 2012)
South Korea	15	Σ_{18} PBDE: 0.78 – 642	(Lee et al., 2014b)

Total HBCDD levels in treatment plants of this study demonstrated similar values with European sludge samples (Table 4-13). The treatment plants given in table were municipal and industrial plants. When the maxiumum concentrations observed so far was evaluated, it can be clearly seen that the highest level was in USA (La Guardia et al., 2010).

Table 4-13. Comparison of total HBCDD levels in sludge samples from world studies.

Country	no. of WWTPs	Total HBCDD Concentration (ng/g dw)	Reference
Turkey	*4*	*13.1 – 612.2*	*This study*
Madrid, Spain	19	5.8 – 24.9	(García-Valcárcel and Tadeo, 2009b)
Switzerland (2003-2005)	19	39 – 597	(Kupper et al., 2008)
Czech Republic (2007)	2	23.9 and 19.6	(Stiborova et al., 2015b)
Czech Republic	6	2 – 23	Pulkrabova et al. 2007 cited in Stiborova et al., 2015b
Netherlands	8	<0.6–1300	
England (2002)	5	531–2683	(Morris et al., 2004)
Cork, Ireland (2002)	6	153 – 9120	
Mid-Atlantic U.S. publicly owned WWTPs (2002-2008)	1	324.8 – 400000	(La Guardia et al., 2010)
Korea	11	1.55 – 29604	(Hwang et al., 2012)
Shangai, China (2010)	27	0.10 – 37.2	(Xiang et al., 2015)
Pearl River Delta, China	1	112 – 136	(Feng et al., 2012)
China (2010-2013)	62	0.09 – 65.8	(Zeng et al., 2014)

Studies on the determination of organic contaminants in sludge samples are of significance in terms of compliance with regulatory limits, and proposing new criteria for emerging contaminants. Organic micropollutants such as nonyphenols, PAHs, PCBs, PCDD/Fs, have limit values in sludge regulations of nine out of 27 EU countries (Kelessidis and Stasinakis, 2012). Also, USA has limit values for pesticides and PCBs in biosolids monitoring guidance for disposal methods (USEPA, 1993).

As in other parts of the world, disposal methods of treatment plant sludge in Turkey are landfilling and incineration. In Europe and USA, most frequently used disposal method is application on soil, while that of Turkey is landfilling. For treatment plant sludge to be disposed in landfill, the criteria on the content of sludge given in regulations should be satisfied. These criteria include the metals and inorganic matter content of sludge to be disposed for class I (hazardous) and II (municipal), additionally PCBs, BTEX and phenols for class III (inert wastes) (Ministry of

Environment and Urbanization, 2010b). According to the regulation, treatment plant sludge is admitted to class II type landfills, which do not require investigation on the content of organic pollutants.

The incineration of sludge is also monitored by regulations in Turkey, and emission limit values for organics and dioxins/furans are set for the stack gases of facilities (Ministry of Environment and Urbanization, 2010c).

According to the Regulation on the Application of Domestic and Urban Treatment Plant Sludges on Soil of Turkey, the application of stabilized sludge on soil is permitted when the conditions given in the regulation are satisfied (Ministry of Environment and Urbanization, 2010d). There are limit values for heavy metals, organics, PAHs, PCBs, dioxins/furans, nonylphenols; however there are no limits for BFRs for the land application of stabilized sludge.

Although landfilling has been the mostly preferred method in Turkey so far, personal communication with the plant managers during sampling revealed that treatment plants are currently switching to incineration and/or land application methods. Incineration of PBDE containing wastes lead to emission of polybrominated dibenzodioxins and furans (Söderström and Marklund, 2002). In the last years, Turkey's sludge disposal is geared towards incineration in cement kilns or incineration facilities built for the purpose of sludge disposal. Hence, data produced in this work needs to be taken into account, in order to prevent brominated dioxin/furan formation. On the other hand, application of sludge on soil increases the exposure of humans and animals to BFRs. Another important step in disposal of sludge is drying process. Prior to sending to landfills, sludge can be dried under sunlight, which results in the photodegradation of BFRs possibly into more toxic forms (Ahn et al., 2006; Yu et al., 2015a). Hence, final disposal method for dewatered sludge should be selected based on the results of detailed investigation of the contents of the material so as not to cause further contamination of the environment.

4.4. Conclusion

To conclude, ultrasonic extraction served well for the purposes of this study, as a relatively quick and efficient extraction method for PBDE analysis in solid matrices. Secondly, this preliminary study for the identification of BFR levels in WWTP sludge samples in Turkey revealed the significance of the determination of POPs inventory throughout the country. Specifically, very high PBDE levels found in one of the industrial WWTPs demonstrated the potential severity of the problem. Further studies should be conducted for all major organized industrial district WWTPs and various time intervals, to reveal use and release of PBDEs. Furthermore, contamination of soil and sediments should also be monitored where these WWTPs discharge their wastewaters and dispose their sludges. As regulations come into force for control of POPs, these industries require use of alternative chemicals. Studies like this one acknowledge the importance of inclusion of ubiquitous environmental pollutants in sludge disposal regulations. EU countries and Turkey started adapting the limit levels for micropollutants in sludge, hence it can be suggested that PBDEs may also be included in these disposal regulations.

CHAPTER 5

INVESTIGATION OF BIOREMEDIATION ALTERNATIVES FOR BDE-209 USING SEDIMENT MICROCOSMS: BIOSTIMULATION AND BIOAUGMENTATION

5.1. Introduction

Brominated flame retardants (BFRs) were widely used to protect against fire in most commercial goods throughout the world. One of the most extensively used BFRs was polybrominated diphenyl ethers (PBDEs) (Covaci et al., 2003). PBDEs were used in resin and polymer products that were utilized in electrical and electronic equipments, plastics, automotive industry, insulation materials, furniture and textiles (Alaee et al., 2003; Rahman et al., 2001). This wide usage led PBDEs to become ubiquitous in the environment, especially accumulation in biota and aquatic sediments became a concern. Production and use of some PBDE mixtures was banned and/or voluntarily eliminated in Europe and United States (Abbasi et al., 2015; UNEP, 2010). Although PBDE congeners do not exhibit dioxin-like toxicity, their negative effects on human and wildlife were identified (Dingemans et al., 2016; Stockholm Convention, 2006). Penta- & tetra-BDEs, and hexa- & hepta-BDEs were listed among persistent organic pollutants (POPs) by the Stockholm Convention on POPs due to their persistency, toxicity and long-range transport potential (Stockholm Convention, 2017).

In the last decade, studies were directed towards understanding the fate of PBDEs in the subsurface environment. PBDE transformation has been shown to occur via photodegradation (Ahn et al., 2006; Hua et al., 2003; Söderstrom et al., 2004), zero valent iron reduction (Wu et al., 2016; Xie et al., 2016) and microbial (aerobic and anaerobic) degradation (Huang et al., 2012; Tokarz III et al., 2008) in soil and

sediment systems. Among those, anaerobic degradation mechanism is the focus of this study since a major component of PBDE mixtures, BDE-209 was consistently measured in sediment cores from rivers and bays (Minh et al., 2007; Zhang et al., 2009), where anaerobic conditions prevail.

There are limited number of studies on anaerobic degradation of PBDEs. These studies investigate the degradation kinetics of PBDEs in soil, sediment and sludge under various conditions, e.g. via addition of primers (Gerecke et al., 2005), in the presence of various electron donors (Qiu et al., 2012), and various buffer solutions including mineral salts and vitamins (Huang et al., 2014; Lee and He, 2010; Tokarz III et al., 2008). Anaerobic debromination of PBDEs was also studied in dehalogenating culture media in the absence of a solid phase (He et al., 2006; Robrock et al., 2008; Yen et al., 2009).

All previous studies indicated the importance of revealing PBDE degradation mechanisms in aqueous sediments in order to comprehensively assess remediation of contaminated sites and establish treatment designs. Remediation strategies should take into account the current situation and characteristics of the contaminated sediments, as well as the efficiency of the techniques used regarding the minimization of the risk posed by contamination. Meanwhile, examination of residual toxicity after remediation, and status of bioaccumulative products should be elucidated. The most commonly used remediation techniques include dredging/removal and capping of sediments, which are neither cost-effective nor sustainable (Mulligan et al., 2010). Therefore, in situ chemical and biological treatments are now attracting attention for remediation of POPs contaminated sites. Bioaugmentation and biostimulation are the two techniques frequently used for in situ bioremediation of halogenated organics (Bedard, 2003; Payne et al., 2011). Application of extraneous electron donors, vitamins, or buffer solutions were typically used for biostimulation of PBDE degradation in previous studies. However, introducing a microorganism culture to sediment media for dehalogenation of PBDEs, i.e. bioaugmentation, was not investigated so far.

The aim of the current study is to investigate the effectiveness of bioremediation alternatives for anaerobic debromination of BDE-209. To achieve this aim, sediment microcosms were established to simulate various in situ bioremediation alternatives, which are bioaugmentation, biostimulation and natural attenuation. For bioaugmentation, enrichment culture of *Dehalobium chlorocoercia* strain DF-1 was used, and for biostimulation sodium formate and ethanol were used as the carbon source and electron donor, respectively. DF-1 strain was previously shown to reductively dechlorinate PCBs (Payne et al., 2011), but was not tested for PBDE debromination. Using sodium formate as a carbon source has not been investigated for PBDEs, and it was also used to grow DF-1 in the culture medium. Application of three bioremediation strategies concurrently is expected to provide a comparative assessment of i) the efficiency of each in BDE-209 removal, ii) possible degradation pathways of BDE-209 so that accumulation of product congeners can be evaluated, and iii) rates of degradation.

5.2. Materials and Methods

5.2.1. Chemicals

All solvents (n-hexane (HEX), dichloromethane (DCM), acetone(ACE)) used for analysis, anhydrous sodium sulfate (granular), copper fine powder (<63 μm), and aluminum oxide (0.063-0.200 mm) were purchased from Merck KGaA (Darmstadt, Germany). Individual standards of BDE-209 (2,2',3,3',4,4',5,5',6,6'-BDE), surrogate standard PCB-141 (2,2',3,4,5,5'-CB), internal standard PCB-209 (2,2',3,3',4,4',5,5',6,6'-CB) were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany). PBDE predominant congeners mixture (BDE-CSM) was purchased from AccuStandard (New Haven, USA). A standard mixture of octa-, nona-, and deca-BDEs (BDE-OND), individual standards of BDE-17 (2,2',4-BDE), BDE-49 (2,2',4,5'-BDE) and BDE-66 (2,3',4,4'-BDE) were purchased from Wellington Laboratories (Canada).

5.2.2. Sediment microcosms

The sediments that were used in microcosm studies were collected from a pond in a specially protected forest area (Camkoru National Park) located 110 km northwest of Ankara, Turkey. Sediments were expected to have no previous PBDE contamination as the pond is located close to a passive POPs air monitoring station which consistently show very low POPs concentrations (Jarkovský et al., 2015). Surface sediments under 70 cm water depth were collected from five different points in the pond and were wet-sieved (2 mm) on-site to remove large particles. Samples were transported to the laboratory and stored at 4 °C in glass jars in the dark until use. Moisture content of sediment was analyzed by drying 10 g of sample in 105°C oven for 24 hours, and found to be 36.5±1.53%. Total organic content analysis was then performed via loss-on-ignition procedure, by igniting the sample analyzed for moisture content in 550°C furnace for 4 hours (Heiri et al., 2001), and determined to be 1.43±0.16%.

Some part of collected sediments was air-dried overnight. Air-dried sediments were spiked with 1700 µL of BDE-209 standard in acetone. Spiked dry sediments were mixed completely. Then, wet sediments were added onto spiked dry sediments and a homogeneous mixture was obtained. BDE-209 contaminated sediments were distributed equally among the microcosm bottles to get approximately 3 g wet sediments in each. Similarly, non-spiked control set was established by spiking dry sediment with high grade acetone and mixed with wet sediments. These contamination control microcosm sediments were found to have no PBDE contamination throughout the incubation period. The samples were also confirmed with GC-MS analysis, and hence it can be said that all congeners observed in spiked sediment microcosms were due to degradation of the spiked BDE-209.

Details of microcosm setup are presented in Table 5-1. Microcosms were prepared as sacrificial reactors and operated for 6 months. For each set and each sampling time, duplicate 20 mL reactors were established. All sets were topped with distilled water while the biostimulation set was topped only with an organic medium. In all reactors sediment-to-liquid ratio was kept constant as 3 g:3.5 mL. In bioaugmentation set,

microorganism culture was added in a liquid medium, and volume of added water was adjusted accordingly.

Table 5-1. Details of sediment microcosm sets.

Reactor Type	Reactor Name	BDE-209 spike	Sediment	Topping Liquid Ingredients (volume)
Test microcosms	Natural attenuation	+	+	DI water (3.5 mL)
	Biostimulation	+	+	e- donor & C source rich organic medium (3.5 mL)[a]
	Bioaugmentation	+	+	DF-1 culture (0.5 mL)[b] + DI water (3.0 mL)
Control microcosms	Negative control	+	+	Spent growth medium (0.5 mL)[c] + DI water (3.0 mL)
	Contamination control	-	+	DI water (3.5 mL)
	Sterile	+	+ (Autoclaved)	DI water (3.5 mL)

[a] Prepared as given in Berkaw et al. 1996 and supplied with sodium formate and ethanol.
[b] *Dehalobium chlorocoercia* strain DF-1 culture.
[c] DF-1 medium with no DF-1 cells, obtained by passing the culture medium through 0.22 μm filter.

Organic medium of biostimulation set was prepared by dissolving several vitamins and minerals in water together with various salts under N_2:CO_2 atmosphere, and pH was adjusted to 6.8 (Berkaw et al., 1996). Medium also contained 10 mM of sodium formate and ethanol as the carbon source and electron donor, respectively, to stimulate biodegradation. *Dehalobium chlorocoercia* strain DF-1 used in bioaugmentation set was previously grown in Prof. Dr. Kevin Sowers' Lab of Institute of Marine & Environmental Technology, University of Maryland, Baltimore, MD, USA. This strain was originally enriched from Charleston Harbor sediments, and shown to be successful in reductive dechlorination of PCBs (Payne et

al., 2011). For natural attenuation set, no amendment was made with extraneous substances to sediments.

There were three types of control sets: i) A negative control set was established to serve as a control for bioaugmentation set so that the effects of adding a culture medium without DF-1 cells can be observed. Hence, a spent growth medium was formed by passing DF-1 culture through 0.22 μm filter so that no cells will remain in the medium. ii) A sterile control set was established where microcosms were autoclaved at 120 °C at 1.1 atm pressure for 20 min on three consecutive days to hinder any microbial activity in sediments. iii) A contamination control set was established without any spike of BDE-209 to check for any contamination resulting from incubation conditions in unspiked sediments. All sets were purged with high purity nitrogen stream after closed with Teflon lined septa crimp caps. They were incubated in the dark at 25°C. During sampling, duplicate reactors were opened and all sediments were analyzed. Headspace gas monitoring was not conducted.

The degradation kinetics of BDE-209 was explained by pseudo-first-order model: $C = C_0 e^{-kt}$, where C is the concentration at sampling times (ng/g dry weight), C_0 is the initial BDE-209 concentration (ng/g dw), k is the pseudo-first-order rate constant (d^{-1}), and t is the incubation time (d). Rates are calculated by plotting $ln(C/C_0)$ vs t, and checked using coefficient of determination, R^2. Degradation rate was also evaluated using bromine per diphenyl ether (Br/dp) content of sediments (Eq.1) and calculated as shown in Eq.2. C_i is the concentration of each congener *i*. The number of bromines (n_i) can be total number of bromines for each homolog group or number of *ortho*-/*meta*-/*para*-bromines for each congener. Furthermore, percent reduction in Br/dp is computed as ratio of the difference in Br/dp between days 0 and 180 to Br/dp at time zero.

$$Bromine\ per\ diphenyl\ ether(Br/dp) = \frac{\sum_{i=1}^{10} C_i \times n_i}{\sum_{i=1}^{10} C_i} \quad \text{Eq. (1)}$$

$$Bromine\ removed\ per\ diphenyl\ ether\ per\ day\left(\frac{Br}{dp.day}\right) = \frac{(Br/dp)_{t1} - (Br/dp)_{t2}}{|t_1 - t_2|}$$

Eq. (2)

5.2.3. PBDE extraction and analysis

US EPA method 3550C Ultrasonic Extraction was followed for the extraction of PBDEs from sediments (USEPA, 2007a). Two grams of sample was mixed with equal amount of anhydrous sodium sulfate in 40 mL vials, and spiked with surrogate standard. Samples were extracted in an ultrasonic bath with 30 mL DCM: HEX: ACE mixture (7:7:1 v/v) for 30 minutes twice, after being soaked in the solvent mixture overnight. Sulfur removal was achieved with the addition of copper powder into the extraction solvents. The two extracts were combined and concentrated to 2-5 mL via rotary evaporator. To remove possible interfering organic compounds, the colored extract after concentration step was treated with concentrated sulfuric acid (U.S. EPA Method 3665A). The top clear extract was purified with 0.5 g of alumina (deactivated to 6%) topped with anhydrous sodium sulfate, and eluted with 2 mL of HEX. The collected extract was concentrated to 2 mL by high purity nitrogen stream.

One mL of this extract was spiked with internal standard and analyzed with gas chromatography coupled with micro-cell electron capture detector (Agilent 6890N GC-μECD) with DB-5 MS capillary column (15 m x 0.25 mm ID x 0.10 μm). Instrumental conditions were as follows: Helium was used as the carrier gas with 1.8 mL/min flowrate using a constant flow mode. The make-up gas for the detector was nitrogen with a flowrate of 30 mL/min. The injector and detector temperatures were 250°C and 350°C, respectively. The sample injection was carried out at 1 μL with splitless injection mode. Oven temperature program started at 90 °C, raised at 20°C/min to 310 °C, and hold there for 6 min. PBDE congeners 17, 28, 47, 49, 66, 99, 100, 153, 154, 183, 194,195, 196, 201, 202, 198/199/200/203 (co-eluting peak), 197/204, 205, 206, 207, 208, and 209 were identified according to the retention times given with certificates of standards and previous studies.

Analysis of BDE-209 standard (CAS No:1163-19-5) in GC-μECD revealed nona-BDE peaks, increasing linearly with the concentration of standard analyzed. Hence, analysis of 100 ppb, 200 ppb, and 400 ppb BDE-209 standard solutions five times consecutively resulted in the derivation of ratios for each nona-BDE in the standard. Average ratios were found to be 0.0097, 0.0191, and 0.0214 for BDE-208, -207, and

-206, respectively, all of which has RSDs lower than 15%. Hence, a correction in nona-BDE quantitation was performed by subtracting the value found by multiplying the corresponding ratio with BDE-209 concentration from the chromatogram result of nona-BDE.

5.2.4. QA/QC

Laboratory control samples were analyzed to check the extraction efficiency, and results yielded recoveries of 96.0±6.07% (range: 86-105.7% for *n*=7) for BDE-209, and between 82.0% and 118.4% for all other congeners (*n*=7). The analytical procedure was further validated with analysis of a certified reference material, which was PCB and PBDE contaminated sandy loam sediment (RTC CNS329). For PBDE congeners 47, 99, 100, 153, 154, and 183, recoveries were 101.3±5.8%, 88.3±2.3%, 84.1±1.5%, 87.7±1.9%, 88.9±2.8%, and 101.4±5.2% (average±standard deviation for triplicate samples), respectively. Method detection limits for single congeners were in the range of 0.06 ppb (BDE-47) to 3.94 ppb (BDE-209), and limit of quantitation were in the range of 0.19 ppb (BDE-47) to 12.53 ppb (BDE-209). Blanks were analyzed in every batch of 12 samples, and no peaks were detected during analysis. Average surrogate recovery for the whole data set was 95.5±9.55% (range: 64.8 – 120.5%). No surrogate and/or blank correction was deemed necessary.

5.3. Results and Discussion

5.3.1. Degradation of BDE-209 in sediments

Initial BDE-209 concentrations of microcosms were in the range of 625 – 725 ng/g dry sediment. The change in BDE-209 concentrations in different microcosm sets and corresponding ratios of BDE-209 remaining and total products formed in reactors is given in Figure 5-1.

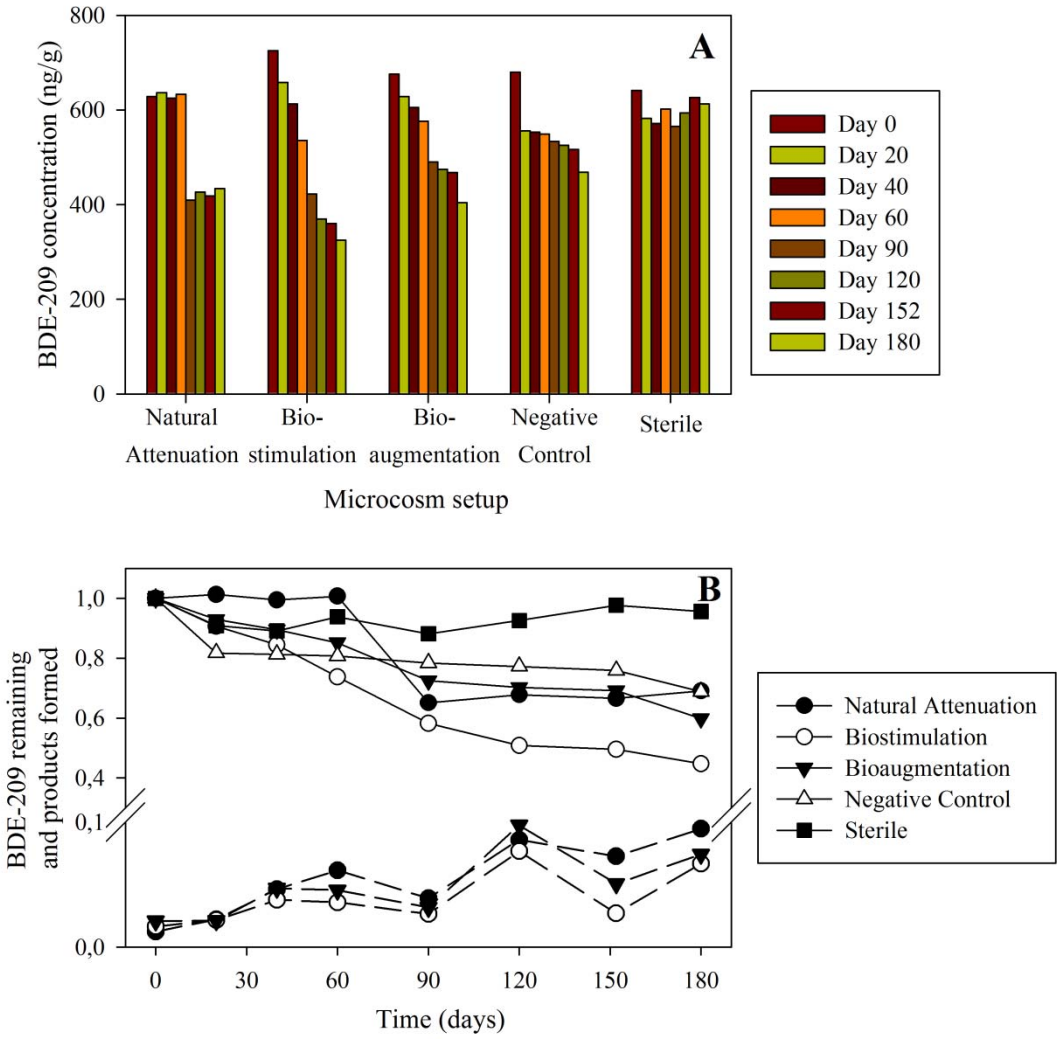


Figure 5-1. A) BDE-209 concentration (ng/g dw) with respect to time for each microcosm, B) ratio of remaining BDE-209 to initial concentration (given as solid lines) and ratio of total products formed to initial BDE-209 concentration (given as dashed lines), in each microcosm set. Values represent average of duplicate reactors.

There was no decrease in BDE-209 level in natural attenuation set up to 90 days, which was interpreted as an acclimation period for indigenous species in the sediment. Since the sediments were not historically contaminated with PBDEs, the indigenous species needed to adapt PBDEs as an electron acceptor for microbial degradation. However, at t=90 days, a reduction in concentration was observed which remained unchanging on the following sampling days, achieving a total of 30.9% decrease. A similar observation of plateau BDE-209 level, after an initial degradation, was reported by Shin et al. (2010) in sewage sludge reactors. On the other hand, in biostimulation and bioaugmentation sets, there was a steady BDE-209 decrease throughout the course of the incubation period reaching 55.3% and 40.2% after 180 days, respectively. Similar reduction percentages were observed in the literature. For example, Song and colleagues (2015) showed 39.7% reduction of BDE-209 in soil microcosms with lactate as carbon source after 90 days. Also, 11% reduction of BDE-209 in 90 days was observed by Qiu and colleagues with and without addition of ethanol as an electron donor, with the highest decrease observed in the first 30 days for methanol and ethanol (Qiu et al., 2012).

Biostimulation and bioaugmentation are preferred strategies for bioremediation of contaminated sediments, when intrinsic capabilities of sediments are not sufficient for microbial degradation. Overall, natural attenuation set of this study exhibited a decline in PBDE levels, yet the set could not maintain continuous degradation. This behavior in sediments results in accumulation of PBDEs in environment, as observed in core sediment samples (Minh et al., 2007; Zhang et al., 2009). Hence, natural attenuation may not be an effective bioremediation strategy. Among all, biostimulation showed the highest percent reduction in BDE-209 level at the end of incubation period, and can be speculated to be most effective bioremediation alternative under these conditions.

For all three sets, the greatest percent removal of BDE-209 was achieved between days 60 and 90. For biostimulation and bioaugmentation, reduction percentage declined after 90 days, and rised again between days 152 and 180. As can be observed from Figure 5-1, formation of products followed a somewhat similar

pattern: products showed an increasing trend in the reactors reaching a peak at day 120. Then, a reduction in product formation was observed for biostimulation and bioaugmentation, followed by a repeated increase at day 180. This behavior can be explained by the attack of microorganisms on BDE-209 initially until sufficient lower brominated congeners were accumulated, after which microorganisms possibly preferred to attack lower brominated BDEs. Hence, BDE-209 decrease slowed down after day 90, and when lower brominated congeners were consumed, microorganisms again started attacking BDE-209. In the literature, there is no clear indication that lower brominated BDEs are degraded faster (Huang et al., 2014; Zhu et al., 2014) or preferentially, when compared to BDE-209. For natural attenuation set, on the other hand, accumulation of product congeners had a somewhat different trend where after an initial incline, product congeners did not accumulate after day 120.

The negative control set, prepared to distinguish the effect of organic media added with the microbial culture in bioaugmentation set, demonstrated a sharp decline at the beginning, but changed slightly afterwards. The initial reduction can be attributed to the triggering effect of organic media added. Consumption of substrates after 20 days may explain the following modest decrease of BDE-209 levels. The reduction in BDE-209 reached 31.2% at t=180 days in this control set. Total percent reduction in negative control and natural attenuation sets were similar, nevertheless their trends differed possibly due to the addition of culture medium without bacterial cells to the negative control set. Although BDE-209 concentration showed small variations in sterile reactors, statistical analysis yielded no significant change in time (analysis of variance -within subject design, $F(1,7) = .35$, $p > .05$). Furthermore, the variation of BDE-209 concentration in sterile reactors is within the variation observed (RSD<15%) in a mixing test performed to give information on homogeneity of concentrations during preparation of sacrificial microcosms. No BDE-209 or its products were detected at any time in the contamination control set.

5.3.2. Debromination pathways of BDE-209

BDE-209 debromination in microcosms resulted in the formation of nona- through tri-BDEs in 180 days. Debromination pathways proposed for the bioremediation

alternatives tested are presented in Figure 5-2. Formation of lower brominated congeners followed different patterns for each treatment. As can be seen from Figure 5-2, some of the products were observed in only one set, such BDE-183 (observed in biostimulation set only), while others, such as BDE-99 or BDE-100 was observed in all sets. In addition, there was a hepta-BDE peak observed in all sets however, it could not be identified due to lack of individual standards. It is expected to be one of the previously identified hepta-BDEs: BDE-180, BDE-182 or BDE-184 (Huang et al., 2014; Robrock et al., 2008; Tokarz III et al., 2008), all of which can lead to the formation of either BDE-153 or BDE-154. For the first time in this study, a new pathway for the formation of BDE-194, i.e., BDE-206 to BDE-194, was identified in all reactors. Additionally, BDE-208 transformation to BDE-202 was confirmed as Gerecke and colleagues (2005) previously proposed. Formation of BDE-201 from either BDE-207 or BDE-208 was also shown in sediments, similar to the observations of Orihel et al. (2016). Other debromination pathways observed were also shown to occur in previous sediment microcosms (Huang et al., 2014; Orihel et al., 2016; Tokarz III et al., 2008) and anaerobic culture media (He et al., 2006; Robrock et al., 2008).

At the end of incubation period, it was observed that the accumulated congeners were different in the microcosm sets. In the natural attenuation set, penta-BDEs (99 & 100) accumulated starting from day 90. These were noted to be the congeners that are observed to be dominant in the environment, following BDE-209 (ATSDR, 2004; Hale et al., 2006). Apart from their wide usage due to their presence in the penta-BDE mixture (Alaee et al., 2003), our results indicate that their abundance in the environment can also be attributed to anaerobic debromination of BDE-209. In the biostimulation set, on the other hand, the accumulated congener was identified to be BDE-47. Its first appearance was at day 40, and its concentration showed an increasing trend throughout the incubation period. Lastly, the bioaugmentation set indicated accumulation of tri-BDEs, i.e., BDE-28 and BDE-17. BDE-28 appeared at day 60 initially, but then disappeared until reappearing on day 180. Hence, it can be speculated that BDE-28 further debrominated, but its product(s) could not be identified in this study. BDE-17, on the other hand, only emerged at day 180. As the

accumulated congeners and identified pathways in Figure 5-2 indicate, bioaugmentation set yielded the greatest extent of debromination among all sets, although it showed a lower percent degradation of BDE-209 compared to the biostimulation set. It was the first time that *Dehalobium chlorocoercia* strain DF-1 was investigated for reductive debromination of a PBDE congener, and results indicate that DF-1 can indeed have a potential to be used for bioaugmentation of PBDE contaminated sediments.

For the bioremediation scenarios evaluated in this study, various accumulating congeners were identified, all of which are bioaccumulative congeners. According to Stockholm Convention's screening criteria for bioaccumulation (Annex D), congeners having bioconcentration or bioaccumulation factor greater than 5000 are called "bioaccumulative" in aquatic species (Stockholm Convention, 2017). Except BDE-85, all tri-, tetra-, and penta-BDEs exceed this criteria (USEPA, 2010). Hence, in most aquatic and also terrestrial species, PBDE congeners predominantly found are BDE-47, 99, 100, 153, and 154. They may also biomagnify in some species, for instance in marine food web (Shao et al., 2016). BDE-47 was also shown, in recent studies, as one of the predominant congeners in human serum (Makey et al., 2016) and blood (Fromme et al., 2016). Therefore, identification of PBDE biodegradation pathways and accumulative congeners in sediments facilitates in-depth examination of the fate of bioaccumulative congeners and perhaps risk associated with such contaminated sites.

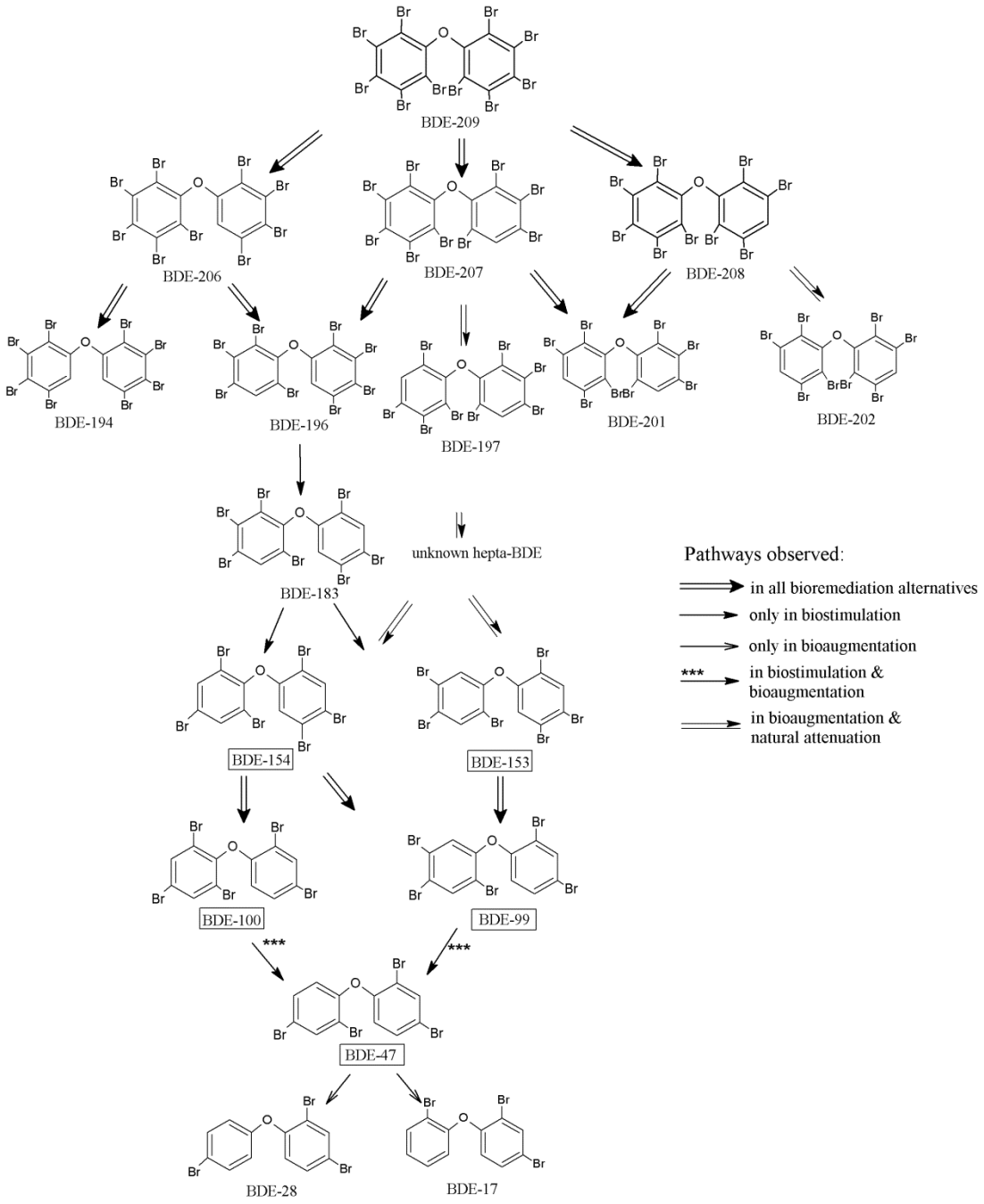


Figure 5-2. Reductive debromination pathways of BDE-209 and its product congeners. Different arrows show pathways occurring in various bioremediation alternatives. Congener numbers in boxes indicate bioaccumulative congeners (Stockholm Convention, 2006).

Previous degradation studies could not agree on the preference of bromine position to be removed. For example, Robrock and colleagues (2009) concluded that although different consortium showed different products in anaerobic cultures, they observed preferential removal of *para* and *meta* bromines. Similarly, formation of BDE-101 via *para*-Br removal from BDE-153 had the highest fraction among other product congeners (Zhu et al., 2014). Nevertheless, they also observed that removal of *ortho* and *para* substituted Br was higher in freshwater sediments than that in marine sediments, indicating the ability of bacterial species to remove *ortho*-Br from BDE-153 in freshwater sediments (Zhu et al., 2014). Hence, different microorganism species and/or different conditions of sediments can demonstrate distinct debromination position selectivity. Within the scope of this study, a total of 20 pathways were identified (Figure 5-2). Among those, debromination followed the order *meta*-> *ortho*-> *para*-Br removal based on the number of pathways. This was consistent with other sediment biodegradation studies revealing preferential removal from *ortho* and *meta* positions (Orihel et al., 2016; Tokarz III et al., 2008), and also with an abiotic degradation study (Wu et al., 2016). Most specific example of low preference of *para*-Br removal was the formation of BDE-208. In all sets, BDE-208 was observed below detection limit until day 120. Hence, it can be speculated that *para* Br removal from BDE-209 was much less preferred compared to *ortho* and *meta* Br removal for formation of the first products.

The emergence of homolog concentrations of PBDE congeners throughout the incubation period is presented in

Figure 5-3 for each bioremediation alternative. Note that in each set, nona-BDEs were present even at day 0. This is due to their presence in deca-BDE standard used in spiking sediments. The standard was found to contain 0.97% BDE-208, 1.91% BDE-207, and 2.14% BDE-206, which is similar to commercial deca-BDE content, as was also mentioned by Alaee et al. (2003) and Tokarz et al. (2008). Therefore, a correction was performed prior to presentation of products to exclude nona-BDEs resulting from the standard.

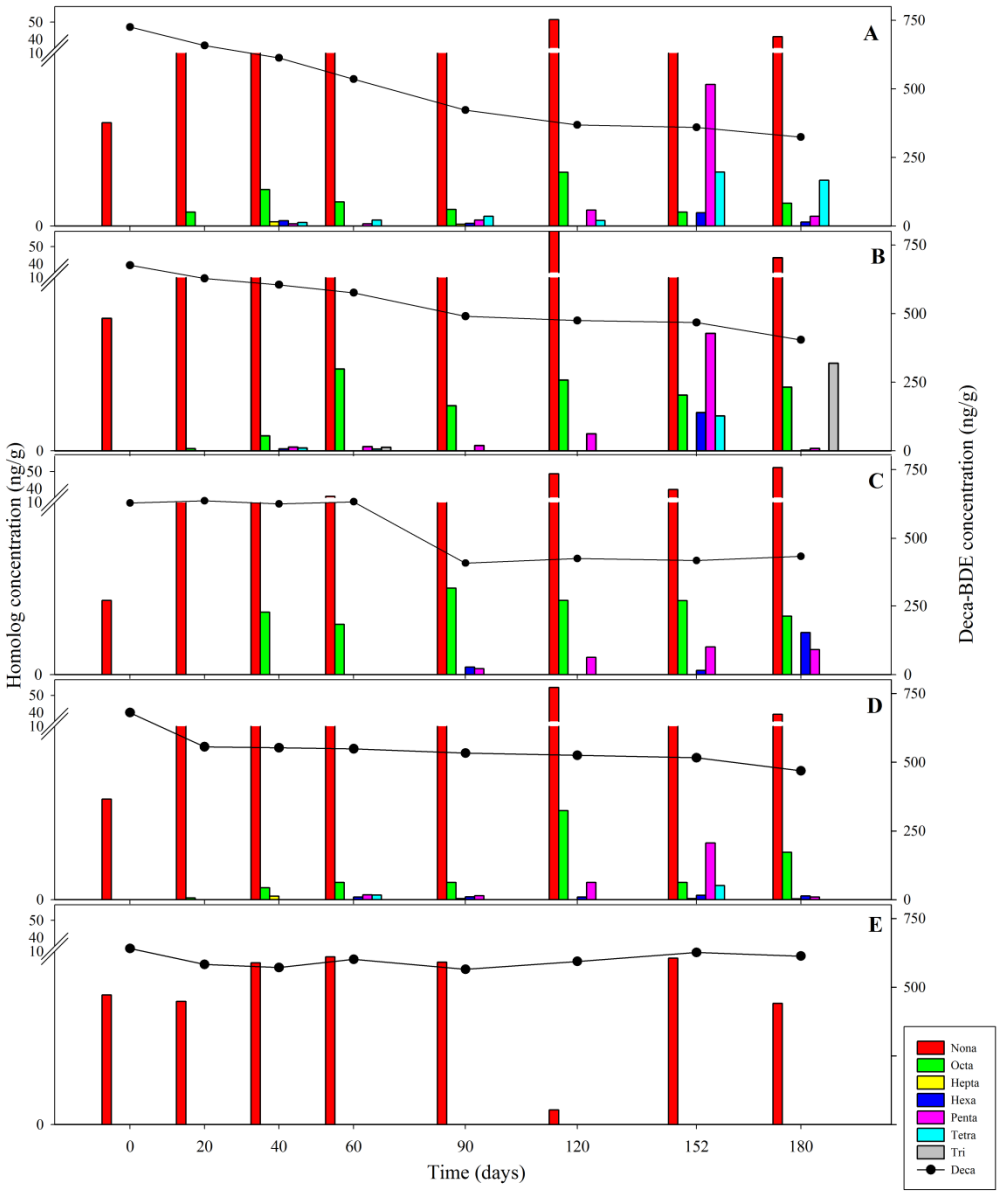


Figure 5-3. Homolog concentrations (ng/g dw) PBDE congeners throughout the incubation period for A) biostimulation, B) bioaugmentation, C) natural attenuation, D) negative control, and E) sterile microcosms. Deca-BDE concentration is presented by solid line with circles and can be read from the right axis. Nona-BDEs= 206+207+208, Octa-BDEs= 194+195+196+197/204+ 198/199/200/203+201+202+205, Hepta-BDE= 183, Hexa-BDEs= 153+154, Penta-BDEs= 99+100, Tetra-BDEs= 47+49+66, Tri-BDEs=17+28.

As can be seen in

Figure 5-3, products of BDE-209 degradation emerged in all reactors to some degree (except the sterile set), with increasing proportion of octa- and lower brominated BDEs as incubation time progressed. In all sets, there was an accumulation of nona-BDEs at day 120, which were then converted to lower brominated congeners on following days. In the biostimulation set, the occurrence of octa-BDEs started at day 20 while lower brominated congeners were produced by day 40. Although penta-BDEs were steadily formed during incubation, they reached a peak at day 152. A similar trend in products formation was observed in the bioaugmentation set. In this set, at day 60, tri-BDEs were formed, reaching the highest level at day 180. In the natural attenuation set, although BDE-209 reduction was apparent at day 90, occurrence of octa-BDEs started at day 40. Lower brominated congeners, i.e. hexa- and penta-BDEs, observed at day 90 onwards. Negative control set showed an increase in lower brominated homologs at day 152, however the extent of debromination and the level of lower brominated homologs at day 180 was much smaller when compared to all other microcosms. Hence, comparison of negative control and bioaugmentation clearly indicate that DF-1 (as opposed to the culture media) is responsible for the greater extent of debromination of BDE-209 all the way to tri-BDEs, which was not observed in any other microcosm. Although a general increasing trend was evident, total concentration of debromination products did not consistently increase with time in the reactors. The main reason for this observation could be that considerable differences in debromination rates of BDE-209 when compared to debromination rates of lower brominated congeners are expected. Furthermore, some of the congeners, e.g. BDE-196 and BDE-197, had their highest concentration at day 120, whereas others (e.g. BDE-201, BDE-194, BDE-153, BDE-154) had higher concentrations at day 152. Hence, variations in formation and transformation rates of lower brominated congeners might be causing the observation of homolog products that do not necessarily have a consistent increasing/decreasing trend. Another reason can be that only a selected number of congeners are analyzed for each homolog. Therefore, accumulation of other congeners belonging to the same homolog group, if any, was not monitored.

5.3.3. BDE-209 degradation rates

Rate of BDE-209 degradation in sediment microcosms can be explained by pseudo-first-order reaction kinetics. The calculated rate constants are given in Table 5-2, together with all available relevant literature values. Highest rate observed in this study was for the biostimulation set. Bioaugmentation and natural attenuation sets demonstrated similar debromination rates, however natural attenuation set yielded a lower R^2 value for the fit of data to pseudo-first-order kinetics.

When previous studies conducted in sediments were examined, it can be observed that the reported range for degradation of BDE-209 had two orders of magnitude variation between minimum and maximum values (0.00013 – 0.041 d^{-1}). Furthermore, Karakas and Imamoglu (2017) estimated debromination rate constants associated with laboratory soil BDE data via an anaerobic dehalogenation model, and reported a range of 0.0003 to 0.0241 d^{-1}, where a min of 0.001 d^{-1} to a max of 0.024 d^{-1} was concluded as advisable to be used in modeling studies (Karakas and Imamoglu, 2017). The rates found in the present study agree well with these studies such that rates observed in this study correspond to the middle of the reported ranges in terms of order of magnitudes. Variations in incubation conditions and presence of microbial species, as well as the characteristics of sediments can be effective in achieving degradation. On the other hand, environmental sediments can also demonstrate distinct degradation rates than laboratory studies. For instance, Orihel et al. (2016) state that sediment microcosms operated under environmental conditions show so little degradation that a rate constant cannot be calculated. Furthermore, the rates of BDE-209 degradation in this study were slightly higher than the ones estimated by modeling studies (Puzyn et al., 2011). Therefore, the rates identified in this study can provide valuable input to environmental fate and transport modeling studies that incorporate biodegradation in sediments (Karakas, 2016), or to explain debromination patterns in environmental sediments (Zou et al., 2014).

Table 5-2. Pseudo-first-order degradation rate constants for anaerobic debromination of BDE-209 in different studies.

Media & Conditions	***k*** **(d^{-1})**	**$t_{1/2}$ (d)**	**R^2**	**Reference**
Lake sediment microcosms without any substrates	0.0025	277.2	0.71	*This study*
Lake sediment microcosms with organic medium (sodium formate+ethanol)	0.0049	141.4	0.96	
Lake sediment microcosms with *Dehalobium chlorocoercia* strain DF-1	0.0028	247.5	0.96	
Lake sediment microcosms with phosphate buffer + methanol+dextrose	0.00013	5162	-	Tokarz III et al., 2008
River sediment microcosms without substrates	0.022	31.5	0.85	Huang et al., 2014
River sediment microcosms with substrates (surfactants, electron donors, zero valent iron, etc.)	0.025 – 0.041	16.9 – 27.7	0.79 – 0.98	
Environmental sediments (estimated by modeling)	0.001 – 0.002	416 – 1250	-	Puzyn et al., 2011
Sewage sludge incubated with a culture medium	0.0011 – 0.0012	577.5 – 630	0.99	Shih et al., 2012
Sewage sludge incubated with primers	0.001	693	-	Gerecke et al., 2005

Another way to examine anaerobic degradation rate in sediments is to examine bromine per diphenyl ether (Br/dp) change with time. Monitoring Br/dp in microcosms also clearly show debromination during incubation, except for the sterile set (Figure 5-4). The Br/dp calculation was also performed for different bromine substitutions; i.e. *ortho*-, *meta*-, and *para*-Br/dp, which yield interesting results. Biostimulation set revealed only 0.82% removal of *para*-Br/dp, while that of *meta*- and *ortho*- was 2.11% and 2.08%, respectively. On the other hand, bioaugmentation set yielded 1.02%, 1.27%, 1.55%, removal for *para*-, *meta*- and *ortho*-Br/dp removal, respectively. Similarly, natural attenuation microcosms showed 1.05%, 1.44% and 1.58% removal in *para*-, *meta*- and *ortho*-Br/dp removal, respectively. Hence, it is interesting to note that in bioaugmentation and natural attenuation sets, *para*-Br removal, although less, was comparable to removal of Br from other positions, while in the biostimulation set, it was much lower. Biostimulation was observed to clearly enhance Br/dp removal from *meta*- and *ortho*- positions. This finding indeed indicate that tailoring of debromination pathways using selected extraneous substances is possible and warrants further study.

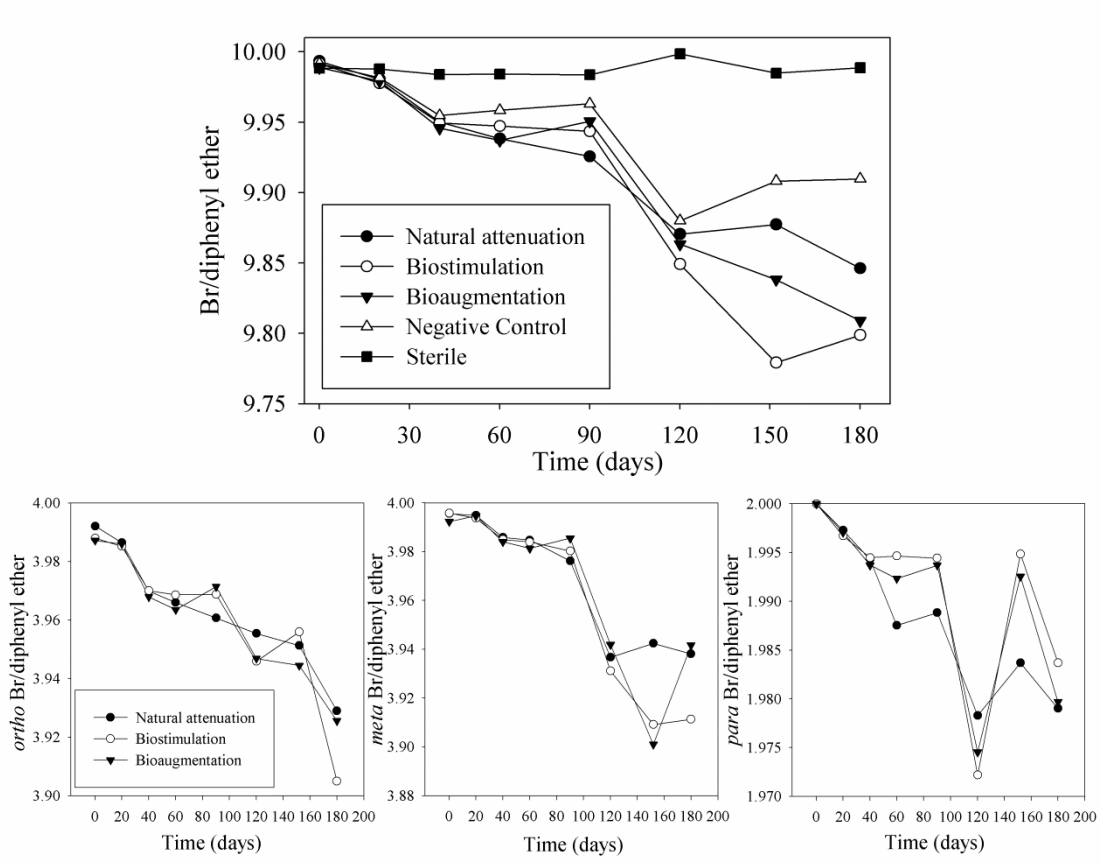


Figure 5-4. Bromine per diphenyl ether change during incubation period. The above graph gives total bromines and the below graphs show *ortho*-, *meta*-, and *para*-bromines per diphenyl ether in three bioremediation alternatives.

Additionally, anaerobic debromination rate in sediments can be calculated with Br/dp/day, as frequently reported for anaerobic dechlorination of PCBs (Abramowicz et al., 1993; Magar et al., 2005). The results yielded 0.0011, 0.0010, and 0.0008 Br/dp/day, for biostimulation, bioaugmentation and natural attenuation sets, respectively. The highest rates were observed between days 90 and 120. The rates for this period were calculated as 0.0031, 0.0029, and 0.0018 Br/dp/d for biostimulation, bioaugmentation and natural attenuation, respectively.

Br/dp/day calculation was not performed in any PBDE studies so far, hence rates were compared with those associated with anaerobic dechlorination of PCBs, i.e., chlorines per biphenyl per day (Cl/bp/d). Freshly spiked sediment microcosm studies

like ours yielded 0.00497 Cl/bp/d for Hudson river sediments (Abramowicz et al., 1993), and 0.0043 Cl/bp/d for Baltimore Harbor sediments (Demirtepe et al., 2015), which are 4 to 5 times higher than our biostimulation set. On the other hand, aged environmental sediments exhibited 0.0019 Cl/bp/d for Hudson river sediments (Abramowicz et al., 1993), and 0.000258 Cl/bp/d for Lake Hartwell sediments (Magar et al., 2005). As discussed earlier, laboratory studies are expected to demonstrate a higher rate than environmental sediments due to presence of more favorable environmental conditions, such as constant temperature and organic content. Therefore, for PBDEs even lower degradation rates may be expected in natural sediments.

5.4. Conclusions

In this study, three remediation approaches were investigated at laboratory scale, namely, natural attenuation, biostimulation and bioaugmentation. Tested bioremediation strategies yield BDE-209 degradation efficiency for biostimulation, bioaugmentation, natural attenuation in decreasing order. The degradation rates calculated for each strategy follow the same order and they fall within the ranges reported in the limited number of studies present in the literature. Assessment of bioremediation strategies for contaminated sites is facilitated via degradation studies, like this study, with freshly spiked sediments under controlled laboratory conditions. However, environmental conditions would probably result in lower degradation rates and require further inquiry.

A detailed evaluation of debromination pathways for each bioremediation strategy was put forth, where new debromination pathways are identified for the first time while others previously identified/proposed are confirmed. This evaluation enabled not only an in-depth assessment for change of concentration of various PBDE congeners, but also revealed the possibility that specific extraneous substances can divert debromination from one position to the other. Implications of these findings could be that debromination pathways may be fostered throughout bioremediation application on a contaminated site to transform bioaccumulative congeners to less brominated and less bioaccumulative ones.

Biodegradation of highly brominated congeners to form lower brominated ones can also potentially increase toxicity. Residual toxicity in sediments can be a major concern following bioremediation of contaminated sites, hence, elucidation of PBDE degradation pathways and degradation kinetics of alternative remediation scenarios provide valuable information for such assessments.

Since fate of halogenated organic pollutants in subsurface environment is a complex issue, modeling studies have been initiated to better understand the behavior of these compounds. However, such modeling studies require extensive data on degradation pathways and kinetics under various treatments. This study, therefore, can also provide valuable input to modeling efforts for the prediction of environmental fate of PBDEs in aquatic sediments.

CHAPTER 6

INVESTIGATION OF BIOTIC DEGRADATION OF HEXABROMOCYCLODODECANE IN SEDIMENT MICROCOSMS

6.1. Introduction

Hexabromocyclododecane (HBCDD) is an additive brominated flame retardant (BFR), which is widely used in extruded, expanded and high impact polystyrene foams for thermal insulation in buildings, and secondarily used in upholstery furniture, automobile interior textiles, car cushions and electrical equipments (Covaci et al., 2006; Marvin et al., 2011). Being produced since 1960s, HBCDD became the mostly used cycloaliphatic BFR (Marvin et al., 2011). The consumption rate of HBCDD was 12500 tons annually in 2013 in Europe (VECAP, 2014), where it is the second highest used BFR (Covaci et al., 2006). The wide usage of HBCDD has led to its occurrence in environmental matrices, i.e. soils, sediments, sewage sludge, dust and atmosphere, and in biota, such as fish, birds, aquatic species including mammals (Covaci et al., 2006). In 2014, HBCDD was included in the Persistent Organic Pollutants list of the Stockholm Convention to eliminate its production, subject to specific exemptions, i.e. exemption in use in expanded and extruded polystyrene in buildings (Stockholm Convention, 2017). HBCDD was also listed as a priority hazardous substance in Water Framework Directive (European Commission, 2013).

Due to its occurrence in environmental matrices, the fate of HBCDD in the environment became a concern. Yet, there are limited studies on the degradation of HBCDD. Biodegradation of HBCDD in soil, sediment and sludge was investigated under various conditions (Davis et al., 2006, 2005; Gerecke et al., 2006; Stiborova et al., 2015b). Davis and colleagues (2006) examined aerobic and anaerobic

degradation in sludge in the presence of appropriate mineral salts medium, while Gerecke and colleagues (2006) and Stiborova et al. (2015b) used nutrients (starch and yeast) for anaerobic degradation of HBCDD in sludge. Gerecke and colleagues (2006) also tested primers (such as 2,6-dibromobiphenyl, 4-bromobenzoic acid, decabromobiphenyl) in their degradation studies. For anaerobic degradation in aquatic sediments, on the other hand, no extraneous substances were added (Davis et al., 2006, 2005). Davis et al. (2005) also investigated the land application of sludge onto soil. These studies revealed HBCDD half-lives in the range of 0.66 to 115.5 days under anaerobic conditions, and 11 to 63 days under aerobic conditions (Davis et al., 2006, 2005; Gerecke et al., 2006), whereas Stiborova and colleagues (2015b) were not able to report a half-life since they observed levels below detection limit at the end of three months. Furthermore, bacterial culture isolates were used to monitor HBCDD degradation under aerobic (Yamada et al., 2009) and anerobic conditions (Peng et al., 2015) in the absence of a solid phase. Previous studies suggested that HBCDD followed dihaloelimination reactions, i.e. elimination of two bromines at each step, leading to the formation of cyclododecatriene (Davis et al., 2006; Peng et al., 2015), having no bromines. Peng et al. (2015) also identified 2-dodecene, which is the product formed as a result of ring cleavage.

Since HBCDD can enter water bodies via waste streams, discharges and etc., it was frequently detected in aquatic sediments due to its hydrophobicity. Therefore, elucidation of their degradation under various conditions is imperative to understand the fate of HBCDD, and to assess remediation strategies in contaminated sediments. The aim of the current study is to investigate biotic degradation of HBCDD in sediments so that the efficiency of various bioremediation strategies could be assessed. The target compound of this study is γ-HBCDD, since it has been predominantly present in the commercially used technical mixture among other isomers (Covaci et al., 2006). The bioremediation alternatives tested in this study are biostimulation, bioaugmentation and natural attenuation. Bioaugmentation and biostimulation are the two techniques frequently used for in situ bioremediation of sediments contaminated with halogenated organics (Bedard, 2003; Payne et al., 2011). For bioaugmentation, enrichment culture of *Dehalobium chlorocoercia* strain

DF-1 was used, and for biostimulation sodium formate and ethanol were used as the carbon source and electron donor, respectively. DF-1 strain was previously shown to reductively dechlorinate PCBs (Payne et al., 2011). Introducing an extraneous carbon source and electron donor rich medium, and a microorganism culture in sediments for degradation of HBCDD were investigated for the first time in this study.

6.2. Materials and Methods

6.2.1. Chemicals

All solvents (n-hexane (HEX), dichloromethane (DCM), acetone(ACE)) used for analysis, anhydrous sodium sulfate (granular), copper fine powder (<63 μm), and aluminum oxide (0.063-0.200 mm) were purchased from Merck KGaA (Darmstadt, Germany). Individual γ-HBCDD isomer was purchased from AccuStandard (New Haven, USA). Internal standard PCB 209 (2,2',3,3',4,4',5,5',6,6'-CB) was supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and surrogate standard BDE-208 was purchased from Wellington Laboratories (Canada).

6.2.2. Sediment microcosms

The sediments that were used in microcosm studies were collected from a pond in a specially protected forest area (Camkoru National Park) located 110 km northwest of Ankara, Turkey. Sediments were expected to have no previous HBCDD contamination as the pond is located close to a passive POPs air monitoring station which consistently show very low POPs concentrations (Jarkovský et al., 2015). Surface sediments under 70 cm water depth were collected from five different points in the pond and were wet-sieved (2 mm) on-site to remove large particles. Samples were transported to the laboratory and stored at 4 °C in glass jars in the dark until use. Moisture content of sediment was analyzed by drying 10 g of sample in 105°C oven for 24 hours, and found to be 36.5±1.53%. Total organic content analysis was then performed via loss-on-ignition procedure, by igniting the sample analyzed for moisture content in 550°C furnace for 4 hours (Heiri et al., 2001), and determined to be 1.43±0.16%.

Some part of collected sediments was air-dried overnight. Air-dried sediments were spiked with γ-HBCDD standard in toluene. Spiked dry sediments were mixed completely. Then, wet sediments were added onto spiked sediments and a homogeneous mixture was obtained. Spiked sediments were distributed equally among the microcosm bottles to get approximately 3 g wet sediments in each. Similarly, non-spiked control set was established by spiking dry sediment with high grade toluene and mixed with wet sediments. These contamination control microcosm sediments were found to have no HBCDD contamination throughout the incubation period.

Details of microcosm setup are presented in Table 6-1. Microcosms were prepared as sacrificial reactors and operated for 36 days. For each set and each sampling time, duplicate 20 mL reactors were established. All sets were topped with distilled water while the biostimulation set was topped only with an organic medium. In all reactors sediment-to-liquid ratio was kept constant as 3 g wet sediment:3.5 mL liquid. For abiotic control set, 5 mL of distilled water was added onto dry abiotic media first to provide wetting of media, and then to provide similar medium/liquid ratio with others containing wet sediments. In bioaugmentation set, microorganism culture was added in a liquid medium, and volume of added water was adjusted accordingly.

Table 6-1. Details of HBCDD sediment microcosm sets.

Reactor Type	Reactor Name	HBCDD spike	Sediment	Topping Liquid Ingredients (volume)
Test microcosms	Natural attenuation	+	+	DI water (3.5 mL)
	Biostimulation	+	+	e- donor & C source rich organic medium (3.5 mL)[a]
	Bioaugmentation	+	+	DF-1 culture (0.5 mL)[b] + DI water (3.0 mL)
Control microcosms	Negative control	+	+	Spent growth medium (0.5 mL)[c] + DI water (3.0 mL)
	Contamination control	-	+	DI water (3.5 mL)
	Sterile[d]	+	+	DI water (3.5 mL)
	Abiotic control[e]	+	-	DI water (5 mL)

[a] Prepared as given in Berkaw et al. 1996 and supplied with sodium formate and ethanol.
[b] *Dehalobium chlorocoercia* strain DF-1 culture.
[c] DF-1 medium with no DF-1 cells, obtained by passing the culture medium through 0.22 μm filter.
[d] Sterile set was poisoned with mercury chloride and then autoclaved on three consecutive days.
[e] Abiotic control set included kaolinite as the solid media instead of sediment.

Organic medium of biostimulation set was prepared by dissolving several vitamins and minerals in water together with various salts under N_2:CO_2 atmosphere, and pH was adjusted to 6.8 (Berkaw et al., 1996). Medium also contained 10 mM of sodium formate and ethanol as the carbon source and electron donor, respectively, to stimulate biodegradation. *Dehalobium chlorocoercia* strain DF-1 used in bioaugmentation set was previously grown in Prof. Dr. Kevin Sowers' Lab of Institute of Marine & Environmental Technology, University of Maryland, Baltimore, MD, USA. This strain was originally enriched from Charleston Harbor sediments, and shown to be successful in reductive dechlorination of PCBs (Payne et al., 2011). For natural attenuation set, no amendment was made with extraneous substances to sediments.

There were four types of control sets: i) A negative control set was established to serve as a control for bioaugmentation set so that the effects of adding a culture medium without DF-1 cells can be observed. Hence, a spent growth medium was formed by passing DF-1 culture through 0.22 μm filter so that no cells will remain in the medium. ii) A sterile control set was established where microcosms were supplied with mercury chloride (0.5 mg $HgCl_2$/g sediment) and also autoclaved at 120 °C at 1.1 atm pressure for 20 min on three consecutive days to hinder any microbial activity in sediments. iii) A contamination control set was established without HBCDD spike to check for any contamination resulting from incubation conditions in unspiked sediments. iv) An abiotic control set was operated which included an abiotic solid medium that resembles sediments structurally. For this purpose, kaolinite ($Al_2Si_2O_5(OH)_4$) was used as the abiotic media. It was used in microcosms after it was washed with distilled water and dried in oven at 105°C overnight, three times. Similar to sediments, kaolinite (in dry form) was spiked with HBCDD, mixed vigorously, and distributed to microcosm bottles to involve 2 g dry weight kaolinite. The dry kaolinite was then wetted with the distilled water added on top of it.

All sets were purged with high purity nitrogen stream after closed with Teflon lined septa crimp caps. They were incubated in the dark at 25°C. During sampling, duplicate reactors were opened and all sediments were analyzed. Headspace gas monitoring was not conducted. Within the scope of this study, HBCDD biodegradation products were not investigated.

The statistical analysis was conducted with SPSS 21. Paired t-test was used to evaluate the difference between two microcosm sets, and analysis of variance (ANOVA) within subject design was used to identify the variance within the data set of a microcosm set. The degradation kinetics of γ-HBCDD was explained by pseudo-first-order model: $C = C_0 e^{-kt}$, where C is the concentration at sampling times (ng/g dry weight), C_0 is the initial γ-HBCDD concentration (ng/g dw), k is the pseudo-first-order rate constant (d^{-1}), and t is the incubation time (d). Rates are calculated by plotting $ln(C/C_0)$ vs t, and checked using coefficient of determination, R^2.

6.2.3. Extraction and analysis

Extraction of HBCDD from sediment samples was based on US EPA method 3550C Ultrasonic Extraction (USEPA, 2007a). Two grams of sample, mixed with two grams of anhydrous sodium sulfate in 40 mL vials, were extracted ultrasonically in an ultrasonic bath with 30 mL dichloromethan:n-hexane:acetone mixture (7:7:1 v/v) for 30 minutes twice, after being soaked into the solvent mixture overnight. Sulfur removal was achieved with the addition of copper powder into the extraction solvents at the beginning of extraction. The two extracts were combined and concentrated to 2-5 mL via rotary evaporator. To remove the possible interfering organic compounds, the colored extract after concentration step was treated with concentrated sulfuric acid (U.S. EPA Method 3665A) (USEPA, 1996b). The top clear extract was purified with 0.5 g of alumina (deactivated to 3%) topped with anhydrous sodium sulfate, and eluted with 5 mL of n-hexane, followed by 2 mL of n-hexane:dichloromethane mixture (1:1). The collected extract was concentrated to 2 mL via rotary evaporator.

The extract was then divided into two and each one mL extract was spiked with internal standard. Agilent 7890A GC 5975C inert mass spectrometry (GC-MSD) in EI mode with DB5-MS column (15 m x 0.25 mm ID x 0.10 μm) was used for the instrumental analysis of HBCDD. Injection temperature was 200 °C, ion source temperature was 230 °C and quadrupole temperature was 150 °C. Helium was used as the carrier gas at a constant rate of 1.5 mL/min. Oven program was as follows: 60 °C for 1 min, raised to 200 °C at 15 °C/min, to 310 °C at 10 °C/min and held there for 5 min. Analysis in scan mode revealed that primary/secondary ions (m/z) used for confirmation are 79/159.1 for HBCDD, 497.8/427.8 for PCB-209 (internal standard) and 721.6 for BDE-208 (surrogate standard). These ions were then used to analyze samples in SIM mode.

6.2.4. QA/QC

Laboratory control samples were analyzed to check the extraction efficiency, and results yielded an average recovery of 89.3±7.92% (range: 81.4-97.2% for n=3) for

γ-HBCDD. γ-HBCDD recovery for laboratory control samples with kaolinite was 90.6 ± 8.14% (*n*=4). Method detection limits were 34.6 ppb and 80.3 ppb, and LOQs were 110.1 ppb and 255.5 ppb, for γ-HBCDD and the surrogate standard BDE-208, respectively. Blanks were analyzed in every batch of 15 samples, and no peaks were detected during analysis. Average surrogate recovery for the whole data set was 86.5±14.3% (range: 62.4 – 122.9%). No surrogate and/or blank correction was deemed necessary.

6.3. Results and Discussion

6.3.1. Degradation of HBCDD in sediments

Initial γ-HBCDD concentrations ranged from 730 to 862 ng/g dw in sediment microcosms, and it was 827 ng/g dw in abiotic control microcosm. The change in γ-HBCDD levels in microcosm sets is presented in Figure 6-1, together with percent remaining γ-HBCDD in reactors. As can be seen from the figure, natural attenuation set remained at the same concentration in the first 4 days, and then started to decrease achieving 36.9% reduction at the end of 36 days. Since the sediments were not historically contaminated with HBCDD, the indigenous species needed to adapt it as an electron acceptor for microbial degradation. The highest reduction in γ-HBCDD was observed in the biostimulation set, with a total of 86.3% decrase in initial concentration. Bioaugmentation with DF-1 strain exhibited an initial sharp decline, but could not follow a net trend afterwards. At the end of incubation period, 34.9% reduction in γ-HBCDD was achieved, while the lowest concentrations were observed in samples at day 16 and 24 (38.8% reduction). At each sampling time, duplicate bottles were sacrified and analyzed as in Davis et al. (2005). Hence, observation of such variations in γ-HBCDD levels might be caused due to the sacrificial operation of microcosms, similarly observed in anaerobic sediment microcosms of Davis et al. (2005).

The results were compared with limited literature on HBCDD degradation. Previous studies on HBCDD degradation showed varying reduction ratios in various solid media and conditions. Commercial HBCDD mixture reached to not detect levels in 7

days in anaerobic aquatic sediments, where initial HBCDD concentrations were 30 – 40 ng/g dw (Davis et al., 2005). On the other hand, aquatic sediments, which had 100 times higher initial concentrations, demonstrated 61.5% reduction in 113 days with no extraneous substances (Davis et al., 2006). The reduction was approximately 27% at around 30 days (as read from the graph given in Davis et al., 2006), which was similar with the percent reduction observed in natural attenuation set of this study. Digester sludge incubated with a mineral salts medium also showed a comparable reduction percentage with the biostimulation set of this study, with 90% reduction in 28 days (Davis et al., 2006). As also discussed by Davis and colleagues (2006), the initial concentration of the contaminant have an impact on degradation ratios.

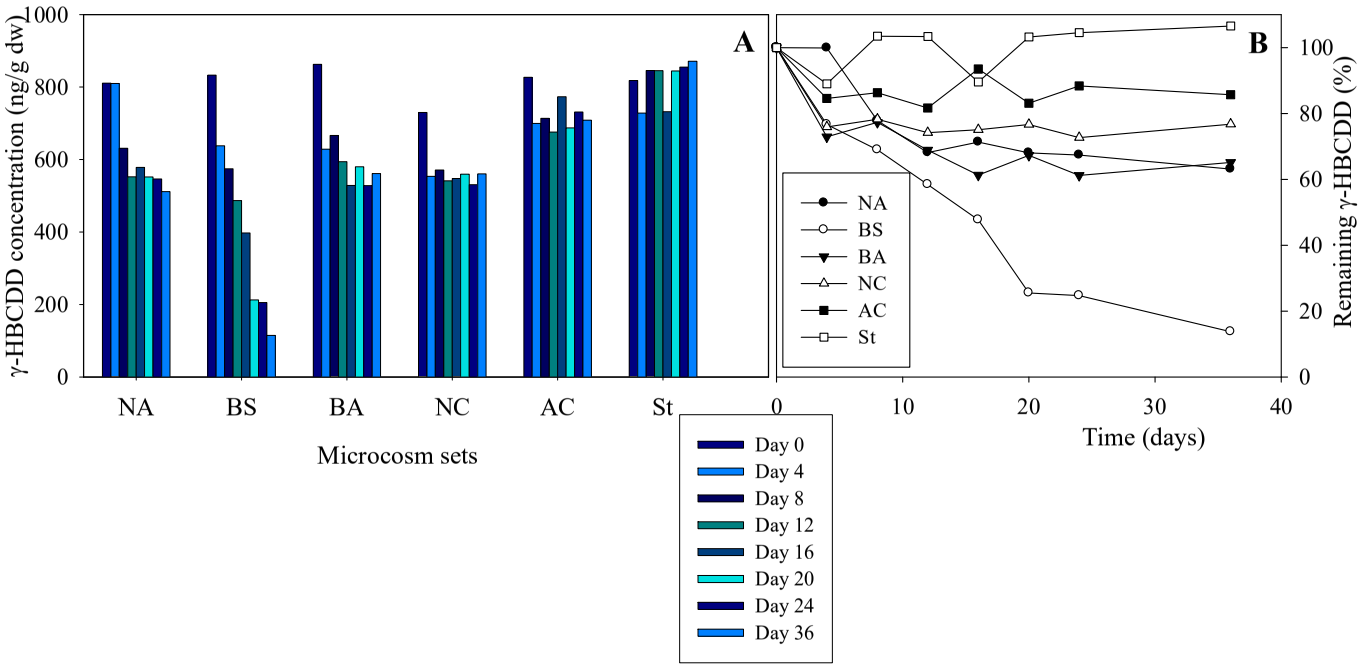


Figure 6-1. A) Change in γ-HBCDD concentration (ng/g dw), B) Percent remaining γ-HBCDD in microcosm sets. NA: Natural attenuation, BS: Biostimulation, BA: Bioaugmentation, NC: Negative control, AC: Abiotic control, St: Sterile. Values represent average of parallel microcosms. Contamination control did not yield any γ-HBCDD.

Negative control set, prepared to distinguish the effects of organic media added with DF-1 cells, showed a sharp decline at the beginning, and remained unchanged afterwards. This trend was very similar with the one observed in PBDE anaerobic degradation studies, presented in Chapter 5. The initial decline was attributed to the triggering effect of organic media added. However, after the consumption of nutrients, γ-HBCDD degradation nearly stopped. The overall reduction in γ-HBCDD was 23.2% at the end of 36 days. The abiotic control set, consisting of kaolinite instead of sediments, demonstrated a variation between sampling times, but yielded no net decrease in concentration. Statistical analysis yielded no significant change in time for abiotic control set (ANOVA -within subject design, $F(1,7) = 1.99$, $p > .05$). Although γ-HBCDD concentration showed small variations also in the sterile reactor, statistical analysis for sterile set yielded no significant change in time either (ANOVA -within subject design, $F(1,7) = .05$, $p > .05$). No γ-HBCDD was detected at any time in the contamination control set.

An interesting observation from the literature is that, in three major studies on HBCDD degradation (i.e. Davis et al. 2005, 2006, and Gerecke et al. 2006) showed degradation of HBCDD to some extent in their sterile reactors. For instance, in Davis et al. study (2005) HBCDD reached to not detect levels in 60 days in sterile sediments, while 33% reduction was observed in 113 days in the same research group's 2006 study. They observed remarkable decrease in concentration also in sterile soil and sludge microcosms (Davis et al., 2006, 2005). The control reactors showed no viable cells in cell count, therefore they concluded that abiotic degradation mechanisms were acting on HBCDD reduction in these reactors (Davis et al., 2006). The presence of coenzymes, e.g. vitamin B12, might cause the autoclaved reactors to show abiotic degradation. Nevertheless, the present study showed that addition of mercuric chloride and autoclaving the sediments concurrently successfully inhibited any microbial activity and the reactivity of enzymes. The abiotic kaolinite medium also demonstrated that abiotic degradation mechanisms were not active under the provided laboratory conditions. Hence, it was

confirmed that the observed decrease in HBCDD levels in test microcosms were only due to anaerobic biodegradation of HBCDD.

To elucidate the differences between reactors, statistical analysis was conducted with paired sample T-test. The results are presented in Table 6-2. In this table, *t* scores represent paired T-test statistic, and *p* scores (given in paranthesis) present the statistical significance of difference between samples, corresponding to the given test statistic *t*. If $p < .05$, this means that there is a significant difference between samples. As can be seen from the table, there is a significant difference between all sets and abiotic control set, and between all sets and sterile set, which means these control reactors simulated the absence of microbial activity as planned. The difference between these two control reactors, additionally, was significant. *p* greater than .05 obtained between natural attenuation (NA) and bioaugmentation (BA) set indicated that these reactors showed no significant difference. Therefore, it can be speculated that introducing DF-1 strain in sediments had no effect on HBCDD degradation, and that DF-1 was not able to degrade HBCDD in sediment microcosms. Negative control set also showed no significant difference with NA set; on the other hand, it showed a significant difference with BA set. All other pairs, except the ones mentioned above, revealed significant difference between microcosm sets.

Table 6-2. Paired sample t-test scores between microcosm pairs[a]. Paired T-test statistic *t* is presented with *p* scores given in paranthesis[b].

	NA	BS	BA	NC	AC
NA					
BS	3.51 (.01)				
BA	0.18 (.86)	-3.11 (.02)			
NC	1.52 (.17)	-1.91 (.05)	2.35 (.05)		
AC	-2.75 (.03)	-3.66 (.01)	-3.44 (.01)	-10.53 (.00)	
St	-3.48 (.01)	-3.94 (.01)	-4.58 (.00)	-8.14 (.00)	-3.03 (.05)

[a] NA: Natural Attenuation, BS: Biostimulation, BA: Bioaugmentation, NC: Negative control, AC: Abiotic control, St: Sterile.

[b] When $p < .05$, there is a significant difference between pairs.

6.3.2. HBCDD degradation rate

Rate of γ-HBCDD degradation in sediment microcosms can be explained by pseudo-first-order reaction kinetics. The calculated rate constants are given in Table 6-3, together with all available relevant degradation rates reported in the literature. Highest rate observed in this study was for the biostimulation set. Natural attenuation and bioaugmentation sets revealed low R^2 values for pseudo-first-order reaction kinetics; however they did not fit to zero-order reaction kinetics, either (data not shown). When compared with literature studies, it can be clearly observed that the rates found in this study were lower, except for the anaerobic sediment microcosms of Davis and colleagues (2006). Freshwater sediment microcosms without any additional substrates revealed very similar rates with natural attenuation set of this study (Davis et al., 2006).

High rates observed with pure culture isolates were an expected result (Peng et al., 2015), while in the presence of a solid phase, degradation rates were anticipated to be lower due to the sorption of compound on solid phase. However, anaerobic degradation in soil and sediments (Davis et al., 2005), and sewage sludge (Davis et al., 2006; Gerecke et al., 2006) was much faster than that in bacterial strains. This might be due to the initial contaminant concentration as mentioned in Section 6.3.1. The highest concentration was studied by Peng and colleagues (2015), with 500 μg/L, among others. Therefore, investigating degradation at environmentally relevant contaminant concentrations is crucial while commenting on the fate of HBCDD in the environment.

Table 6-3. Degradation rate constants for pseudo-first-order reaction kinetics of this study and comparison with literature.

Media & Conditions	isomer	k (d^{-1})	$t_{1/2}$ (d)	R^2	Reference
Lake sediment microcosms without any substrates	γ	0.0155	44.7	0.75	*This study*
Lake sediment microcosms with carbon source + electron donor	γ	0.0542	12.8	0.97	
Lake sediment microcosms with *Dehalobium chlorocoercia* strain DF-1	γ	0.0123	56.3	0.56	
Anaerobic soil microcosms with activated sludge simulating land application of sludge	All	0.10[a]	6.9	-	(Davis et al., 2005)
River sediment microcosms without any substrates	All	0.45[a]	1.5	-	
Creek sediment microcosms without any substrates	All	0.61[a]	1.1	-	
Digester sludge microcosms with mineral salts medium	All	0.1278	5.4	-	(Davis et al., 2006)
	α	0.1327	5.2		
	β	0.1522	4.6		
	γ	0.1248	5.6		
Freshwater sediment microcosms without any substrates	All	0.0109	63.6	-	
	α	0.0060	115.5		
	β	0.0140	49.5		
	γ	0.0113	61.3		
Digester sludge microcosms with nutrients and primers	All	1.1	0.66	-	(Gerecke et al., 2006)
Mixed liquor reactors with inoculums of two individual isolated strains[b]	α	0.1286/0.1217	5.4 / 5.7	0.94/0.96	(Peng et al., 2015)
	β	0.0847/0.0726	8.2 / 9.6	0.89/0.96	
	γ	0.0789/0.0721	8.8 / 9.6	0.96/0.98	

[a] Calculated from the half-lives given.

[b] Results obtained from two different strains given as separated with "/".

6.4. Conclusion

Anaerobic degradation of HBCDD in sediments was investigated under various conditions to assess the efficiency of bioremediation alternatives: natural attenuation, biostimulation and bioaugmentation. Since HBCDD is an emerging pollutant, studies on its environmental fate are scarce. This study, therefore, contributed to the limited literature on application of various bioremediation alternatives for HBCDD removal.

Biostimulation of sediments with a carbon source and electron donor rich organic medium resulted in the highest rate of degradation. Investigation of bioremediation strategies for contaminated sites via degradation studies, like this study, is crucial. Monitoring the contaminants in freshly spiked sediments under controlled laboratory conditions facilitates the assessment of strategies. However, environmental conditions would probably result in lower degradation rates and require further inquiry.

Bioaugmentation with *Dehalobium chlorocoercia* strain DF-1 yielded no statistically significant difference with the natural attenuation set, revealing the limited ability of this strain to degrade a cycloaliphatic compound. However, bioaugmentation with microorganism species which are shown to degrade HBCDD should be examined further to investigate their efficiency to be used in bioremediation efforts.

Abiotic degradation mechanisms were found to have an impact while examining biodegradation in previous studies. However, in the present study, statistically no significant change in time was observed in control microcosms. Hence, degradation rates presented in this study represent only anaerobic biodegradation of HBCDD.

The present study focused on the HBCDD isomer which is the abundant one in commercial mixtures, and hence predominantly found in environmental matrices. Further studies might follow the degradation of commercial mixtures to assess the efficiency of biostimulation on other HBCDD isomers.

CHAPTER 7

ABIOTIC DEGRADATION OF BROMINATED FLAME RETARDANTS IN SEDIMENTS VIA CATALYZED HYDROGEN PEROXIDE PROPAGATIONS

7.1. Introduction

In situ chemical oxidation processes have been investigated and applied for several years for effective remediation of soils and groundwater. One of these processes is the use of Fenton's reagent in which hydroxyl radical generated decomposes the recalcitrant contaminants (Fenton, 1894). Application of Fenton's reagent for contaminant removal in subsurface systems may become infeasible due to scavenging of hydroxyl radical with the constituents in soil, such as natural organic matter (Watts and Teel, 2005). Therefore, modifications on Fenton's reagent have attracted attention for the transformation of organic contaminants. The catalyzed hydrogen peroxide propagations (CHP) is a modified Fenton's reagent by the use of high hydrogen peroxide concentrations (2 – 20%) and various catalysts e.g. Iron (III), iron chelates, or iron minerals (Watts and Teel, 2005). While iron (II) is the most effective catalyst at dilute peroxide concentrations, iron (III) is effective at high peroxide concentrations (Watts and Teel, 2005). Thus, CHP is initiated with equation 1 (Barb et al., 1951):

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\bullet + \mathrm{H}^+ \qquad k = 2 \times 10^{-3} s^{-1} \ (\mathrm{pH} = 3) \qquad (1)$$

Iron (II) produced with this reaction is then used to initiate classical Fenton's reactions as given in equation 2. The hydroxyl radical generated in equation 2 reacts with hydrogen peroxide and the following propagation reactions occur:

$$H_2O_2 + Fe^{2+} \xrightarrow{k} OH\bullet + \mathrm{OH}^- + Fe^{3+} \qquad k = 76\ M^{-1}s^{-1} \qquad (2)$$

$$OH\bullet + H_2O_2 \xrightarrow{k} H_2O + OOH\bullet \qquad k = 2.7 \times 10^7 M^{-1}s^{-1} \qquad (3)$$

$$OOH\bullet \leftrightarrow O_2\bullet^- + H^+ \qquad pK_a = 4.8 \qquad (4)$$

$$R\bullet + H_2O_2 \xrightarrow{k} ROH + OH\bullet \qquad k = 10^6 - 10^8 M^{-1}s^{-1} \qquad (5)$$

$$OOH\bullet + Fe^{2+} \rightarrow {}^-OOH + Fe^{3+} \qquad k = 1.2 \times 10^6 M^{-1}s^{-1} \qquad (6)$$

The hydroxyl radical produced with reaction (2) is an electrophile, and can commonly participate into (i) electrophilic substitution reactions in aromatic compounds, (ii) hydrogen abstraction reactions from saturated compounds (Watts and Teel, 2005), and (iii) addition to double bonds. The formation of nonhydroxyl radicals (i.e. $OOH\bullet$, $O_2\bullet^-$, ${}^-OOH$) in the presence of high H_2O_2 concentrations leads to an increased range of reactivity. Perhydroxyl radical ($HO_2\bullet$) is a relatively weak oxidant, superoxide radical anion ($O_2\bullet^-$) is a weak reductant and nucleophile in aqueous systems, and hydroperoxide anion (HO_2^-) is a strong nucleophile (Watts and Teel, 2005). This mixture of radicals is believed to help degrade organic chemicals.

The effectiveness of CHP reactions depends on system parameters, such as concentrations of reagents, pH and temperature (Manzano et al., 2004; Watts and Teel, 2005). Previous studies demonstrated the necessity to determine the optimum conditions of parameters for a specific test system. For example, varying concentrations of iron and hydrogen peroxide were tested, and optimum concentrations were determined to maximize contaminant removal efficiency (Manzano et al., 2004). On the other hand, Ahmad and co-workers (2011) used no additional iron since the soil had adequate amounts of iron oxide. They also observed high demand for hydrogen peroxide (up to 50%) due to the high organic content of soil when testing ex-situ application of CHP (Ahmad et al., 2011). The effect of pH has also been reported in many studies. At low pH (below 3), iron is maintained in soluble form (Snoeyink and Jenkins, 1980) and this provides effective catalysis to CHP reactions. However, due to the strong buffering capacity of the subsurface environment, adjustment of pH becomes an important concern in order not to form iron precipitates.

Previous studies on degradation of PCBs and PAH showed CHP to be useful in soil systems (Manzano et al., 2004; Quiroga et al., 2009; Venny et al., 2012a). CHP was never tested for the degradation of PBDEs or HBCDD in soils and sediments. The aim of this study is to investigate the applicability of CHP for remediation of aquatic sediments contaminated with PBDEs and HBCDD, and to compare CHP performance with other abiotic degradation studies for these contaminants.

7.2. Materials and methods

7.2.1. Chemicals

All solvents (n-hexane (HEX), dichloromethane (DCM), acetone(ACE)) used for analysis, anhydrous sodium sulfate (granular), copper fine powder (<63 μm), and aluminum oxide (0.063-0.200 mm) were purchased from Merck KGaA (Darmstadt, Germany). Individual standards of PBDE 209 (2,2',3,3',4,4',5,5',6,6'-BDE), surrogate standard PCB 141 (2,2',3,4,5,5'-CB), internal standard PCB 209 (2,2',3,3',4,4',5,5',6,6'-CB) were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Individual HBCDD isomer (γ-) was purchased from AccuStandard (New Haven, USA). BDE-99 (2,2',4,4',5-BDE) was purchased from CPA (Bulgaria). H_2O_2 solution (34.5-36.5%) and iron(III)sulfate hydrate (97%) were purchased from Sigma Aldrich.

7.2.2. Sediments

The sediments that were used in microcosm studies were collected from a pond in a specially protected forest area (Camkoru National Park) located 110 km northwest of Ankara, Turkey. Sediments were expected to have no previous PBDE contamination as the pond is located close to a passive POPs air monitoring station which consistently show very low POPs concentrations (Jarkovský et al., 2015). Surficial sediments under 70 cm water depth were collected from five different points in the pond and were wet-sieved (2 mm) on-site to remove large particles. Samples were transported to the laboratory and stored at 4 °C in glass jars in the dark until use. Moisture content of sediment was analyzed by drying 10 g of sample in 105°C oven for 24 hours, and found to be 36.5±1.53%. Total organic content analysis was then performed via loss-on-ignition procedure (Heiri et al., 2001), by igniting the sample

analyzed for moisture content in 550°C furnace for 4 hours, and determined to be 1.43±0.16%.

7.2.3. Single-fill microcosms experimental setup

For abiotic degradation of PBDEs and HBCDD, preliminary sets were established with single-fill 24-hour experimental setups to observe the response of these chemicals to CHP reactions, and to detect the effects of changing operational parameters; such as hydrogen peroxide concentrations, pH, and contaminant concentration. Initial experiments were conducted with BDE-209, and then HBCDD set was operated with the selected CHP process and parameters.

The details of preliminary sets are presented in Table 7-1. In each reaction set, 40 mL amber bottles were used as the reactors and operated sacrificially for each sampling time. Controls for single-fill microcosms were i) no H_2O_2: using distilled water instead, ii) no contaminant: to check any contamination/interference caused from CHP reactions, starting from "H_2O_2-repeat" experiment set. Except the first set in which duplicate reactors were operated, all sets included triplicate test reactors and duplicate control reactors. In all sets, 3 g wet sediment to 10 mL total solution volume ratio was kept constant. Sampling was performed at t = 0, 1, 4, and 24 hours. H_2O_2 concentration in reaction vessels were monitored with Quantofix H_2O_2 strips. Fe(III)sulfate hydrate and Fe(II)sulfate were used to prepare iron catalyst solutions. For the first three sets (i.e. "pH unadjusted", "H_2O_2", "H_2O_2-repeat"), t=0 samples were taken without the addition of any solution onto sediments. However, in "BDE-high", "Fenton" and "HBCDD" sets, solutions were also added to t=0 sample bottles, and discarded right after peroxide addition.

Percent reduction in contaminant concentration was calculated based on the average concentrations of t=0 and t=24 h for no H_2O_2 controls.

Table 7-1. Details of single-fill CHP experimental setup.

Experiment name	Contaminant	pH[a]	H_2O_2	Fe(III)	Monitoring of	
					H_2O_2	T
pH unadjusted	BDE-209	-	2 M	100 ppm	-	+
H_2O_2	BDE-209	+	10 M	100 ppm	+	+
H_2O_2-repeat	BDE-209	+	10 M	100 ppm	+	+
BDE-high	BDE-209	+	10 M	100 ppm	+	-
Fenton	BDE-209	-	10 M	Fe(III): - Fe(II):300 ppm	+	+
HBCDD	γ-HBCDD	+	10 M	300 ppm	+	+

[a] pH adjustment (+) was done to pH=2 by addition of sulfuric acid.

Temperature was not controlled but monitored during reactions. In all experimental CHP setups, temperature change followed similar trends, increasing in first 2 – 3 hours up to at most 26°C, then decrasing to room temperature at the end of 24 hours. For Fenton set, temperature raised to 42°C at first hour, then decreased gradually. Previous studies showed at higher temperatures degree of degradation of organic compounds increases, although this reaction does not require heat (Manzano et al., 2004). On the other hand, at very high temperatures like 40 – 50 °C, H_2O_2 decomposition is promoted (Ahmad et al., 2011), hence this may not be preferred and/or applicable during in-situ applications.

pH was initially unadjusted, however, this may lead to precipitation of iron and ineffective catalysis of CHP (Watts and Teel, 2005). Hence, in the following experimental sets, except for Fenton set, pH was adjusted with sulfuric acid at the beginning of reaction to maintain pH below 2. Fenton reactions do not require the pH adjustment, since H_2O_2 reactivity sustains low pH. At each set, pH was monitored with pH strips, and observed to remain below 2 during the reaction.

H_2O_2 concentrations in previous studies were between 20 g/L and 500 g/L (Ahmad et al., 2011; Watts and Teel, 2005). First experiment, therefore, targeted H_2O_2 concentration near the lower end, i.e. 68 g/L (2 M). Due to the observation of no net

trend in BDE-209 concentration, H_2O_2 concentration was changed to 300 g/L (10 M) in the following sets. H_2O_2 concentration reached nearly 100 g/L at the end of 24 hours, except for Fenton set in which concentration decrased to 1 g/L.

Fe(III)sulfate was used as the catalyst in reactions. Fe(III) concentration was kept constant at 100 ppm during preliminary BDE-209 reactions which was within the ranges given in literature for PCBs (Ahmad et al., 2011; Manzano et al., 2004) and chloroaliphatic compounds degradation (Watts et al., 1999). For Fenton set, on the other hand, 300 ppm Fe(II) was used. This concentration was chosen to provide a better catalysis of Fenton reaction but not to cause aggressive conditions at the same time. After these was observed for Fenton set, HBCDD set was prepared with 300 ppm Fe(III) concentration.

BDE-209 concentration was the last parameter to be changed. In the first three sets, concentration was kept at low. Due to the observation of concentrations close to the detection limit of BDE-209 in first hours of reaction, the concentration was increased in the next sets. Also for HBCDD set, concentration of γ-HBCDD was at high level.

7.2.4. Fill-and-draw (F&D) microcosms experimental setup

Fill-and-draw (F&D) operation means withdrawal of reaction solution at predetermined time intervals and refilling the reactor with fresh solution. The experimental setup of F&D treatments are given in Table 7-2, while operation of control reactors is presented in Table 7-3.

Similar to the single-fill microcosms, 40 mL amber bottles consisting of 3 g wet sediments and 10 mL solution were employed, and operated sacrificially. F&D process included 5 treatments. At each F&D treatment, sacrificial bottles were collected for sediment extraction so that withdrawal and refilling was conducted 5 times for the last sample. The test systems and spike control were operated in triplicate, while no H_2O_2 and contaminant control systems were in duplicate. Time zero samples were taken right after they were amended with solutions. At each

sampling, approximately 0.5 mL of ethanol was added onto solution before withdrawal to quench CHP reactions.

Table 7-2. Details of fill-and-draw (F&D) CHP experimental setup.

Contaminant	Concentration of contaminant (ng/g dw)	H_2O_2 (M)	Fe(III) (ppm)	F&D period	pH	Monitoring of	
						H_2O_2[a]	T[b]
BDE-209	1000	10	300	1 day	2	+	+
BDE-209 & BDE-99	1000 each	10	300	8 days	2	+	+
γ-HBCDD	1000	10	300	8 days	2	+	+

[a] H_2O_2 was monitored only at fill-and-draw treatment times.
[b] Temperature was monitored only at first few hours of reaction.

Table 7-3. Details of F&D CHP control reactor setup.

	H_2O_2	Fe(III)	Contaminant	Sampling time
No-H_2O_2 control	-	+	+	t=0, F&D-1, F&D-5
Contaminant control	+	+	-	t=0, F&D-5
Spike control	-	-	+	t=0
LOI control	+	+	-	t=0, F&D-1, F&D-5

No-H_2O_2 controls were established using distilled water instead of H_2O_2 to observe contaminant concentration change without CHP reactions. Contaminant controls were operated to check for a contamination resulting from the CHP process or during extraction/analytical procedure. Spike controls were prepared to determine the contaminant concentration in sediments without addition of CHP solutions. The purpose of LOI controls was to observe the change in organic content of sediments during CHP process.

Percent reduction in contaminant concentration was calculated based on the average concentrations of t=0, F&D-1 and F&D-5 for no H_2O_2 controls.

7.2.5. BFR extraction and analysis

US EPA method 3550C Ultrasonic Extraction was followed for the extraction of PBDEs and HBCDD from sediments (USEPA, 2007a). Two grams of sample was mixed with equal amount of anhydrous sodium sulfate in 40 mL vials, and spiked with surrogate standard. Samples were extracted in an ultrasonic bath with 30 mL DCM: HEX: ACE mixture (7:7:1 v/v) for 30 minutes twice, after being soaked in the solvent mixture overnight. Sulfur removal was achieved with the addition of copper powder into the extraction solvents. The two extracts were combined and concentrated to 2-5 mL via rotary evaporator (Heidolph, Hei-Vap Advantage HL/G1). To remove possible interfering organic compounds, the colored extract after concentration step was treated with concentrated sulfuric acid (U.S. EPA Method 3665A). For PBDE extraction, the top clear extract was purified with 0.5 g of alumina (deactivated to 6%) topped with anhydrous sodium sulfate, and eluted with 2 mL of HEX. The collected extract was concentrated to 2 mL by high purity nitrogen stream. For HBCDD extraction, the top clear extract after sulfuric acid treatment was purified with 0.5 g of alumina (deactivated to 3%) topped with anhydrous sodium sulfate, and eluted with 5 mL of HEX, followed by 2 mL of HEX:DCM (1:1). The collected extract was concentrated to 2 mL by a rotary evaporator.

For PBDE analysis one mL of extract was spiked with internal standard and analyzed with gas chromatography coupled with micro-cell electron capture detector (Agilent 6890N GC-μECD) with DB-5 MS capillary column (15 m x 0.25 mm ID x 0.10 μm). Instrumental conditions were as follows: Helium was used as the carrier gas with 1.8 mL/min flowrate using a constant flow mode. The make-up gas for the detector was nitrogen with a flowrate of 30 mL/min. The injector and detector temperatures were 250°C and 350°C, respectively. The sample injection was carried out at 1 μL with splitless injection mode. Oven temperature program started at 90°C, raised at 20°C/min to 310°C, and hold for 6 min.

For total HBCDD analysis, Agilent 7890A GC 5975C inert mass spectrometry (GC-MSD) in EI mode with DB5-MS column (15 m x 0.25 mm ID x 0.10 μm) was used. Injection temperature was 200°C, ion source temperature was 230°C and quadrupole temperature was 150°C. Helium was used as the carrier gas at a constant rate of 1.5 mL/min. Oven program was as follows: 60°C for 1 min, raised to 200°C at 15 °C/min, to 310°C at 10 °C/min and held for 5 min. Analysis in scan mode revealed that primary/secondary ions (m/z) used for confirmation are 79/159.1 for HBCDD, 497.8/427.8 for PCB-209 (internal standard) and 403.8/563.6 for BDE-99 (surrogate standard). These ions were then used to analyze samples in SIM mode.

7.2.6. QA/QC

A six-point internal calibration was performed for PBDEs in GC-ECD, yielding relative standard deviations (RSD) lower than 20% and R^2 greater than 0.99. PCB-141 and BDE-77 was used as surrogate standards. Method detection limit (MDL) and limit of quantitation (LOQ) were determined according to U.S. EPA (USEPA, 2016). MDLs were 0.10 ppb for BDE-99 and 3.94 ppb for BDE-209, and LOQ were 0.30 ppb for BDE-99 and 12.53 ppb for BDE-209.

A five-point internal calibration in GC-MS was performed for γ-HBCDD, yielding RSD lower than 20% and R^2 greater than 0.995. MDLs were 34.6 ppb and 66.7 ppb, and LOQs were 110.1 ppb and 212.0 ppb, for γ-HBCDD and BDE-99, respectively.
Laboratory control samples were analyzed to check the extraction efficiency, and results yielded recoveries of 96.0±6.07% (range: 86-105.7% for *n*=7) for BDE-209, 105.0±2.33% (range: 101.6-108.5% for *n*=7) for BDE-99, and 89.3±7.92% (range: 81.4-97.2% for *n*=3) for γ-HBCDD.

Blanks were analyzed in every batch of 10 to 12 samples, and no peaks were detected during analysis. Average surrogate recoveries for the whole data set for PBDE samples were 88.2±10.1% (range: 64.4 – 117.5%), and 96.2±5.52% (range: 87 – 105.3%) for HBCDD samples. No surrogate and/or blank correction was deemed necessary. No contamination was detected in contaminant controls for all experimental sets.

7.3.Results and Discussion

7.3.1. Single-fill microcosms

Preliminary experiments were conducted to identify parameters that affect system performance. As mentioned in introduction part, the effective system parameters are concentration of reagents (H_2O_2 and iron), pH and temperature. Within the scope of this study, the altered parameters were pH, H_2O_2 concentration and BDE-209 concentration. Additionally, a Fenton set was established to observe the behavior of BDE-209 to classical Fenton process.

When the change in BDE-209 concentrations in time was examined from Figure 7-1, it can be said that regardless of the alteration of parameters in CHP systems, BDE-209 trend was similar, i.e. sharp decline in first hours, then increase at 24^{th} hour. The reason for this observation was attributed to the sorptive interaction of BDE-209 molecules and fine particulates of sediment. As reported in previous studies, size of particles in sediments/soils is the second important factor after organic matter content for sorption of hydrophobic compounds (Site, 2001). As the particle size decreases, organic content of soil increases exponentially, and it is stated that small sized particles are typically organic in nature (Voice and Weber, 1983). Hence sorption of BDE-209 molecules on fine particles would be expected. During CHP reactions, the fine particulates are suspended in solution due to action of H_2O_2. Since sampling was carried out by drawing the solution from the top of sediments without centrifugation, it is possible that the suspended fine particulates might also be drawn, causing some of the BDE-209 molecules to be lost with the discarded solution. After 24 hours, quiescent conditions were achieved in sediment/solution system when the reactions terminate so that all particles settle in sediment phase. Therefore, samples at the first hours of reaction may not fully represent the effect of degradation. For the Fenton set, on the other hand, a continuous decline was observed throughout the reaction. This may be because H_2O_2 decomposition was faster when compared to CHP reactions, which shortens the time for radical formation reactions in the Fenton process (remaining H_2O_2 was 10 g/L at t=1 h). Hence, effect of fine particulate loss would be expected to be at minimum in Fenton set so that gradual decrease of BDE-209 level was clearly observed for this set.

Overall reduction at the end of 24 hours in BDE-209 concentration was comparable between sets. The highest degradation was in "H_2O_2" set with 38.5% reduction. Other sets followed the order 31.6%, 23.1% and 17.4% reduction for "pH unadjusted", "H_2O_2-repeat", and "BDE-high", respectively. Fenton set exhibited a reduction in BDE-209 of 43.5%. Although lowest reduction was observed in "BDE-high" set, it is not possible to conclude that there was a net trend in percent reduction depending on contaminant concentration.

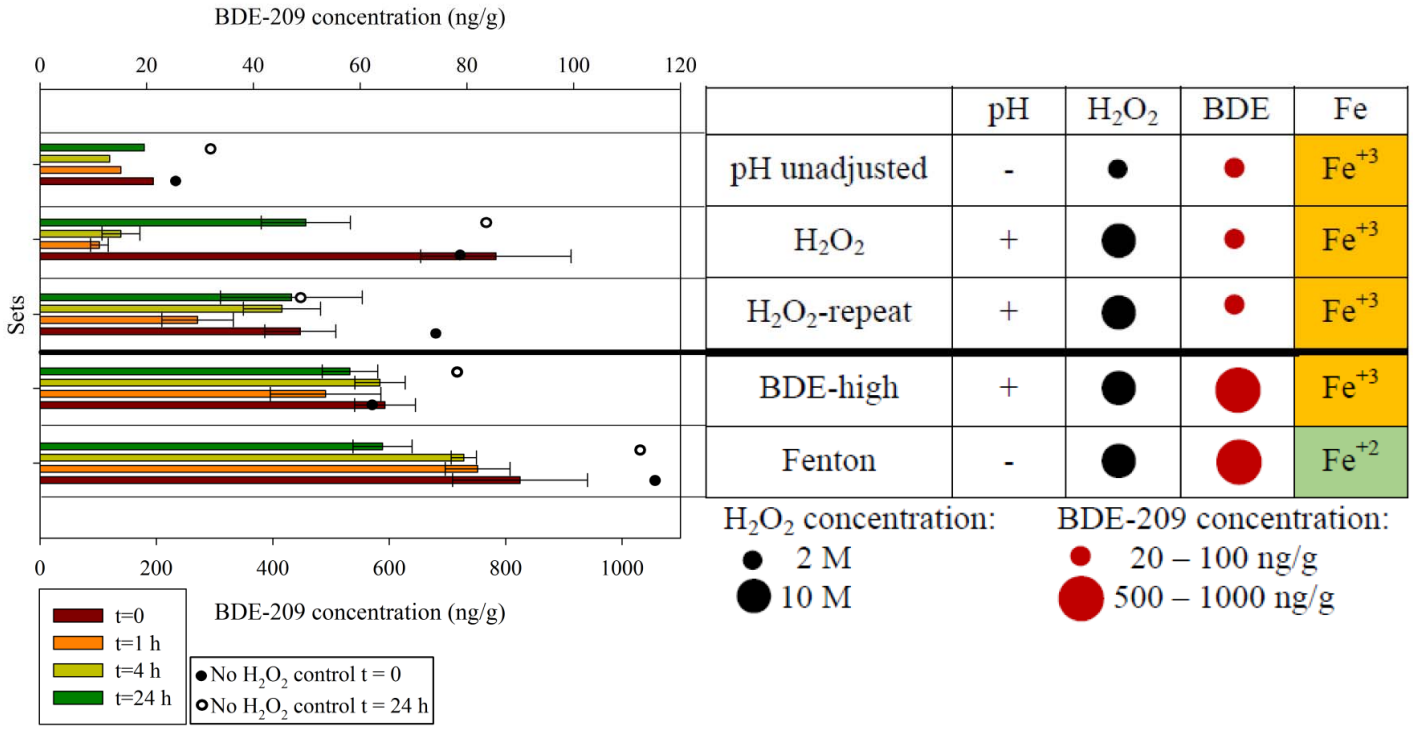


Figure 7-1. BDE-209 vs time for single-fill microcosms. The experimental conditions are given on the right hand panel, and corresponding results are presented on the left hand side. The BDE-209 concentrations can be read from the top and bottom axis, above and below the thick line, respectively.

Using the experience gained from BDE-209 single-fill microcosms, a set was prepared for HBCDD spiked sediments using the favorable operational conditions observed for CHP (i.e., 10 M H_2O_2, pH=2, Fe(III)= 300 ppm). The results for this set are presented in Figure 7-2. As can be observed from figure, change in γ-HBCDD level resembled that of BDE-209, with an apparent reduction at first hour and gradual increase afterwards. Nevertheless, overall reduction in 24 hours reached 46.4% with respect to average concentrations of control reactors. This was slightly higher as compared to the percent reduction achieved in BDE-209 sets. Here, there was an apparent difference in γ-HBCDD concentrations between t=0 and control reactors. This was barely observed for BDE-209 preliminary experiments. One reason for this observation might be that drawing the solution right after addition from t=0 bottles caused some γ-HBCDD lost with fine particulates. However, the distilled water on no H_2O_2 control sediments were also discarded in the same manner. Hence, this is not very likely to explain this observation. Another, and more possible, reason may be the higher solubility of γ-HBCDD in water (i.e. $3.4*10^{-3}$ mg/L) (European Commission, 2008) when compared to that of BDE-209 (i.e. $1.3*10^{-8}$ mg/L) (Palm et al., 2002). Higher solubility of γ-HBCDD might cause this compound to become more available for oxidative attacks of reactive species formed during CHP. Therefore, as soon as the solutions were added, the reactions started, yielding the observation of a quick decline in time zero concentrations. This may also explain the higher percent reduction of HBCDD achieved with CHP reactions when compared to BDE-209.

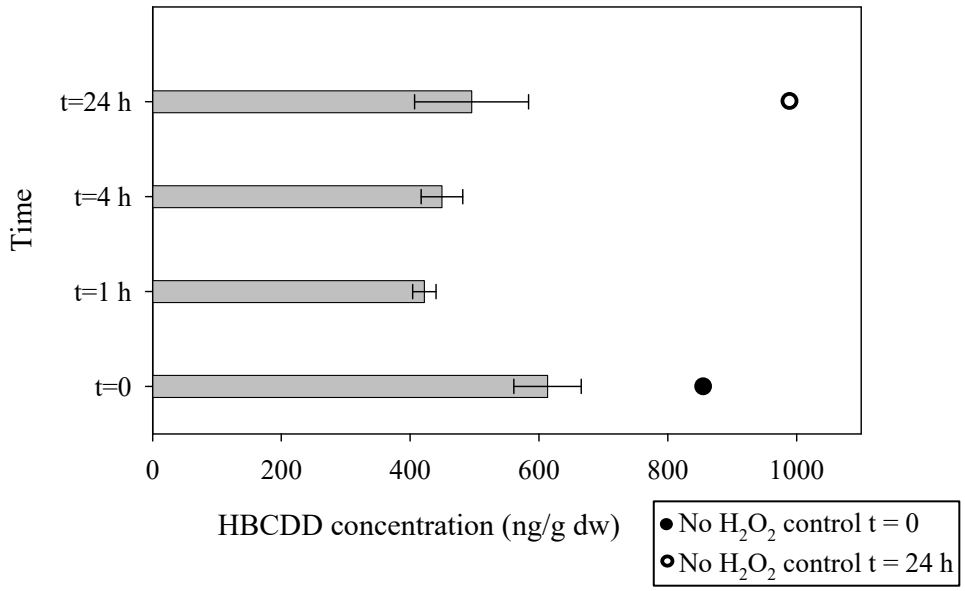


Figure 7-2. γ-HBCDD change in time for single-fill microcosms.

Single-fill microcosm experiments gave valuable information about response of PBDEs and HBCDD to CHP reactions. Lessons learned from these experiments were:

- Monitoring contaminant level in the first hours of reaction might lead to erroneous contaminant concentration in sediments.
- Application of CHP for 24 hours was evaluated to not be feasible due to observation of inefficient use of H_2O_2, especially on sediment organic matter instead of on degradation of contaminant.

As a result, application of CHP was planned to follow fill-and-draw (F&D) treatments, i.e. solution will be drawn after a predetermined time, and then refilled with a fresh solution of H_2O_2 and Fe(III) at same concentrations. This would provide continuous formation of reactive species with CHP reactions. This treatment process was previously applied once for PCB destruction in soil (Ahmad et al., 2011). The researchers investigated H_2O_2 longevity in soil with stabilizers, and optimum H_2O_2 concentrations for effective removal. Using the optimum conditions, F&D

application of CHP was shown to reduce total PCB levels in soil up to 94% (Ahmad et al., 2011).

A preliminary F&D set was established to see its effectiveness. F&D treatments were done on every 24th hour. H_2O_2 was monitored by measuring it in solution prior to withdrawal of solution. There was no decrease in H_2O_2 after treatment 3. The BDE-209 change with treatments, and H_2O_2 concentrations at withdrawal was shown in Figure 7-3. Although in treatment 3, an increase in concentration was observed, overall reduction in BDE-209 concentration was 47.7% at the end of 5 treatments, which was higher than those observed in single-fill microcosms. Therefore, application of CHP as F&D treatments was evaluated to be effective in PBDE removal from sediments.

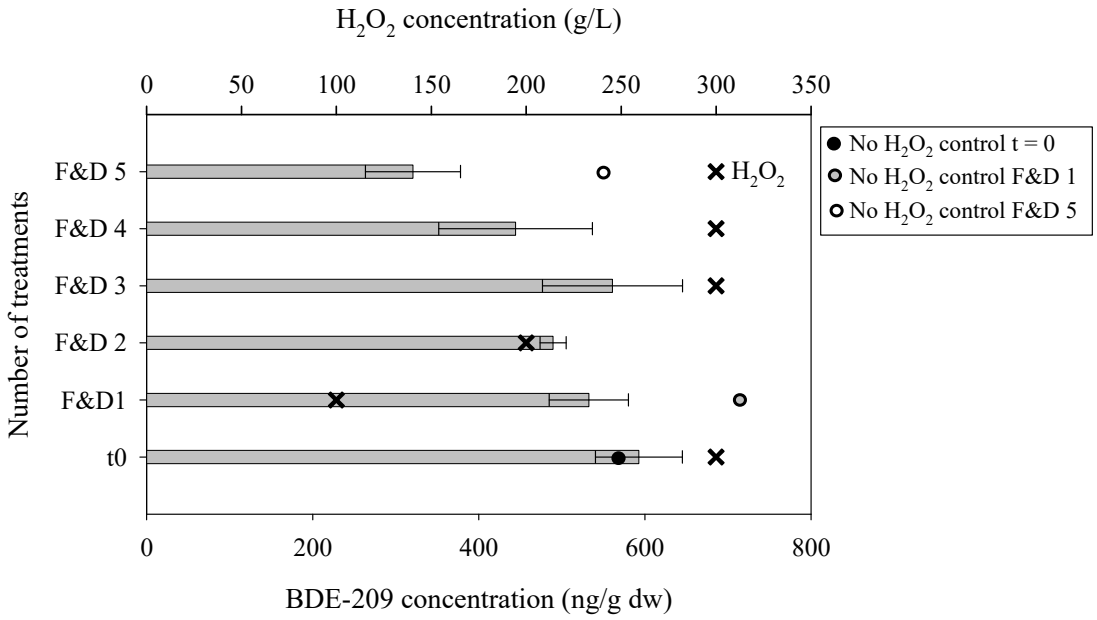


Figure 7-3. Change in BDE-209 concentration (below axis) with fill-and-draw treatments. Cross signs indicate H_2O_2 concentrations (top axis) measured prior to solution withdrawal.

Since this application showed no change in H_2O_2 concentrations after third treatment, and Ahmad and colleagues (2011) exchanged solutions after all H_2O_2 was diminished, a separate test was performed to determine the applicable F&D treatment times. A simple reaction set was established with unspiked sediments. H_2O_2 concentration was 300 g/L initially, and measured every day. At the end of seven days, it reached zero. Hence, minimum of seven days were to be used in F&D treatment sets.

7.3.2. Abiotic degradation of BDE-209 and BDE-99 in sediments

CHP was applied as F&D treatment to sediments simultaneously spiked with BDE-209 and BDE-99. Even though 7 days was found to be adequate time for H_2O_2 attenuation in unspiked sediment test, this was not the case for spiked reactors. H_2O_2 concentration monitoring showed 97% decrease after 8 days. Therefore, F&D treatment time was decided as 8 days for this application. In every treatment, H_2O_2 concentration was found to be reduced by the same reduction ratio.

Together with the F&D microcosms that were used to observe contaminant reduction, LOI controls were employed to observe reduction in organic content of sediment at time 0, treatment 1 and treatment 5. Organic content of the sediment was 1.44% without addition of any solutions. Sediment samples prepared and treated similarly with test systems yielded organic contents of 1.02%, 0.85% and 0.83% for time zero, treatment 1 and treatment 5, respectively. These results showed that addition of H_2O_2 solution resulted in an almost instant decomposition of organic content in sediments, and after 8 days of exposure to CHP reactions, 41% of organic matter in sediments were decomposed. The reactive species of CHP reactions occurring after first treatment may then be expected to engage in contaminant degradation. Furthermore, it was previously revealed that decomposition of soil organic matter by H_2O_2 could cause the soil to have more hydrophilic sites, thus sorption of hydrophobic organic compounds would decrease (Site, 2001; Z. Zhang et al., 2014), and the organic compounds would then be expected to more prone to attacks of reactive species in CHP system.

Change in concentration for BDE-209 and BDE-99 with respect to treatment times are presented in Figure 7-4. For this system, spike controls were also employed, which did not include any solution, to observe the instantaneous effect of solution addition onto time zero samples. As can be clearly seen from the figure, at t=0, BDE-209 concentrations agreed well with no H_2O_2 control and spike control levels, while BDE-99 concentrations were lower. This was also observed in single-fill HBCDD microcosm set. As discussed in Section 7.3.1, differences in solubilities of BDE-209 and BDE-99 would lead to the observation of instantaneous effect of H_2O_2 in BDE-99 levels in sediments.

The CHP F&D treatments achieved 31.8% removal of BDE-209 at the fourth treatment at best, and overall 24.1% reduction was observed at fifth treatment. On the other hand, there was a continuous decrease in BDE-99 throughout the treatment system, reaching an overall 83% reduction at treatment 5. Hence, it can be evaluated that CHP is more effective in BDE-99 removal in sediments when compared to BDE-209.

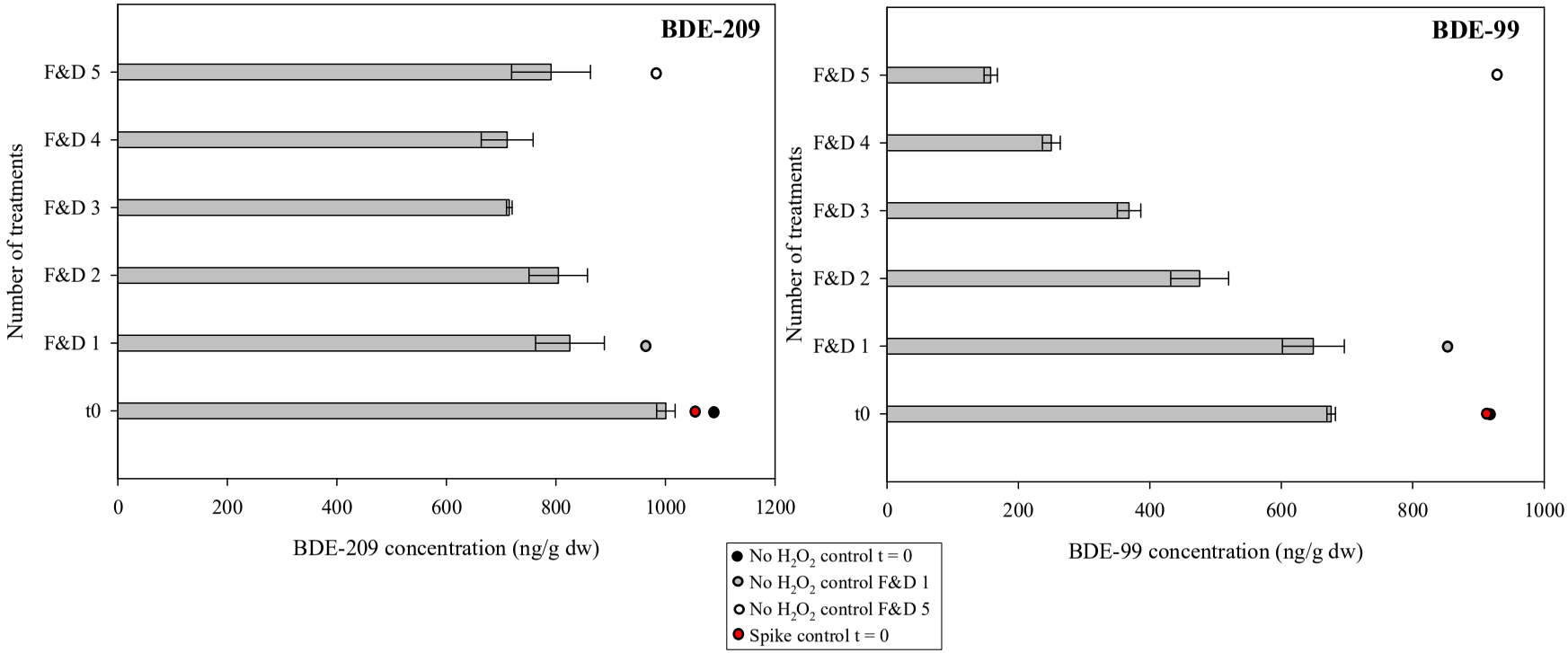


Figure 7-4. Change in BDE-209 (left figure) and BDE-99 (right figure) concentrations in sediments with respect to number of F&D treatments.

The difference in physico-chemical properties of BDE-99 and BDE-209 are believed to be effective in the observation of different reduction percentages. A summary of these properties are presented in Table 7-4. BDE-99 has at least one (and at most five) order of magnitude higher water solubility, and two orders of magnitude lower Kow compared to BDE-209. The sorption of PBDEs on sediments is due to van der Waals forces and thermodynamic gradient, which result in nonpolar, low soluble congeners to prefer to bond organic matter (Voice and Weber, 1983). When the organic matter is oxidized, their sorption is reduced, thus a retarded removal of BDE-209 in CHP was observed as compared to BDE-99. Another reason for reduced removal rate of BDE-209 might be steric hindrance occurring for BDE-209 due to its fully brominated structure. The atoms of this molecule occupy more space to hinder attack of reactive species.

Table 7-4. Physico-chemical properties of BDE-99 and BDE-209.

	BDE-99	**BDE-209**
Water solubility (mg/L)	$9*10^{-7} – 2.4*10^{-3}$ [Ψ]	$1.3*10^{-8}$ [Ψ]
Log Kow	6.53 – 7.66 [Ψ]	9.97[ξ], 11.15 [Ψ]

[Ψ] (Palm et al., 2002), [ξ] (Stockholm Convention, 2013)

7.3.3. Abiotic degradation of HBCDD

CHP was applied as F&D treatment to sediments spiked with γ-HBCDD. As in PBDE's case, F&D treatment time was 8 days. In every treatment, H_2O_2 concentration was monitored, but its concentration was found to be changing at each F&D time, such that H_2O_2 level reduced by 97 – 99% in various F&D times.

The F&D microcosms included the LOI controls with distilled water and H_2O_2 to observe reduction in organic content of sediment in treatments. Sediment samples with distilled water yielded organic contents of 1.29%, 1.20% and 1.15% for time zero, treatment 1 and treatment 5, respectively. Although there is a slight decrease in organic content, this is much less when compared to those observed for PBDE sets.

On the other hand, sediment samples with H_2O_2 yielded organic content of 1.11%, 0.83% and 0.95% for time zero, F&D - 1 and F&D - 5, respectively. A net decreasing trend is not evident in organic matter, yet results indicated that H_2O_2 can decompose the organic content of Çamkoru sediments up to 0.83%, and the reactive species of CHP reactions occurring after first treatment may then be expected to engage in HBCDD degradation as in PBDEs.

Change in concentration of HBCDD with respect to treatment times is presented in Figure 7-5. As also observed for single-fill HBCDD microcosms and F&D BDE-99 microcosms, spike controls and no H_2O_2 control at time zero showed higher HBCDD levels than time zero samples. The instantaneous effect of CHP reactions on HBCDD concentration in sediments was obvious.

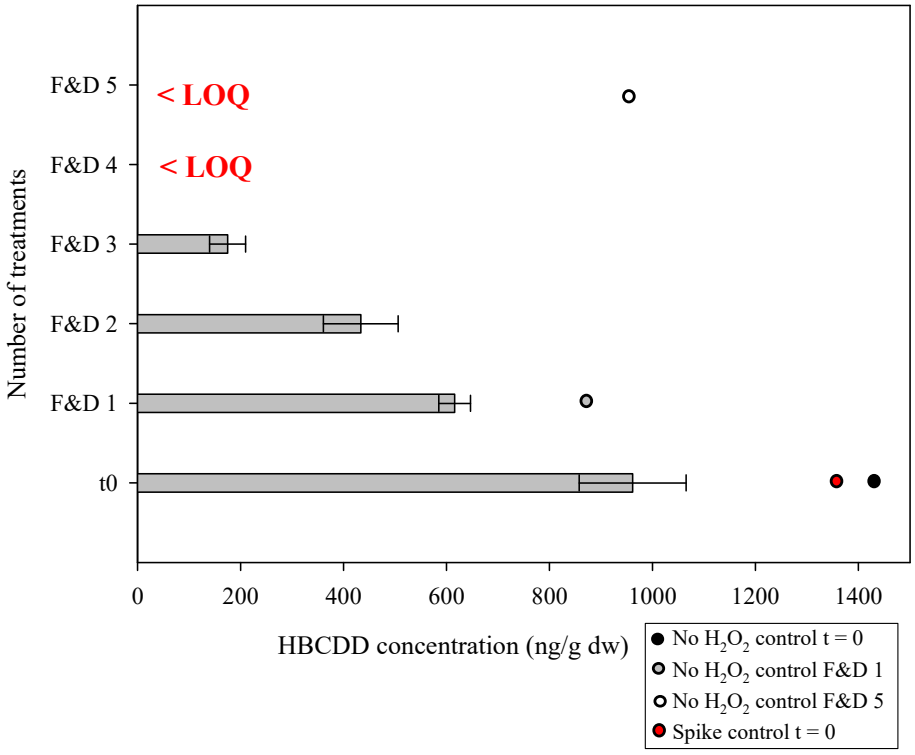


Figure 7-5. Change in HBCDD concentration in abiotic degradation of sediments with respect to number of F&D treatments.

The CHP F&D treatments achieved almost complete removal of HBCDD from sediments after five treatments. At fourth treatment, HBCDD remaining in sediments fell below the limit of quantitation (but above MDL), reaching over 90.5% reduction. Hence, it can be evaluated that CHP effectively removed HBCDD in sediments.

When compared to PBDEs, the behavior of HBCDD in sediments treated with CHP reactions resembles that of BDE-99, both demonstrating a continuous decline with treatment times. At the end of five treatments, HBCDD removal was greater than BDE-99. The physico-chemical properties of these two compounds differ in terms of water solubility and log Kow. Water solubility of HBCDD is higher, and its log Kow is lower than BDE-99. These properties make the chemical more favorable for CHP reactions to be effective, especially for those that are sorbed onto a solid media. Furthermore, the structural difference between PBDEs and HBCDD may also play an important role in abiotic degradation mechanisms of the compounds.

7.3.4. Evaluation of CHP degradation of BFRs and comparison with abiotic degradation studies from literature

CHP was found to be effective for the degradation of BFRs in aquatic sediments with microcosm reactors operated in this study. Kinetics of BFR degradation could not be performed since application of CHP was based on withdrawal and refilling of solutions onto sediments. There are no studies on removal of BFRs using CHP. Comparison with other abiotic studies is performed based on percent removal of BFRs. Table 7-5 summarizes abiotic degradation studies conducted for PBDE and HBCDD in soil and sediments. As can be seen from the table, there is only one study that investigates abiotic degradation of HBCDD in a solid matrix. There were studies for photodegradation (Yu et al., 2015b; Zhou et al., 2014, 2012), ultrasound assisted Fenton reaction (Ye et al., 2014), and degradation with nano-ZVI particles (Tso and Shih, 2014) of HBCDD in aqueous single-phase solutions. However, these were not included in comparison since they would not be comparable to degradation in solid media.

Table 7-5. Performance comparison between abiotic degradation mechanisms for PBDEs and HBCDD.

Media	Contaminant	Abiotic degradation mechanism	% removal	Ref
Lake sediments	BDE-209	CHP with F&D microcosm	31.8% in 4 treatments (32 d)	*This study*
Lake sediments	BDE-99	CHP with F&D microcosm	83% in 5 treatments (40 d)	
Lake sediments	γ-HBCDD	CHP with F&D microcosm	>90.5% in 4 treatments (32 d)	
Humic acid coated sand particles	BDE-209	Photodegradation under sunlight	10.9% in 96 h	(Hua et al., 2003)
Sand	BDE-209	Photodegradation under sunlight Photodegradation under UV-light	64% in 96 h 79% in 32 h	(Söderstrom et al., 2004)
Sediment	BDE-209	Photodegradation under sunlight Photodegradation under UV-light	57% in 96 h 57% in 32 h	(Söderstrom et al., 2004)
Sediment	BDE-209	Photodegradation under UV-light Photodegradation under sunlight	6.24% in 14 d 6.83% in 101 d	(Ahn et al., 2006)
Sediment	BDE-209	Debromination with ZVI	90% in 56 d	(Huang et al., 2014)
Soil	BDE-209	Ni/Fe bimetallic nanoparticles	72% in 70h	(Xie et al., 2014)
Soil	BDE-209	Debromination with nano-ZVI	78% in 120 h	(Xie et al., 2016)
Soil	BDE-209	Biochar supported Ni/Fe bimetallic nanoparticles	87.7% in 72h	(Wu et al., 2016)
Soil	HBCDD	Mechanochemical degradation with various co-milling agents	75 – 99% in 4 h	(K. Zhang et al., 2014)
Soil	PBDEs BDE-99	Modified Fenton with tourmaline	53% in 70 d 49% in 70 d	(J. Li et al., 2016)
Fungi treated soil	BDE-99	Treatment with two doses of H_2O_2	50 & 55% in 50 d	(Zhang et al., 2014)
Sediments	BDE-209	Oxidation with UV/H_2O_2	90% in 10 h	(Feo et al., 2014)

When the results of previous studies were examined, the highest degradation was observed for advanced oxidation of BDE-209 with UV/H_2O_2 system. Photodegradation generally resulted in greater removal especially with artificial UV-light compared to the present study. However, Ahn and colleagues (2006) found lower degradation percentage in sediments under sunlight. Debromination with zerovalent iron particles also showed remarkable reduction in BDE-209 levels within hours. Modified Fenton reaction with tourmaline, and application of H_2O_2 to fungi treated soil demonstrated comparable reduction with the present study.

As mentioned, CHP was never applied for PBDEs and HBCDD, therefore, a comparison can be made with PCB contaminated soil treated with CHP via F&D treatment process to evaluate its applicability to these compounds. Application of CHP by using similar conditions with the present study (i.e. pH adjustment, concentration of H_2O_2) for total PCB degradation in soil resulted in 70% and 85% reduction for 4 and 8 treatments (F&D period = 4 days), respectively (Ahmad et al., 2011). It is important to note that commercial PCB mixtures did not include PCB-209 congener in their composition (Frame et al., 1996). Therefore, application of CHP to PCBs in Ahmad and colleagues' study (2011) does not involve the possible response of a fully chlorinated biphenyl structure. On the other hand, the PCB removal percentages were similar with BDE-99 and HBCDD removal of this study. As a result, it can be concluded that application of CHP with F&D treatments may have a potential for use in treatment of BFR contaminated sediments.

7.4.Conclusion

Application of CHP to BFR contaminated sediments was investigated for the first time in this study. F&D CHP process was found to be more effective in removal of BFRs from contaminated sediments when compared to single-fill 24-hour microcosms. This was clearly observed especially for BDE-99 and γ-HBCDD. BDE-209 showed an inconsistent behavior between F&D treatment periods of 1 day vs 8 days. The physicochemical properties of BDE-209 make this chemical behave substantially differently when compared to the other BFRs of concern in this study. In order to increase efficiency for abiotic degradation of BDE-209, the F&D period

may be further optimized or the number of F&D treatments may be increased. Additionally, higher H_2O_2 concentrations may be tested since there are reports of use of much higher levels in literature studies compared to levels used in this study.

When compared to other abiotic degradation mechanisms such as debromination with bimetallic nanoparticles, advanced oxidation with hydrogen peroxide under UV, and artificial photodegradation, CHP can be evaluated as relatively slow. However, it reached similar reduction percentages with modified Fenton and natural photodegradation. While evaluating remediation strategies, the applicability of techniques should be assessed in terms of appropriateness to site conditions, and formation of products, other than contaminant removal percentages. For instance, photodegradation, as a natural process, might not be very effective in deep sediments, compared to surface sediments and soil. Furthermore, advanced oxidation processes requiring UV light cannot be applied for in situ remediation. On the other hand, debromination via nanoparticles may produce more bioavailable or more toxic products. Although the products of CHP cannot be identified within the scope of this study, PBDEs and HBCDD may be transformed via ring cleavage. In order to make a comprehensive discussion on the possible consequences of CHP, products should also be identified.

CHP can be used to degrade PBDEs and HBCDD in aquatic sediments via application of fill-and-draw treatment of reagents into system. Regarding remediation of contaminated sediment, this system can potentially be applied under both in situ and ex situ conditions. In situ applications can be performed via direct injections in sediments by using injection well-type equipments. For in situ remediation, drawing of solution cannot be possible; however, when residual peroxide reaches very low levels, reagents can be reinjected. Additionally, environmental conditions should be monitored not to cause temperature increases, which may affect the ecosystem. Ex situ remediation of sediments, on the other hand, can be performed similarly to the system operated with this study. However, pilot scale reactors should be operated to observe the system performance at larger scale, and these need further inquiry.

CHAPTER 8

REDUCTIVE DEBROMINATION OF PBDES IN SEDIMENT MESOCOSMS VIA BIOSTIMULATION

8.1.Introduction

The fate of PBDEs in the subsurface environment has attracted attention of researchers in the last decade. PBDE transformation has been shown to occur via photodegradation (Ahn et al., 2006; Hua et al., 2003; Söderstrom et al., 2004), zero valent iron reduction (Wu et al., 2016; Xie et al., 2016) and microbial (aerobic and anaerobic) degradation (Huang et al., 2012; Tokarz III et al., 2008) in soil and sediment systems. Among those, anaerobic degradation mechanism is the focus of this study since PBDEs were consistently measured in sediment cores from rivers and bays (Minh et al., 2007; Zhang et al., 2009), where anaerobic conditions prevail.

There are limited number of studies on anaerobic degradation of PBDEs. These studies investigate the degradation kinetics of PBDEs in soil, sediment and sludge under various conditions, e.g. via addition of primers (Gerecke et al., 2005), in the presence of various electron donors (Qiu et al., 2012), and various buffer solutions including mineral salts and vitamins (Huang et al., 2014; Lee and He, 2010; Tokarz III et al., 2008). Currently, there are no studies examining anaerobic degradation of PBDEs in large scale laboratory reactors. Orihel and colleagues (2016) studied BDE-209 degradation in environmental mesocosms, however, they concluded with a slight degradation of BDE-209 after 2 years (Orihel et al., 2016).

Formerly within the scope of this study, three bioremediation alternatives were investigated in sediment microcosms, and it was revealed that biostimulation showed

the highest degradation rate for anaerobic debromination of BDE-209 (Chapter 5). The aim of this part of the study is to examine anaerobic debromination of PBDEs in mesocosms to simulate environmental aquatic sediments, when biostimulation is applied as a bioremediation strategy. To achieve this aim, sodium formate and ethanol was added on sediments as the carbon source and electron donor, respectively. Different than microcosm reactors of the previous study, sediments received a reduced amount of carbon source and electron donor in order not to provide great amount of chemical oxygen demand into the aquatic sediment system. The target compounds of this study were the PBDE congeners observed at the highest concentrations in environmental media among others: BDE-209 and BDE-99 (Hites, 2004). Application of biostimulation in large scale reactors is expected to provide i) the efficiency of this technique in the simulated environmental sediments, ii) rates of degradation when reduced amount of carbon source and electron donor is supplied, iii) the degradation mechanism of BDE-99.

8.2.Materials and Methods

8.2.1. Chemicals

All solvents (n-hexane (HEX), dichloromethane (DCM), acetone(ACE)) used for analysis, anhydrous sodium sulfate (granular), copper fine powder (<63 μm), and aluminum oxide (0.063-0.200 mm) were purchased from Merck KGaA (Darmstadt, Germany). Individual standards of surrogate standard PCB-141 (2,2',3,4,5,5'-CB), internal standard PCB-209 (2,2',3,3',4,4',5,5',6,6'-CB) were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany). PBDE predominant congeners mixture (BDE-CSM) was purchased from AccuStandard (New Haven, USA). A standard mixture of octa-, nona-, and deca-BDEs (BDE-OND), individual standards of BDE-17 (2,2',4-BDE), BDE-49 (2,2',4,5'-BDE) and BDE-66 (2,3',4,4'-BDE) were purchased from Wellington Laboratories (Canada). BDE-209 (2,2',3,3',4,4',5,5',6,6'-BDE), and BDE-99 (2,2',4,4',5-BDE) were supplied from CPA (Bulgaria).

8.2.2. Sediment mesocosms

The sediments that were used in mesocosm studies were collected from a pond in a specially protected forest area (Camkoru National Park) located 110 km northwest of Ankara, Turkey. Sediments were expected to have no previous PBDE contamination as the pond is located close to a passive POPs air monitoring station which consistently show very low POPs concentrations (Jarkovský et al., 2015). Surficial sediments under 70 cm water depth were collected from five different points in the pond and were wet-sieved (2 mm) on-site to remove large particles. Samples were transported to the laboratory and stored at 4 °C in glass jars in the dark until use. Moisture content of sediment was analyzed by drying 10 g of sample in 105°C oven for 24 hours, and found to be 36.5±1.53% (*n*=3). Total organic content analysis was then performed via loss-on-ignition procedure, by igniting the sample analyzed for moisture content in 550°C furnace for 4 hours (Heiri et al., 2001), and determined to be 1.43±0.16% (*n*=3).

Mesocosm reactors of approximately 2400 mL total volume (LxWxH: 21 cm x 5.7 cm x 20.4 cm) made up of monolithic glass were used. Preparation of mesocosms was performed completely under N_2:CO_2:H_2 environment in an anaerobic glovebox (PlasLabs 818GB/Exp). PBDE spike was done similarly as the microcosms: spike of BDE-209 and BDE-99 onto dried sediments, thorough mixing until solvent evaporation, addition of wet sediments and complete mixing as a whole. Target contaminant concentration was 1000 ng/g dw for both BDE-209 and BDE-99. However, in one of the sterile sets, volume of BDE-99 added was accidentally smaller compared to others, which resulted in the concentration at that specific reactor to be 750 ng/g dw. Unspiked control sets were prepared by adding the same volume of solvent (isooctane:toluene mixture of 9:1) as BDE spikes. After mixing provided under anaerobic atmosphere, sediments were placed into mesocosm reactors. On top of sediments resazurin was added as a redox indicator. For each set, parallel reactors were operated. The details of sediment mesocosms are presented in Table 8-1.

Table 8-1. Details of PBDE sediment mesocosms.

Reactor	Sediment	Spike	Carbon source & electron donor
BDE-209 Biostimulation	+	BDE-209	+
BDE-209 Sterile	+ *	BDE-209	-
BDE-99 Biostimulation	+	BDE-99	+
BDE-99 Sterile	+ *	BDE-99	-
Contamination Control	+	-	-

* Reactors were autoclaved at 120°C at 1.1 atm pressure for 20 min on three consecutive days.

Each reactor contained 720 g of wet sediments, and total of 550 mL liquid. For biostimulation sets, 50 mL of organic medium was added onto sediments, mixed gently, and then 500 mL of distilled water, purged with nitrogen, was added. Although in microcosms topping liquid included only the organic medium, in mesocosms a diluted organic medium was provided. The reason for this was to supply an acceptable chemical oxygen demand (COD) in sediments. The organic medium had a COD of 2500 mg/L. According to Water Pollution Control Regulation, receiving water discharge criteria for many industries has COD values around 200 – 250 mg/L (Ministry of Environment and Urbanization, 2004). Additionally, surface water classification criteria for COD lies between less than 25 mg/L and greater than 70 mg/L (Ministry of Environment and Urbanization, 2004). Therefore, COD load to the sediments was kept as low as possible by diluting the organic medium with a ratio of 1/11. Accordingly, for sterile and control sets 550 mL distilled water was added.

After preparation, sediment mesocosms were placed into the incubator at 25°C in the dark. Sterile sets were autoclaved at 120°C at 1.1 atm pressure for 20 min on three consecutive days before placing into the incubator. Although they were prepared under anaerobic atmosphere, this atmosphere could not be provided during incubation. The reactors were allowed to contact with normal atmosphere to mimic the environmental conditions, hence the overlying water was exposed to the

atmosphere. The mesocosm reactors have a glass lid on top, only opened when sampling was done.

Triplicate sediment samples were taken from each reactor with minimum disturbance to sediments (i.e. as a core sample using a glass pipette with tip cut-off). Sediment sampling was performed on days 0, 15, 30, 60, 90, and 120 for BDE-209, and on days 0, 15, 30, 45, 60, 75, 90, 120 for BDE-99. Nona- to tri-BDEs were monitored for BDE-209 degradation products, and tetra- and tri-BDEs were monitored for BDE-99 degradation.

Percent remaining PBDEs in mesocosms was calculated by taking the initial concentration as that detected in day zero samples. The degradation kinetics of PBDEs was explained by pseudo-first-order model: $C = C_0 e^{-kt}$, where C is the concentration at sampling times (ng/g dry weight), C_0 is the initial BDE-209/99 concentration (ng/g dw), k is the pseudo-first-order rate constant (d^{-1}), and t is the incubation time (d). Rates are calculated by plotting $ln(C/C_0)$ vs t, and checked using coefficient of determination, R^2.

8.2.3. PBDE extraction and analysis

The extraction of PBDEs from sediments was based on US EPA method 3550C Ultrasonic Extraction (USEPA, 2007a). One gram of sample was mixed with equal amount of anhydrous sodium sulfate in 40 mL vials, and spiked with surrogate standard. Samples were extracted in an ultrasonic bath with 30 mL DCM: HEX: ACE mixture (7:7:1 v/v) for 30 minutes twice, after being soaked in the solvent mixture overnight. Sulfur removal was achieved with the addition of copper powder into the extraction solvents prior to extraction. The two extracts were combined and concentrated to 2-5 mL via rotary evaporator. To remove possible interfering organic compounds, the colored extract after concentration step was treated with concentrated sulfuric acid (U.S. EPA Method 3665A). The top clear extract was purified with 0.5 g of alumina (deactivated to 6%) topped with anhydrous sodium

sulfate, and eluted with 2 mL of HEX. The collected extract was concentrated to 2 mL by high purity nitrogen stream.

One mL of this extract was spiked with internal standard and analyzed with gas chromatography coupled with micro-cell electron capture detector (Agilent 6890N GC-μECD) with DB-5 MS capillary column (15 m x 0.25 mm ID x 0.10 μm). Instrumental conditions were as follows: Helium was used as the carrier gas with 1.8 mL/min flowrate using a constant flow mode. The make-up gas for the detector was nitrogen with a flowrate of 30 mL/min. The injector and detector temperatures were 250°C and 350°C, respectively. The sample injection was carried out at 1 μL with splitless injection mode. Oven temperature program started at 90 °C, raised at 20°C/min to 310 °C, and hold there for 6 min. PBDE congeners 17, 28, 47, 49, 66, 99, 100, 153, 154, 183, 194,195, 196, 201, 202, 198/199/200/203 (co-eluting peak), 197/204, 205, 206, 207, 208, and 209 were identified according to the retention times given with certificates of standards and previous studies.

Analysis of BDE-209 standard (CAS No:1163-19-5) in GC-μECD revealed nona-BDE peaks, increasing linearly with the concentration of standard analyzed. Hence, analysis of 100 ppb, 200 ppb, and 400 ppb BDE-209 standard solutions five times consecutively resulted in the derivation of ratios for each nona-BDE in the standard. Average ratios were found to be 0.0097, 0.0191, and 0.0214 for BDE-208, -207, and -206, respectively, all of which has RSDs lower than 15%. Hence, a correction in nona-BDE quantitation was performed by subtracting the value found by multiplying the corresponding ratio with BDE-209 concentration from the chromatogram result of nona-BDE.

8.2.4. QA/QC

Laboratory control samples were analyzed to check the extraction efficiency, and results yielded recoveries of 96.0±6.07% (range: 86-105.7% for *n*=7) for BDE-209, and 105.0±2.33% (range: 101.6-108.5% for *n*=7) for BDE-99. The analytical procedure was further validated with analysis of a certified reference material, which was PCB and PBDE contaminated sandy loam sediment (RTC CNS329). For PBDE congeners 47, 99, 100, 153, 154, and 183, recoveries were 101.3±5.8%, 88.3±2.3%,

84.1±1.5%, 87.7±1.9%, 88.9±2.8%, and 101.4±5.2% (average±standard deviation for triplicate samples), respectively. Method detection limits for single congeners were in the range of 0.06 ppb (BDE-47) to 3.94 ppb (BDE-209), and limit of quantitation were in the range of 0.19 ppb (BDE-47) to 12.53 ppb (BDE-209). A complete list of MDL and LOQ values of each congener is provided in Table 3-4. Blanks were analyzed in every batch of 15 samples, and no peaks were detected during analysis. Average surrogate recoveries of BDE-77 and PCB-141 for the data sets were 87.2±11.28% (range: 64.4 – 126.8%) and 85.3±10.9% (range: 65.7 – 122.4%) for BDE-99 mesocosm samples, respectively, and 91.2±12.0% (range: 65.3 – 125.4%) and 91.6±11.3% (range: 69.8 – 114.6%) for BDE-209 mesocosm samples, respectively. No surrogate and/or blank correction was deemed necessary.

8.3.Results and Discussion

BDE-209 and BDE-99 degradation were observed in sediment mesocosms, operated in parallel reactors for each. Degradation behavior in the parallel reactors showed some variation, especially for BDE-209, although preparation and operation were completely the same. Hence, the results were presented and discussed by presenting results of individual reactors.

8.3.1. Degradation of BDE-209 in sediments

The change in concentration of BDE-209 in parallel reactors and corresponding percent BDE-209 remaining in reactors is presented in Figure 8-1. The BDE-209 concentration decrease was gradual in time, and nearly stopped after 60 days of incubation for biostimulation reactor B. At the end of 120 days incubation, biostimulation reactor A reached 12.2%, and reactor B reached 16.7% reduction in BDE-209 level. These degradation percentages were much lower compared to that observed in biostimulation microcosms of this study which achieved 49.2% reduction in 120 days. The observation of lower degradation ratios in sediment mesocosms is attributed to the reduced amounts of carbon source and electron donor supplied for mesocosms. Amount of sodium formate added per gram of dry sediments was 0.805 mg/g in microcosms, while that in mesocosms was 0.05 mg/g. Likewise; amount of ethanol added per gram of dry sediments was 1.19 mg/g,

whereas that in mesocosms was 0.07 mg/g. Hence, 6% of the carbon source and electron donor added in microcosms was provided in mesocosms.

Some of the previous studies also revealed higher reduction percentages compared to biostimulation mesocosms. For example, soil microcosms demonstrated 39.7% reduction in 90 days with lactate as electron donor (Song et al., 2015), and up to 99.0% decrease was observed with various amendments in sediment slurry at the end of 180 days (Huang et al., 2014). On the other hand, Qiu and colleagues (2012) observed a similar reduction percentage of 11% in 90 days with and without addition of ethanol as an electron donor, with the highest decrease observed in the first 30 days (Qiu et al., 2012). Furthermore, there were studies showing very slight or no decrease in BDE-209 in sediment microcosms. For instance, 3.8% reduction was reported in 300 days with methanol and dextrose amendments (Tokarz III et al., 2008), while no change in concentration was observed in 90 days in sediments amended with mineral salts medium (Zhu et al., 2014). The application of a mesocosm within a lake also showed little change in deca-BDE level after 2 years, in spite of the observation of product congeners (Orihel et al., 2016). Therefore, degradation of BDE-209 is strongly affected by the characteristics of solid media, conditions of incubation, and the amendments provided. Within the scope of this study, sediment media was the same in microcosms and mesocosms, hence the factors influencing the degradation efficiency were believed to be, firstly, the amendments provided and secondly, the incubation conditions that did not allow purely anaerobic conditions in a larger scale.

Sterile sets showed small variations in BDE-209 concentration. The relative percent differences between minimum and maximum BDE-209 concentrations within the data set were 9.5% and 7.8% for sterile reactors A and B, respectively. Those of biostimulation sets were 13.0% and 18.6% for reactors A and B, respectively. Hence, it was concluded that the observed variations in sterile sets were not the result of any degradation activity. No BDE-209 or its products were detected at any time in the contamination control set.

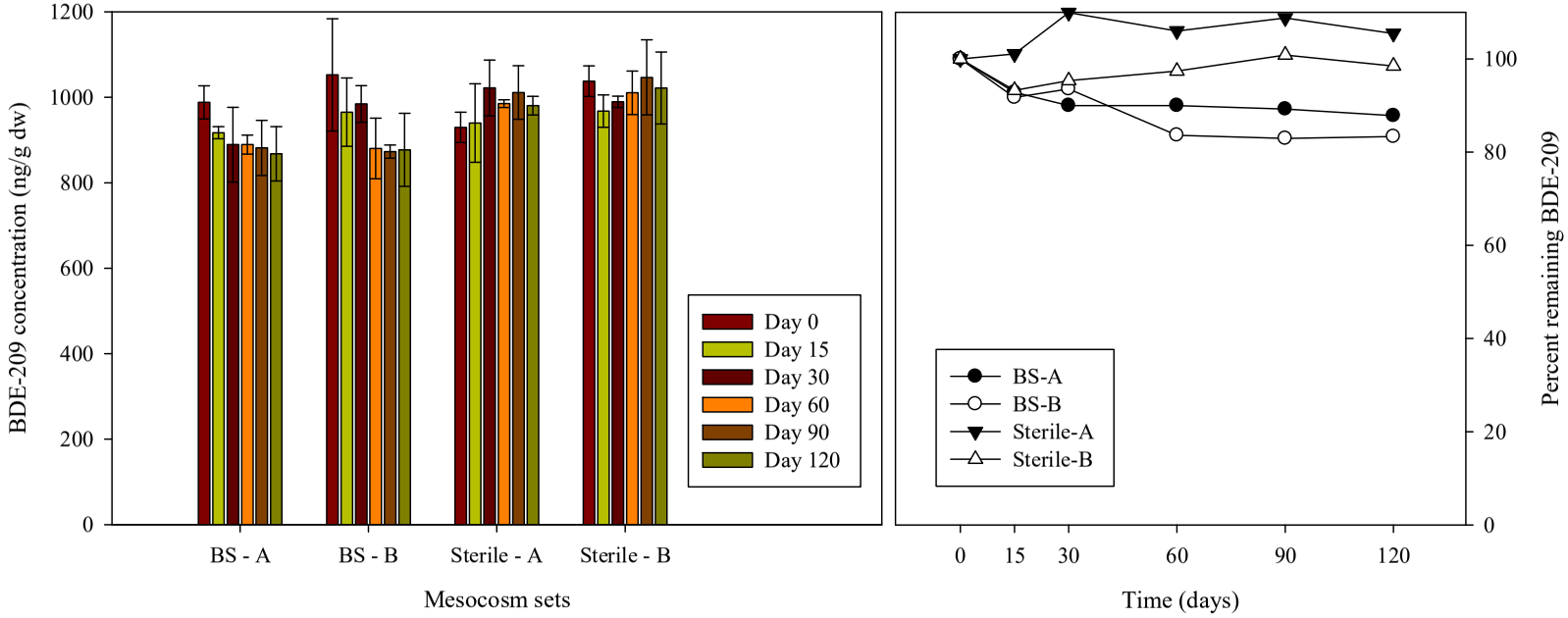


Figure 8-1. BDE-209 change in time (left panel), and percent remaining BDE-209 (right panel) in parallel reactors A & B. BS stands for biostimulation.

8.3.2. Degradation of BDE-99 in sediments

The change in BDE-99 concentration in parallel reactors, and corresponding percent remaining BDE-99 are presented in Figure 8-2. Similar to BDE-209, BDE-99 level changed evenly in time, and reached a steady level after 45 days. At the end of 120 days incubation, biostimulation achieved 18.8% and 23.3% BDE-99 reduction in reactors A and B, respectively. These were slightly higher than BDE-209 reduction percentages observed in mesocosms.

There was only one study in the literature investigating BDE-99 degradation in individual reactors (Tokarz III et al., 2008). With the addition of phosphate buffer, methanol and dextrose, BDE-99 in sediment microcosms was reduced by more than 3% in 240 days (Tokarz III et al., 2008), revealing a lower degradation ratio compared to the current study. A higher degradation percentage was reported in digested sludge reactors involving several other PBDE congeners (Shin et al., 2010). 35% reduction was observed in BDE-99 levels in 100 days, and the level stayed stable through the end of incubation of 238 days (Shin et al., 2010).

As mentioned in Section 8.2.2., volume of BDE-99 standard added in sterile reactor B was lower compared to other reactors that it received a BDE-99 concentration of 750 ng/g dw. Nevertheless, this did not result in a different behavior than the other, sterile-A reactor. Both sterile reactors showed small variations in BDE-99 concentrations in time. The relative percent differences between minimum and maximum BDE-99 concentrations within the data set were 8.2% and 6.0% for sterile reactors A and B, respectively. Those of biostimulation sets were 25.4% and 27.5% for reactors A and B, respectively. Hence, the observed variations in sterile sets were not the result of any degradation activity. No BDE-209 or its products were detected at any time in the contamination control set.

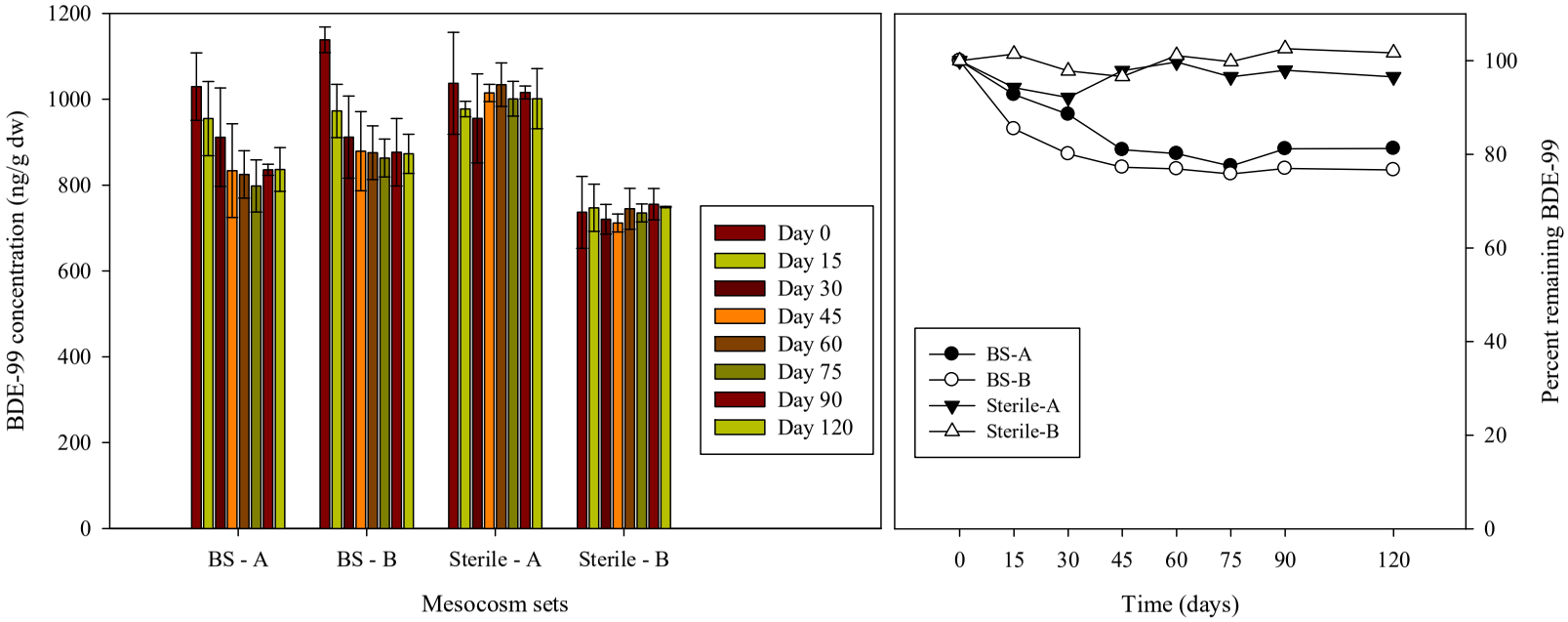


Figure 8-2. BDE-99 concentration change in time (left panel), and percent remaining BDE-99 (right panel) in parallel mesocosms A & B. BS stands for biostimulation.

8.3.3. Debromination pathways of BDE-209

BDE-209 debromination in biostimulation mesocosms resulted in the formation of nona- and octa-BDEs in 120 days. Debromination pathways identified are presented in Figure 8-3. When compared to microcosm results of this study, formation of BDE-196 and BDE-201 were demonstrated similarly with biostimulation microcosms, yet formation of some of the octa-BDEs was observed to be changed here. These observed changes in pathways are: (i) BDE-194, one of the products of BDE-206, did not appear in the mesocosms; whereas this was previously observed in all microcosms (see Figure 5-2 in Chapter 5). Formation of BDE-194 from BDE-206 was reported for the first time in literature, yet it was not observed in the mesocosms. It can be speculated that great amount of sodium formate and ethanol supplied into sediments possibly created a favorable condition for microorganisms to select this debromination pathway, (ii) BDE-197, which was previously observed only in bioaugmentation and natural attenuation microcosms, was this time observed in the mesocosms investigating biostimulation, (iii) BDE-203 was recorded in mesocosms whereas it was previously below method detection limit in all microcosms. This congener co-elutes with BDE-198, -199, and -200 during GC-μECD analysis. Previous studies did not report the formation of any of these congeners, except for two (Gerecke et al., 2005; Orihel et al., 2016). Gerecke et al. (2005) identified the co-eluting peak for BDE-198 and -203 due to the degradation of BDE-209 and BDE-206, individually. Orihel et al. (2016) also proposed formation of BDE-203 from the parent congeners BDE-206 and BDE-207. Hence, the appearance of co-eluting peak in the present study was attributed to BDE-203 among the group.

Within the scope of mesocosms study, a total of 10 pathways were identified (Figure 8-3). Among those, debromination followed the order *meta*-> *ortho*-> *para*-Br removal based on the number of pathways. This was consistent with the sediment microcosms study, and other sediment biodegradation studies revealing preferential removal from *ortho* and *meta* positions (Orihel et al., 2016; Tokarz III et al., 2008).

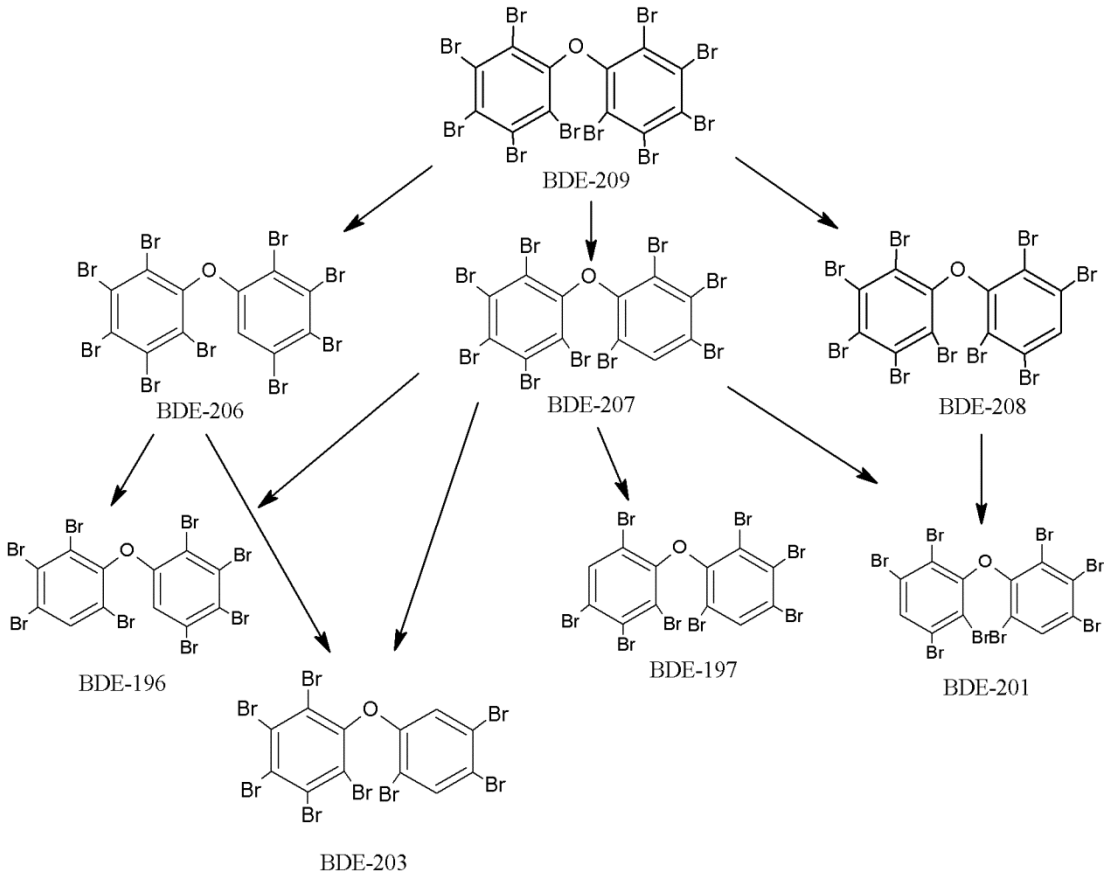


Figure 8-3. BDE-209 debromination pathways observed in biostimulation mesocosms.

The emergence of lower brominated congeners in mesocosms is shown in Figure 8-4, individually for reactors A and B. Note that in each set, nona-BDEs were present even at day 0. This is due to their presence in deca-BDE standard used in spiking sediments. The standard was found to contain 0.97% BDE-208, 1.91% BDE-207, and 2.14% BDE-206, which is similar to commercial deca-BDE content, as was also mentioned by Alaee et al. (2003) and Tokarz et al. (2008). Therefore, a correction was performed prior to presentation of products to exclude nona-BDEs resulting from the standard. However, nona-BDEs were still present at day zero, even after this this correction was made; however, their concentrations were very low. For sterile set, only BDE-206 was quantified throughout the incubation, but it was below

the detection limit at some time points. Other lower brominated congeners were never observed in sterile reactors.

As can be seen in Figure 8-4, biostimulation mesocosms A and B followed different trends, especially regarding emergence patterns of nona-BDEs. At day 30, biostimulation-A reactor showed a decrease in nona-BDEs due to the start of octa-BDEs formation, while in biostimulation-B reactor, nona-BDEs had their highest concentration. Nona-BDEs in reactor A accumulated at day 90, followed by a decrease afterwards. Although BDE-209 debromination was gradual in both reactors, continuous formation of octa-BDEs after 30 days resulted in fluctuation in nona-BDE levels. In reactor B, concentrations of octa-BDEs decreased at day 90, when hepta-BDEs were observed in this reactor. The only hepta-BDE congener (BDE-183) was below detection limit at day 90, and hence it was not included in Figure 8-4.

8.3.4. Debromination pathways of BDE-99

Debromination of BDE-99 in biostimulation reactors resulted in the formation of tetra- and tri-BDEs. The debromination pathways identified are presented in Figure 8-5. In the microcosm study, BDE-99 (appeared as the debromination product of BDE-209) was proposed to form only BDE-47, and this product was identified to accumulate in biostimulation set. Nevertheless, in biostimulation mesocosms, two more products were observed, i.e. BDE-66 and BDE-49. Additionally, BDE-47 and BDE-66 debrominated further to form BDE-28. Due to the lack of individual standards for di-BDEs, their formation could not be identified in this study. Previous studies also demonstrated the same products for BDE-99 debromination (He et al., 2006; Huang et al., 2014; Robrock et al., 2008; Tokarz III et al., 2008).

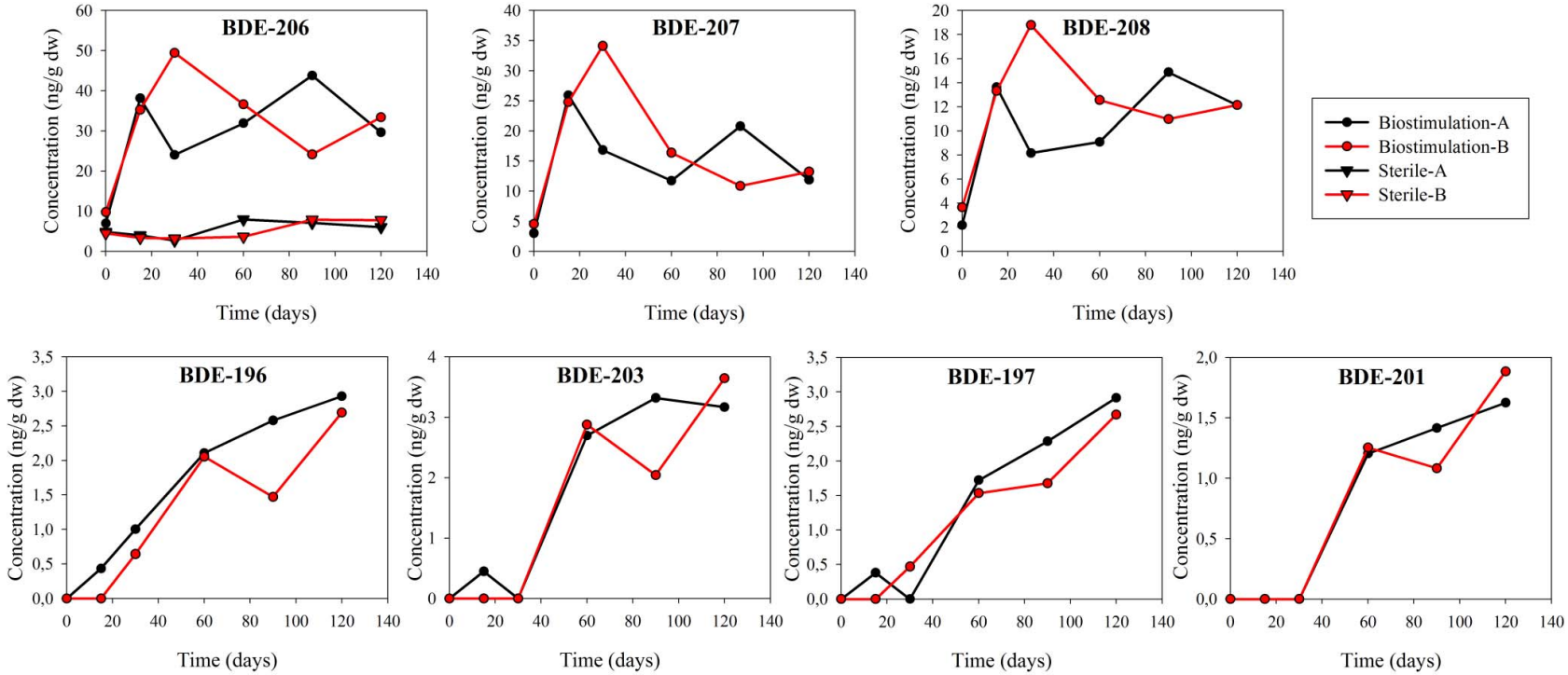


Figure 8-4. Formation of nona- and octa-BDEs from BDE-209 in mesocosms.

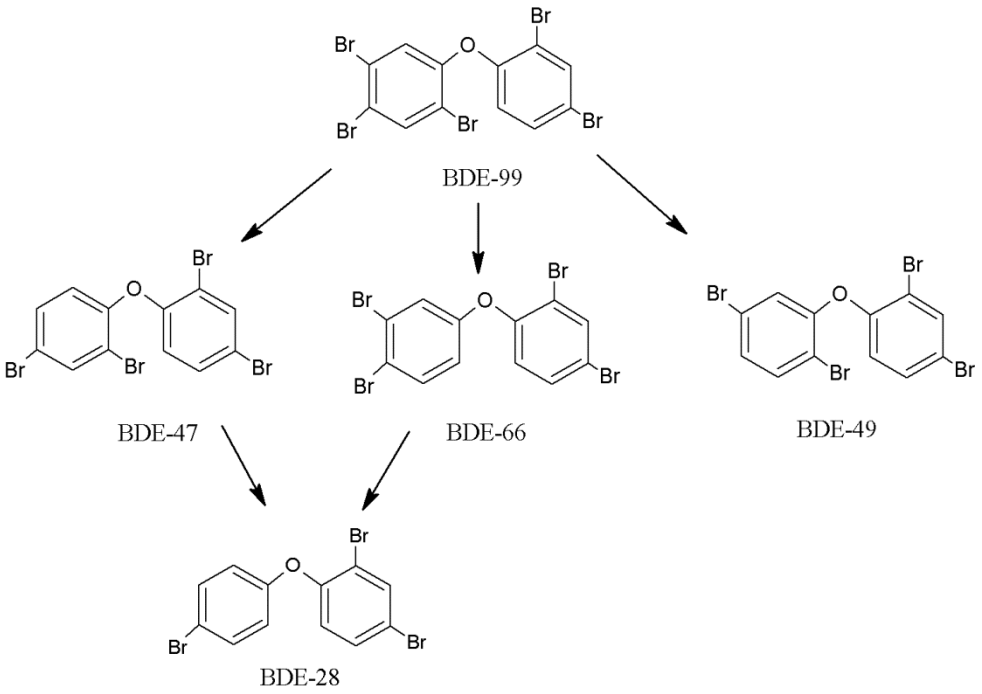


Figure 8-5. Debromination products of BDE-99 in biostimulation mesocosms.

The time varying concentrations of tetra- and tri-BDE in biostimulation reactors are presented in Figure 8-6. As can be seen from the figure, the first product to be formed was BDE-66. Other tetra-BDEs (i.e. BDE-47 and BDE-49) were observed at day 45. As soon as BDE-66 was formed, it was debrominated to form BDE-28. Tokarz et al. (2008) observed the formation of BDE-66 as the major product, and BDE-47 in small amounts in sediment microcosms. Although it was formed earlier than others, BDE-66 was not the major product in this study due to its lower concentrations compared to BDE-47 and BDE-49.

The fluctuations observed in tetra-BDEs were due to their debromination to form lower brominated congeners, while their formation via BDE-99 debromination continued. BDE-49 concentrations decreased at days 60 and 90, indicating debromination of this congener. Possible products of BDE-49 were BDE-31, -25, -18, and -17. Due to the lack of individual standards for these congeners, except for

BDE-17, the products of BDE-49 could not be detected. Among the detectable tri-BDEs, BDE-17 has not appeared throughout the incubation period in mesocosms study. Furthermore, a decline in concentration of BDE-28 was also noticeable, which indicates the formation of lower brominated congeners.

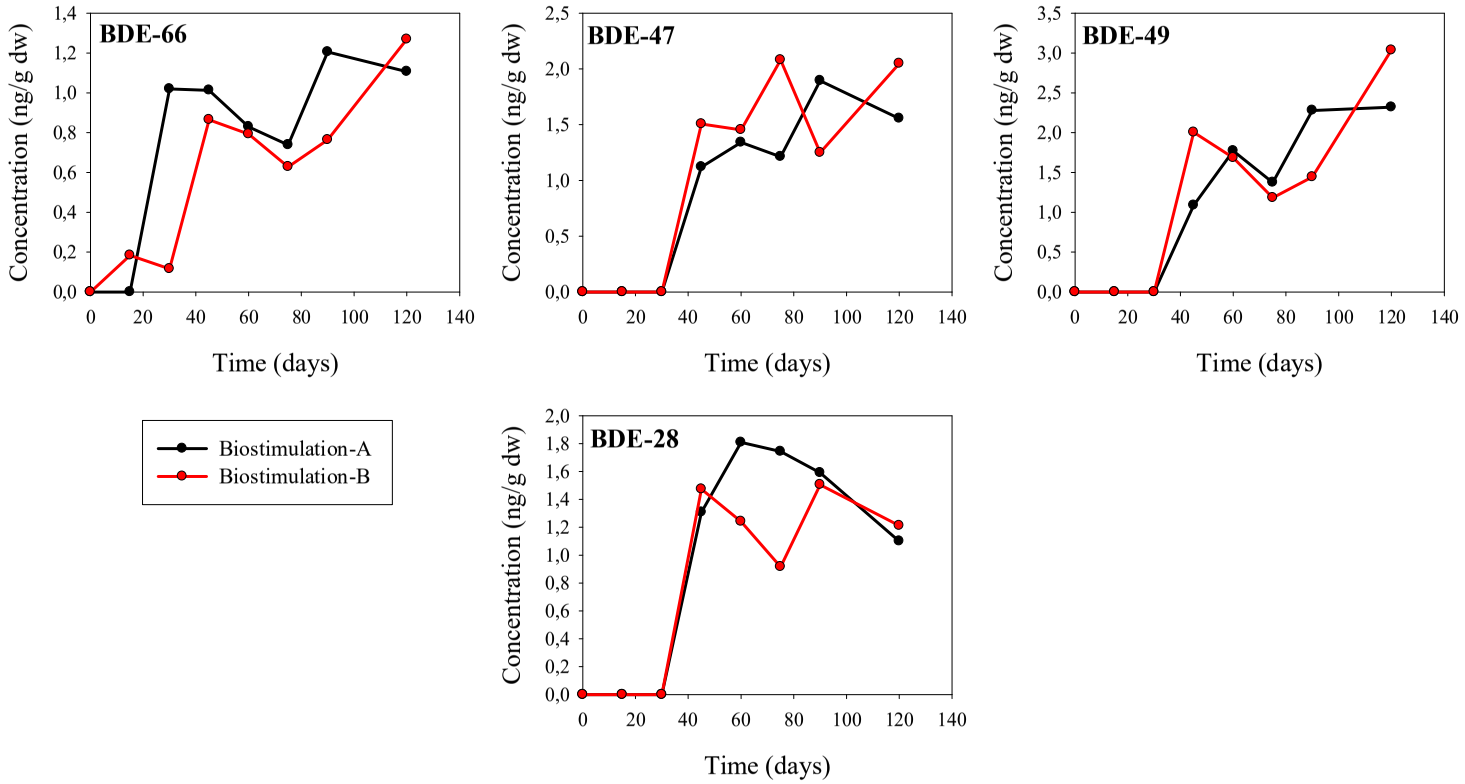


Figure 8-6. Formation of tetra-BDEs and tri-BDE due to debromination of BDE-99 in biostimulation mesocosms.

8.3.5. Degradation rates

Rate of BDE-209 and BDE-99 degradation in sediment mesocosms can be explained by pseudo-first-order reaction kinetics. The calculated rate constants are given in Table 8-2, together with all available relevant literature values. The rate constants determined in this study were presented separately for two parallel reactors operated, since they showed distinct rates for BDE-209. Reactor B had two times higher rate than reactor A. BDE-209 change in time followed a more steep decline in reactor B, compared to A.

When compared with the rates identified in microcosm study, the rates found in BDE-209 mesocosms were observed to be lower. The reduced amount of carbon source and electron donor added in mesocosm sediments is the most obvious reason for this observation. However, the rates were even lower than the natural attenuation microcosm set. This might be attributed to the resemblance of mesocosms to the environmental sediments. Previous literature studies showed degradation rates with a range of 0.00013 – 0.041 d^{-1} for BDE-209. The rate identified in biostimulation mesocosms of this study fell within the given range. Furthermore, sewage sludge reactors showed very similar degradation rates with the present study; such as 0.001 – 0.0012 d^{-1} (Gerecke et al., 2005; Shih et al., 2012b).

For BDE-99 degradation, the parallel mesocosms showed nearly the same rate constants (Table 8-2). There were limited number of studies that explicitly reported rate constants for BDE-99 degradation. The rates presented by the sludge reactors (Shin et al., 2010) and soil microcosms, estimated by modeling, (Karakas and Imamoglu, 2017) agreed well with that of BDE-99 biostimulation mesocosms.

Table 8-2. Degradation rate constants for anaerobic debromination of PBDEs in different studies.

Media & Conditions	T(°C)	k (d^{-1})	$t_{1/2}$ (d)	R^2	Reference
BDE-209					
Lake sediment mesocosms with organic medium (sodium formate+ethanol)	25	0.0008 0.0016	866.3 433.1	0.64 0.79	*This study*
Lake sediment microcosms without any substrates	25	0.0025	277.2	0.71	*This study (Chapter 5)*
Lake sediment microcosms with organic medium (sodium formate+ethanol)		0.0049	141.4	0.96	
Lake sediment microcosms with *Dehalobium chlorocoercia* strain DF-1		0.0028	247.5	0.96	
Lake sediment microcosms with phosphate buffer + methanol+dextrose	22	0.00013	5162	-	Tokarz III et al., 2008
River sediment microcosms with and without substrates	30	0.022 – 0.041	16.9 – 31.5	0.79 – 0.98	Huang et al., 2014
Environmental sediments (estimated by modeling)	-	0.001 – 0.002	416 – 1250	-	Puzyn et al., 2011
BDE-99					
Lake sediment mesocosms with organic medium (sodium formate+ethanol)	25	0.0019 0.0020	364.7 346.5	0.67 0.58	*This study*
Sludge reactors with basal medium	37	0.0028	247.5	-	(Shin et al., 2010)
Model estimation using soil microcosms data of Song et al. (2015)	25	0.001/0.003 [a]	693/231	-	(Karakas and Imamoglu, 2017)

[a] For pathway BDE-99 → BDE-47, k values represent median and maximum rate constants, separated by slash.

8.4.Conclusions

Biostimulation of PBDE contaminated sediments was examined in mesocosms to observe the efficiency of this bioremediation strategy, which was identified to be the most efficient in degradation of BDE-209 in sediment microcosms. Reductive debromination of PBDEs in large scale reactors was investigated for the first time in this study. Results show reduced degradation rates for BDE-209, compared to small scale sediment reactors both in the literature and in this study. Typically, laboratory studies are expected to yield higher degradation rates when compared to environmental sediments due to the presence of more favorable environmental conditions, such as constant temperature and organic content. However, maintaining these favorable conditions in larger scale reactors was harder than in small scale reactors. For example, a more environmentally acceptable concentration of organic medium was injected into mesocosms when compared to the rich organic medium of microcosms. This resulted in lower degradation rates, and even lower ones would be expected in natural sediments. Results indicate a direct relationship between organic content and biodegradation rate of BDE-209. If biostimulation is to be applied in environmental sediments, amendments with relatively high concentrations of carbon source and electron donor would need to be used.

The debromination pathways for BDE-209 and BDE-99 were identified and a detailed evaluation of the change in concentrations of the product congeners was provided. Debromination pathways observed in mesocosms showed variation from its microcosm counterparts, which can be interpreted by the impact of environmental factors on microbial preference during biodegradation. This finding also implicates that degradation pathways may be fostered throughout bioremediation at a contaminated site to transform bioaccumulative congeners to less brominated and less bioaccumulative ones.

Investigation of BDE-99, one of the predominantly found PBDE congeners in environmental matrices, in individual reactors contributed to the relevant literature in terms degradation kinetics and formation of product congeners. The fate of BDE-99 was scarcely evaluated in laboratory or modeling studies, hence revealing possible

consequences of BDE-99 debromination would provide valuable input to further studies on environmental fate of PBDEs in aquatic sediments.

Debromination rates observed for both BDE-209 and BDE-99 fall within the ranges reported in the literature. Reduced organic content has a direct impact on debromination rate of BDE-209 when compared to rates observed in microcosms. These findings will provide valuable information if and when full scale bioremediation systems are designed for contaminated sites.

CHAPTER 9

SUSTAINABLE REMEDIATION OF BFRS IN AQUATIC SEDIMENTS

9.1.Background for contaminated site remediation

Contaminated site is defined as any site that was confirmed by various measurements and/or evaluations to have hazardous pollutants due to human activities and pose significant risk to human health and the environment, and decided to be treated considering the present and future land use. These treatments performed to control or reduce the risk on human health and environment posed by the contaminated sites are defined as remediation activities.

First restoration project on contaminated land was conducted in 1961 at Lower Swansea Valley (Wales), in which the social and economical benefits preceded the environmental considerations (Bardos et al., 2016). Back then, the industries were discharging their wastes into the environment without any treatment (Ellis and Hadley, 2009). Recognition of the severity of contaminated sites started with the contamination incident occurred in 1978 at Love Canal (USA) (USEPA, 2017b). Similar incidents followed in 1980 at Lekkerkerk (Netherlands) and in 1983 at Time Beach (USA) (Bardos et al., 2016). In the USA, Superfund program (The Comprehensive Environmental Response, Compensation, and Liability Act – CERCLA) was established in 1980 to control the release of hazardous substances by the industries (USEPA, n.d.). Between 1979 and 1986, NATO implemented pilot studies for contaminated lands, and continued to conduct studies for remediation (Bardos et al., 2016). During that time, in 1987, a report of World Commission on Environment and Development, known as the Brundtland Report, was published,

which emphasized the significance of sustainable development (United Nations, 1987).

Starting from 1995, several frameworks and projects were carried out on the risk assessment of contaminated lands, considering the economical and environmental merits (Bardos et al., 2016). In that decade, treatments started to be applied at contaminated sites since they became a concern for public and authorities. Remediation industry relied on energy-intensive engineered techniques; such as groundwater pump-and-treat, dredging, soil excavation, incineration, and thermal treatment (Ellis and Hadley, 2009). However, it became evident in the last decades that these techniques cannot achieve acceptable cleanup levels, besides contributing to the climate change due to the energy and raw materials used during cleanup efforts (Ellis and Hadley, 2009). Hence, in early 2000s, studies were initiated for remediation technologies to rely more on transformation and degradation of contaminants, while recycle and reuse of raw materials were regarded (Ellis and Hadley, 2009).

At the beginning of 2000s, an EU project (CLARINET) was implemented on establishment of networks for contaminated land management, in which sustainability was explicitly recognized in risk management (Bardos et al., 2016). Afterwards, at 2006, Sustainable Remediation Forum, namely SuRF, was initiated in the USA to engage sustainability in remediation strategies, and then the forum was expanded in many countries (SURF, 2017). At 2008, the first guidance on green remediation was published by US EPA (USEPA, 2008b), and in the following years several institutions; such as SuRF, ASTM, etc. published their guidance/framework documents for application of sustainability during remediation activities (Bardos et al., 2016).

By examining the history of contaminated site management, it was observed that the perception of contaminated site remediation was improved over the years. Starting from the present decade onwards, remediation practices developed will be evaluated in terms of sustainability before adaption. The aim of this chapter is, therefore, to

assess the remediation alternatives investigated throughout this study for BFR-contaminated sediments from the perspective of sustainability. By this way, the outcomes can contribute to the decision-making processes for strategy development.

9.2.Sustainable remediation

Sustainable remediation is defined as the development of remediation practices, having greater benefits than impacts in terms of environmental, social and economical aspects, after a balanced decision making process (SuRF-UK, 2010). Hence, it includes an assessment of remediation options prior to implementation on a site. The sustainability assessment should proceed with i) setting clear remediation objectives, ii) defining comprehensible boundaries of the system, iii) identifying scope of relevant sustainability indicators, and iv) selection of methodology for comparison of remediation alternatives (Bardos, 2014).

The sustainable remediation practices need to follow several principles. Principles of sustainable remediation defined by SuRF-UK (2010) are explained below:

1. **Protection of human health and environment:** Remediation should include risk management to reduce the risk associated with human health and environment for current and future land-use, and assess the alternative strategies in terms of cost-benefits, effectiveness, feasibility and durability.
2. **Safe working environment:** the activities included in remediation should be safe for the workers and public, as well as for the environment.
3. **Consistent, clear and reproducible evidence-based decision-making:** Remediation practices should incorporate the social, environmental and economic aspects considering current and future implications, while maximizing the potential benefits. During decision-making, benefits and impacts should be aggregated or traded to determine a clear rationale.
4. **Reporting:** A transparent reporting process should be adapted from cradle to grave, including all assumptions and data used.
5. **Stakeholder involvement:** Decisions should be clearly followed by all stakeholders and participation of them should be provided.

6. **Sound science:** use of relevant and accurate data, elaboration of assumptions and uncertainties should be the base for decisions to make them justifiable and reproducible (SuRF-UK, 2010).

While assessing sustainable remediation strategies, performance of various scenarios should be evaluated based on sustainability indicators. These environmental, social and economic indicators are listed in Table 9-1. These indicators can be extended to include several other considerations, as well as all of them may not be necessarily used for qualitative or quantitative assessment (SuRF-UK, 2010).

As given in sustainable remediation principles above, the stakeholder involvement is important for decision-making process of remediation. The framework of SuRF-UK (2010) suggests that assessment of remediation options should proceed by evaluation of environmental, social and economic indicators from every stakeholder's point of view. Therefore, it can be said that sustainability is site-specific, involving the opinions of all stakeholders for the particular site, which makes the assessment subjective rather than objective (Bardos, 2014).

Table 9-1. Sustainable remediation indicators (SuRF-UK, 2010).

Environment	**Issues considered for each indicator**
• Emissions to air	➢ emissions that may affect air quality
• Soil and ground conditions	➢ changes in physical, chemical, biological soil condition
• Groundwater and surface water	➢ changes in the release of contaminants, nutrients, particulates
• Ecology	➢ effects on ecology, flora, fauna and food chains
• Natural resources and waste	➢ impacts or benefits for land resources, use of energy/fuels
Economic	
• Direct economic costs and benefits	➢ Direct financial costs, including capital and operation cost
• Indirect economic costs and benefits	➢ Long term indirect costs, including changes in land property values, taxes
• Employment and employment capital	➢ Job creation, skills before and after, opportunity for education and training
• Induced economic costs and benefits	➢ Creating opportunities for inward investment, ability to affect other projects in the area
• Project lifespan and flexibility	➢ Duration of risk management, ability to respond to changing circumstances
Social	
• Human health and safety	➢ Risk management performance of the project regarding mitigation of risk and duration of work
• Ethics and equity	➢ Social justice and intergenerational equity
• Neighbourhoods and locality	➢ Impacts and benefits to local areas, effects from dust, noise, odour
• Communities and community involvement	➢ Impacts on community functions and services they can access
• Uncertainty and evidence	➢ Robustness, requirements for validation/verification, uncertainties in project

9.3.Evaluation of remediation alternatives regarding sustainability

9.3.1. Methodology used in evaluation

In the literature, there are few studies implementing sustainable remediation assessment for contaminated sites (Chen et al., 2017; Favara et al., 2016; Gill et al., 2015). Governmental and non-governmental agencies propose frameworks for sustainability assessment. A commonly followed framework is the one presented by SuRF-UK (2010). Hence, in this study SuRF-UK framework, and the steps mentioned above will be followed for evaluation of sustainability.

According to SuRF-UK (2010), assessment should start by identifying the scope of evaluation explicitly. Initially, the boundaries including system, lifecycle, spatial and temporal boundaries should be determined. These boundaries will then be used to prepare conceptual models. Also, assumptions, limitations, and uncertainties of evaluation should be clearly stated. Then, sustainability indicators intended to be used should be defined by relevant considerations for each. All available data will then be used for the justification of evaluations. Finally, the method (e.g. qualitative vs. quantitative) to be used is determined, by considering its relations with conceptual models, indicators and available data (Cl:Aire, 2011).

9.3.2. Hypothetical case of PBDE/HBCDD contaminated sediments

To evaluate the remediation alternatives used in the present study in terms of sustainability, a hypothetical case for PBDE/HBCDD contaminated sediments was created. According to the methodology proposed by SuRF-UK (2010), and as applied by Gill et al. (2015), the scope of evaluation was identified for this hypothetical case below:

Setting of remediation objectives:
The objective of remediation is to decrease contaminant concentrations upto regulatory limits in aquatic sediments contaminated with BDE-209 or HBCDD.

Definition of system and lifecycle boundaries:

Within the scope of this study, degradation of BDE-209 and HBCDD was investigated using laboratory microcosm reactors. Degradation mechanisms included biotic and abiotic degradation. These remediation alternatives are explained below:

1. Natural attenuation (NA): This system relies on natural physical, biological and chemical processes to achieve reduction of mass, toxicity or concentration of contaminants in a reasonable time period (Fuchsman et al., 2014). Within the scope of this study, natural attenuation reactors included spiked sediments with distilled water on top. For application on-site, this system is planned to include only monitoring of contaminant levels via sampling from surficial sediments at pre-defined time intervals. System does not involve construction of any permanent facility for monitoring purposes.

2. Biostimulation (BS): This remediation strategy is the addition of extraneous substrates, carbon sources or electron acceptors to enhance the activity of indigenous microorganisms when they show little or no degradation activity (Reible, 2014). In this study, biostimulation was simulated using reactors composed of spiked sediments and an organic medium, rich in carbon source (sodium formate) and electron donor (ethanol). The medium was prepared with the ingredients including several vitamins and mineral salts. Delivery of organic medium into actual contaminated sediments would be realised with injection systems, such as wells. Hence, an injection system would be constructed, and then injection of organic medium into the sediments would be performed. Monitoring of contaminant level would be done similar to natural attenuation strategy.

3. Bioaugmentation (BA): Bioaugmentation is introducing degrading microorganisms enriched from a contaminated site (whether it is the same or a different site), if the microbial activity is insufficient for degradation at the site (Reible, 2014). Within the scope of this study, *Dehalobium chlorocoercia* strain DF-1 was used in microcosms for anaerobic degradation in sediments.

The bacterial strain was grown in an organic medium with the same ingredients of biostimulation medium. For the growth of microorganisms incubation at 30°C for two months is required. The delivery of DF-1 in sediments would be conducted similarly with biostimulation strategy. Likewise, monitoring of contaminant level would be done via sampling from surficial sediments.

4. Catalyzed hydrogen peroxide propagations (CHP): CHP was tested to abiotically degrade organic pollutants in this study. The reactions initiated with the addition of H_2O_2 and Fe^{3+}, at pH=2, onto sediments. The preparation of H_2O_2 and Fe^{3+} solutions does not require energy, since they readily dissolve in water. The injection of solutions in aquatic sediments would be performed via injection well-type systems. Differently from other remediation alternatives, injection of H_2O_2 and Fe^{3+} solutions would have to be conducted 5 times since this was tested under laboratory conditions, as presented in Chapter 7. Monitoring of contaminant levels would be done similarly.

The system and lifecycle boundaries of these alternatives were determined according to the explainations given above, and their corresponding conceptual models are presented in Figure 9-1, Figure 9-2, Figure 9-3, and Figure 9-4. The spatial boundaries could then be identified as the contamination site of the hypothetical case. The temporal boundaries were set as the regulatory limits for these contaminants in sediments, and these were represented by the diamond decision boxes in conceptual models.

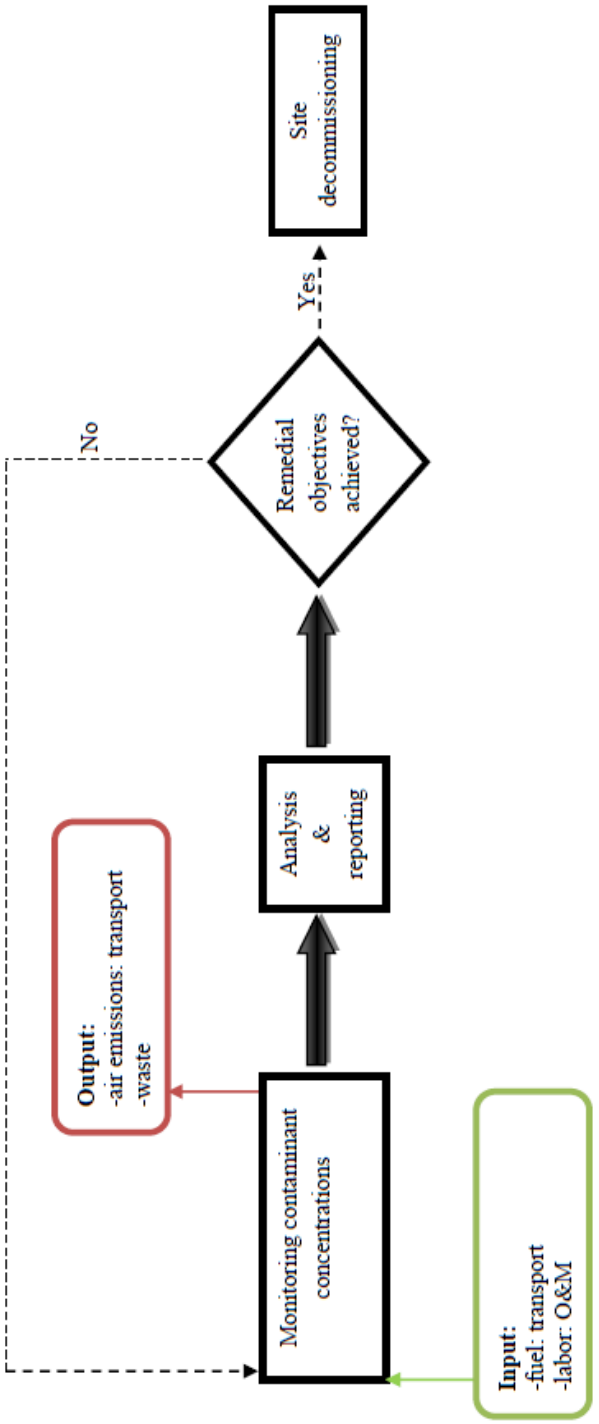


Figure 9-1. Conceptual model for natural attenuation

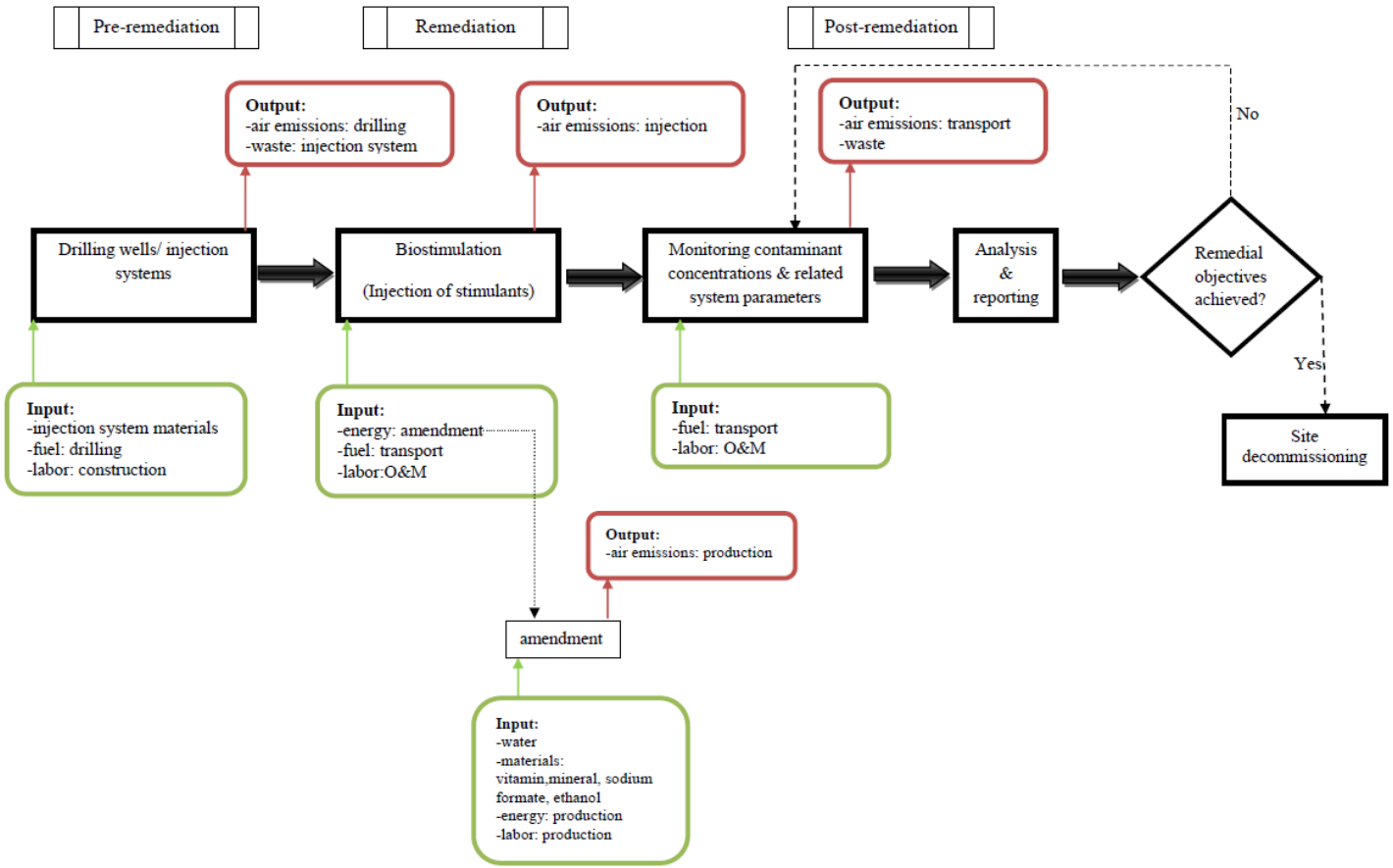


Figure 9-2. Conceptual model for biostimulation

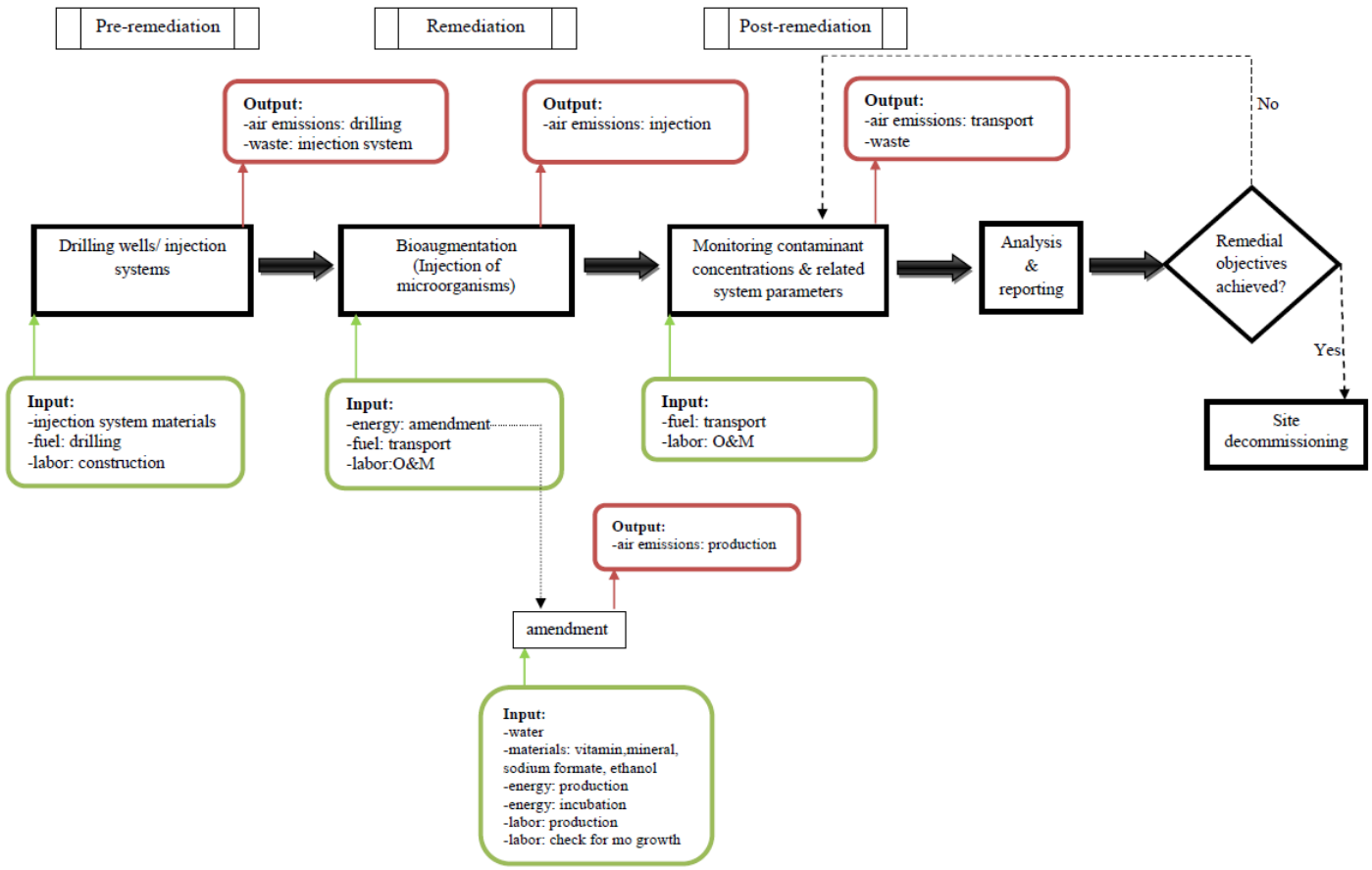


Figure 9-3. Conceptual model for bioaugmentation

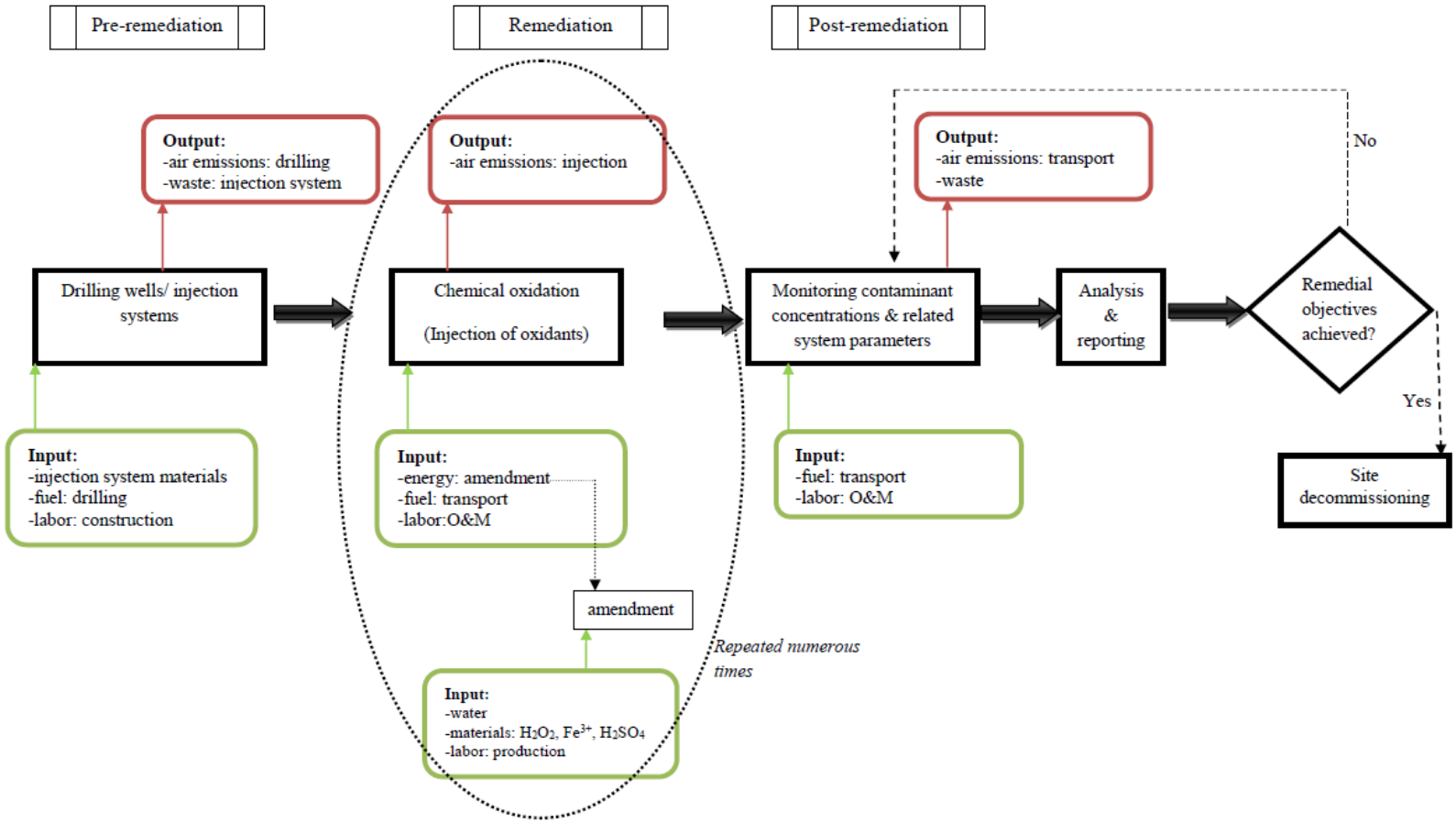


Figure 9-4. Conceptual site model for catalyzed hydrogen peroxide propagations.

Identification of boundaries also involve stating the assumptions, and revealing limitations clearly. The below assumptions hold for the hypothetical case of PBDE/HBCDD contaminated sediments:

- The contaminated site was Çamkoru National Park pond, whose sediments were collected for degradation studies.
- Contamination of the site was 1000 ng/g dry weight of sediments. Contamination was due to BDE-209 or HBCDD, individually.
- No previous remedial actions have been taken at the site.

Limitations of this evaluation would be foreseen as below:

- Since the case is hypothetical, site-specific factors may lead to uncertainties in indicator evaluations.
- The evaluation of environmental, social and economic indicators were only based on the results of laboratory scale experiments, hence the economic and social aspects were based on estimations.
- Spatial and temporal boundaries were uncertain, since the site was assumed to contain only the lake area, and regulatory limits for contaminants were not determined.

Identification of sustainability indicators and available data:

The sustainability indicators proposed by SuRF-UK (2010), and presented in Table 9-1, will be used for evaluation. The issues considered during evaluations for the hypothetical case are also presented in the table. Any prioritization between indicators was not performed.

As mentioned earlier, the evaluations were based on the results of laboratory degradation studies conducted in small scale reactors (microcosms). The removal efficiencies achieved by four remediation strategies for BDE-209 and HBCDD are summarized in Table 9-2.

Table 9-2. Summary of the results of remediation alternatives for BDE-209 and HBCDD degradation.

Remediation strategy*	BDE-209 removal (%)	Treatment time (days)	HBCDD removal (%)	Treatment time (days)
NA	30.9	180	36.9	36
BS	55.3	180	86.3	36
BA	40.2	180	34.9	36
CHP	31.8	32	>90.5	32

* NA: natural attenuation, BS: biostimulation, BA: bioaugmentation, CHP: catalyzed hydrogen peroxide propagations.

Selection of methodology used during assessment:

The sustainability indicators for environmental, economic, and social criteria were assessed for each remediation option: natural attenuation (NA), biostimulation (BS), bioaugmentation (BA), and catalyzed hydrogen peroxide propagations (CHP). During evaluation of options, SuRF-UK framework Annex-1 was used as a guide for category contexes (Cl:Aire, 2011). A tiered approach was described by SuRF-UK (2010) in which Tier 1 is defined as a qualitative ranking, Tier 2 is defined as a semi-quantitative method, and Tier 3 is quantitative. Since the data obtained from laboratory studies can only allow a qualitative assessment, a ranking system was adapted to follow Tier 1 approach. For adaption of this approach, study of Gill et al. (2015) was utilized. Grading between 1 (lowest score) to 5 (highest score) was assigned for each option for every indicator. During ranking, the benefits and impacts of options were aggregated or traded to determine the best and worst option for the specific indicator. The indicators whose benefits or impacts were at same degree for all options, 5 was given as a score to all, as proposed by Gill et al. (2015). At the end, all scores were summed up and the option having the highest score was evaluated as the most sustainable remediation alternative. Since responses of each contaminant, BDE-209 and HBCDD, varied during degradation studies, the assessment was performed individually. The conceptual models were used to count some of the parameters e.g. air emissions, fuel used, etc. while ranking between options.

9.4.Results and Discussion

Sustainability assessment for the hypothetical BDE-209 contamination scenario in Çamkoru pond using remediation alternatives was conducted using a qualitative approach, and scoring is presented in Table 9-3. For each score assignment, conditions given in justification column were taken into account. The cumulative gradings for each option are shown in Figure 9-5. As can be observed from figure, the highest score was obtained by biostimulation, followed by bioaugmentation.

The laboratory studies provided valuable data for the sustainability evaluation, such as removal efficiency, emergence of product congeners, effects of H_2O_2 to sediment organic matter, and duration of remediation. These data were useful to score all of the environmental indicators, and some of the social (e.g. human health and safety, uncertainty), and economic (e.g. indirect costs, project lifespan) indicators. However, further site-specific information was needed to score most of the social and economic indicators. Hence, this may lead the environmental indicators to predominantly affect the result of evaluation. A more complete assessment can only be made for a real contaminated site.

Table 9-3. Scores for sustainability assessment of remediation of BDE-209 contaminated sediments.

Criteria	Indicator	Scores *				Justification
		NA	BS	BA	CHP	
Environmental	Emissions to air	5	3	3	1	• CHP has consecutive treatment cycles (injection of materials). • NA has only monitoring
	Soil and ground conditions	4	5	4	2	• CHP causes sediment organic matter to decrease, also sediment particulate structure to change. But removes contaminant fast. • BS helps sediment get back to non-contaminated state faster (improvement of sediment condition).
	Ground water & surface water	2	3	2	4	• In terms of bioavailable contaminants formation (penta&tetra-BDEs), BS has highest concentration, while BA produced tri-BDEs which are less bioaccumulative. Penta-BDEs accumulated in NA, but in smaller quantity. CHP products were not identified, but it does not produce lower Br bioaccumulative congeners. • In terms of contaminant reduction, BS performed best, followed by BA. CHP has a potential to provide better percent removal than NA, if number of treatments increased. • BS may lead to algal blooms due to nutrients provided, causing surface water quality decrease. • BA may result in formation of unwanted biomass.
	Ecology	4	4	3	4	• NA removes contaminant to some degree, no improvement in lake ecosystem. • BA and BS can cause freshwater ecology to change due to the production of biomass. BA might be worse since it introduces new microorganisms. • Organic matter loss from sediments in CHP will result in negative impact on flora/fauna.
	Natural resources & waste	5	4	3	2	• Evaluated in terms of water and ingredients of solutions used, and waste generated. • Fuel and energy used is highest in CHP due to consequtive application of treatment method. BA consumes higher energy than BS due to the need for biomass growth before injection.

Table 9-3. Cont'd.

Criteria	Indicator	Scores				Justification
		NA	BS	BA	CHP	
Economic	Direct economic costs & benefits	5	4	3	3	• No capital cost for NA. • Generally, biological treatment costs are lower than chemical treatment costs (Juwarkar et al., 2010). • O&M costs for monitoring for all.
	Indirect economic costs & benefits	4	5	5	4	• For NA and CHP reduciton in land value might have impact due to low contaminant removal.
	Employment & employment capital	1	4	5	5	• Labor needed for construction, O&M, preparation of amendments and monitoring taken into consideration. • Skills for mo growth, preparation of chemicals.
	Induced economic costs & benefits	5	5	5	5	• Applicable to all at same degree.
	Project lifespan & flexibility	2	5	4	3	• Ability of the project to changing circumstances is very low for NA, others at same degree. • BS achieve remedial objectives quicker than others. • Duration for contaminant reduction at a determined ratio (e.g. 30%) is longest for NA, and there is an uncertainty for CHP.

Table 9-3. Cont'd.

Criteria	Indicator	Scores				Justification
		NA	BS	BA	CHP	
Social	Human health and safety	2	4	3	4	• Human health risk management of the project depends on the contaminant removal, production of bioavailable compounds with regard to duration of project. • Risk associated with operation is minimum for NA since it has no intrusion in the system by workers. • NA&BA: slow degradation and formation of bioaccumulative congeners impact human health. • CHP provided faster removal than NA.
	Ethics and equity	5	5	5	5	• Applicable to all at same degree, (site-specific)
	Neighbourhoods and locality	5	4	4	4	• Considering noise generated during construction, NA performs better than others. • For conservation of lake ecosystem, all scored same.
	Communities and community involvement	5	5	5	5	• Applicable to all at same degree, (site-specific)
	Uncertainty and evidence	3	5	5	3	• Level of uncertainty is highest for NA & CHP, due to plateu level of contaminant in NA, and varying concentrations achieved in CHP application.

* NA: natural attenuation, BS: biostimulation, BA: bioaugmentation, CHP: catalyzed hydrogen peroxide propagations.

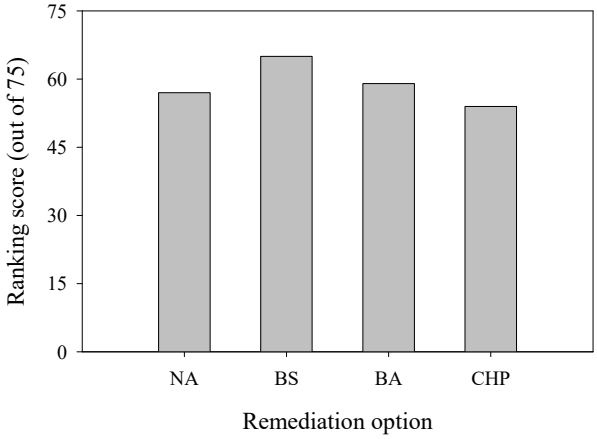


Figure 9-5. Ranking scores of remediation options (NA: natural attenuation, BS: biostimulation, BA: bioaugmentation, CHP: catalyzed hydrogen peroxide propagations) for BDE-209 contaminated sediments.

The results of HBCDD contaminated sediment remediation evaluation using sustainability indicators are presented in Table 9-4, and cumulative scores are shown in Figure 9-6. The highest score was obtained by biostimulation set, similar to BDE-209 assessment. On the other hand, bioaugmentation revealed a lower score for HBCDD. This is because DF-1 strain was found to be incapable of degrading HBCDD in microcosm studies. CHP was the second highest scored remediation alternative for HBCDD.

Table 9-4. Scores for sustainability assessment of remediation of HBCDD contaminated sediments.

Criteria	Indicator	Scores *				Justification
		NA	BS	BA	CHP	
Environmental	Emissions to air	5	3	3	1	• CHP has consecutive treatment cycles (injection of materials). • NA has only monitoring
	Soil and ground conditions	4	5	4	2	• CHP causes sediment organic matter to decrease, also sediment particulate structure to change. But removes contaminant fast. • BS helps sediment get back to non-contaminated state faster (improvement of sediment condition).
	Ground water & surface water	2	4	1	5	• In terms of contaminant reduction, CHP performed the best, followed by BS. BA & NA performed similar. • BS may lead to algal blooms due to nutrients provided, causing surface water quality decrease. • BA may result in formation of unwanted biomass.
	Ecology	4	4	3	4	• NA removes contaminant to some degree, no improvement in lake ecosystem. • BA and BS can cause freshwater ecology to change due to the production of biomass. BA might be worse since it introduces new microorganisms. • Organic matter loss from sediments in CHP will result in negative impact on flora/fauna.
	Natural resources & waste	5	4	3	2	• Evaluated in terms of water and ingredients of solutions used, and waste generated. • Fuel and energy used is highest in CHP due to consequtive application of treatment method. BA consumes higher energy than BS due to the need for biomass growth before injection.

Table 9-4. Cont'd.

Criteria	Indicator	Scores				Justification
		NA	BS	BA	CHP	
Economic	Direct economic costs & benefits	5	4	3	3	• No capital cost for NA. • Generally, biological treatment costs are lower than chemical costs (Juwarkar et al., 2010). • O&M costs for monitoring for all.
	Indirect economic costs & benefits	4	5	4	5	• For NA and BA reduciton in land value might have impact due to low contaminant removal.
	Employment & employment capital	1	4	5	5	• Labor needed for construction, O&M, preparation of amendments and monitoring taken into consideration. • Skills for mo growth, preparation of chemicals.
	Induced economic costs & benefits	5	5	5	5	• Applicable to all at same degree.
	Project lifespan & flexibility	1	4	1	5	• Ability of the project to changing circumstances is very low for NA, others at same degree. • CHP achieve remedial objectives quicker than others. • Duration for contaminant reduction at a determined ratio (e.g. 30%) is longest for NA&BA.

Table 9-4. Cont'd.

Criteria	Indicator	Scores				Justification
		NA	BS	BA	CHP	
Social	Human health and safety	3	4	3	4	• Human health risk management of the project depends on the contaminant removal, with regard to duration of project. • Risk associated with operation is minimum for NA since it has no intrusion in the system by workers. • NA&BA: slow degradation. • CHP provided fastest removal.
	Ethics and equity	5	5	5	5	• Applicable to all at same degree, (site-specific)
	Neighbourhoods and locality	5	4	4	4	• Considering noise generated during construction, NA performs better than others. • For conservation of lake ecosystem, all scored same.
	Communities and community involvement	5	5	5	5	• Applicable to all at same degree, (site-specific)
	Uncertainty and evidence	4	5	4	5	• Level of uncertainty is highest for NA and BA.

* NA: natural attenuation, BS: biostimulation, BA: bioaugmentation, CHP: catalyzed hydrogen peroxide propagations.

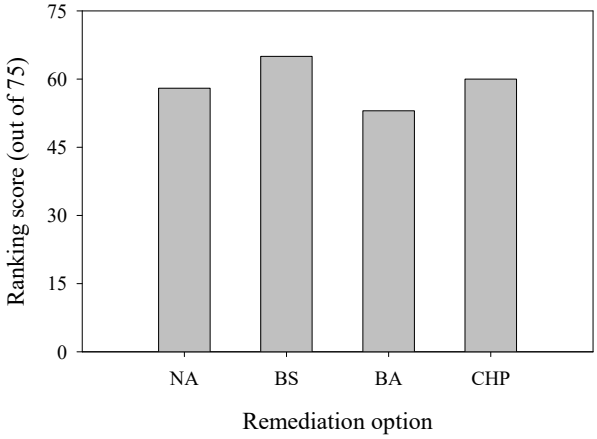


Figure 9-6. Ranking scores of remediation options for HBCDD contaminated sediments (NA: natural attenuation, BS: biostimulation, BA: bioaugmentation, CHP: catalyzed hydrogen peroxide propagations).

9.5.Conclusion

A preliminary attempt was made on the assessment of remediation alternatives investigated throughout this study from the perspective of sustainability. BDE-209 and HBCDD contamination in sediments were targeted in assessing remediation, and the information obtained from microcosm studies were utilized during qualitative grading of options.

The most favorable remediation option for both contaminants was biostimulation. It provided the highest percent removal among all for BDE-209, and among biotic mechanisms for HBCDD in microcosm studies. Due to the similarity between remediation options regarding the construction of injection systems, and the process of injection; biostimulation, bioaugmentation and CHP scored similar in terms of energy used and waste generated. During laboratory studies, CHP was identified to give maximum efficiency when applied in consecutive treatment cycles. Hence, energy and fuel used, and air emissions generated during injection were added up, resulting in CHP scores to be the lowest for environmental indicators.

NA is the least energy consuming and waste generating option among others, and it did not require construction of any facility at the site for this specific case. This may create a bias during selection of sustainable remediation alternative to be implemented. NA would yield the contamination to persist in the environment, which may not be the preferred option when contaminants are toxic to aquatic species.

There were few studies conducting sustainability assessment for remediation strategies, and none for BFR contamination. This study provided an initial assessment of various remediation alternatives for BFR contaminated sediments in terms of sustainability. Nevertheless, more comprehensive assessments should follow with further inquiry on indicators, especially on social and economic indicators. The results of laboratory studies supplied information for qualitative assessment of environmental indicators. For a quantitative assessment of sustainable remediation, costs of equipments, installation, and transport, and analysis of social factors should be elaborated, as well as the environmental indicators, e.g. calculations on CO_2 emissions, amount of materials used, and wastes generated.

There are also best management practices (BMP) proposed by agencies, e.g. US EPA published BMPs for green remediation practices. These include implementation of some measures to minimize waste generated or non-renewable energy used during operation. For example, material usage in amendments of remediation alternatives could be wastes/by-products of relevant industries or organics readily found in nature. These types of practices should also be taken into account during more comprehensive assessments and implementation of strategies.

Stakeholder involvement could not be performed during this study since the assessment was based on a hypothetical contamination scenario. However, during decision-making process for remediation implementation, all stakeholders should be involved in assessment procedure by assigning rankings to options.

CHAPTER 10

OVERVIEW

This study aimed at investigating fate of PBDEs and HBCDD in aquatic sediments under conditions which simulate various remediation strategies. Within this scope, initially, the analytical methods for the determination of PBDEs and HBCDD in solid matrices were validated with laboratory control samples. The validated methods were then used to identify PBDE and HBCDD levels in wastewater treatment plant (WWTP) sludge samples in Turkey. For the investigation of biotic and abiotic degradation mechanisms of PBDEs and HBCDD, sediment microcosms were operated. To test the applicability of biotic degradation mechanism for PBDE debromination in environmental sediments, larger scale reactors (mesocosms) were established. Finally, the remediation strategies investigated throughout this study were assessed in terms of principles of sustainable remediation.

Throughout the course of this study, focus was placed on specific congeners/isomers since PBDEs are a group of compounds consisting of 209 different congeners, and HBCDD can be found as five different isomers. In order to monitor degradation and derive specific characteristics of degradation (e.g. rate and products), specific compounds, namely BDE-209 and BDE-99 among PBDEs and γ-HBCDD were selected for these studies.

Several different analytical methods have been reported in the literature to be efficiently applied for extraction of PBDEs and HBCDD from solid matrices. The objective to validate an analytical method in this study was, to establish an extraction procedure which requires small amount of material for extraction and provides

numerous samples extraction in one batch. For this purpose, three extraction methods were tested: Soxhlet, ultrasonic and vial extraction. Initial method validation studies were conducted with BDE-209 congener. After the method was verified, analysis of other PBDE congeners (22 congeners/congener groups) was tested with the method. As a result, ultrasonic extraction procedure presented high accuracy and precision in laboratory control samples, as well as certified reference material, hence selected to be used in the consecutive studies. Ultrasonic extraction method gained an advantage over Soxhlet extraction due to its low material and time requirements, despite the latter one also presented satisfactory performance in determination of PBDEs in solid matrices. The validated method was then tested for HBCDD extraction, and it was further optimized for this purpose.

Prior to degradation studies, BFR contamination levels in Turkey were aimed to be identified with WWTP sludge samples so that similar concentrations could be used to establish laboratory degradation reactors. Although atmospheric and soil levels of PBDEs were determined in previous studies, there were no reports for BFR levels in WWTP sludges of Turkey. The verified methods for PBDE and HBCDD determination was, therefore, used to reveal WWTP sludge levels for the first time in Turkey. Since PBDEs and HBCDD consumption is probable in industries and they are globally found in commercial goods, industrial and domestic WWTPs can be sinks for these substances. Hence, investigation of levels would indicate the use of BFR-treated products in Turkey. For this purpose, dewatered sludge samples were collected from four WWTPs (two urban, and two organized industrial wastewater treatment plants) from four different geographical regions of Turkey. Two sampling campaigns were employed to represent cold and warm periods. The results revealed the use of BFR-treated products, as well as direct usage of PBDEs in industry in Turkey. Both of the urban WWTP sludges, and one of the industrial WWTP sludge presented PBDE levels that are in similar order of magnitudes with those from other parts of the world. However, one of the industrial WWTP sludges showed thousand times greater BDE-209 level than the highest recorded sludge level worldwide. This finding demonstrated the potential severity of the problem in terms of global usage and distribution of PBDEs. The HBCDD levels of Turkey were observed to be

similar with the European sludge samples, having a range of 13.1 - 616.2 ng/g dw. As a result of this study, PBDE concentrations in sludge samples were identified to have a wide range (up to 6 orders of magnitude difference). Therefore, for the succeeding degradation studies, contamination level was decided to be at levels used in the previous degradation studies in literature.

In the last decade, studies on PBDE degradation have accelerated, yet this mechanism needs further elaboration especially when remediation of contaminated sites is taken into consideration. Sediment microcosms were operated to observe anaerobic debromination of BDE-209 under conditions which represent various bioremediation strategies; namely, biostimulation, bioaugmentation and natural attenuation. The reason for focusing on BDE-209 among other PBDE congeners was that it was used widely as a flame retardant (Alaee et al., 2003), and although it was voluntarily phased out in some countries, it has not been the subject of any legislation yet, which means that its usage may still be continuing. Additionally, it is the most abundant congener identified in environmental matrices. To simulate biostimulation, a defined mineral medium rich in carbon source and electron donor was added into sediments. Bioaugmentation, which was applied for PBDEs for the first time, was established by adding into sediments an enrichment culture of *Dehalobium chlorocoercia* strain DF-1, previously shown to dechlorinate polychlorinated biphenyls. No extraneous substances were added to represent the case of natural attenuation. Findings from the three bioremediation alternatives were evaluated in terms of percent reduction of BDE-209, identity of debromination pathways and rate of debromination reactions. Greatest reduction percentage of BDE-209 and highest degradation rate was observed for biostimulation. A total of 20 different debromination pathways were proposed, some of which were identified for the first time in literature with this study. These pathways revealed the preferential debromination from *ortho* and *meta* positions on the diphenyl ether structure. At the end of incubation, products for bioaugmentation, biostimulation and natural attenuation were tri-BDEs, tetra-BDEs and penta-BDEs, respectively. Greatest extent of debromination, hence, was observed in bioaugmentation. With this study, the differences in efficiency and formation of product congeners for bioremediation

alternatives were revealed so that these would provide valuable information for on-site applications of these alternatives in the future. Furthermore, identification of product congeners was imperative for monitoring bioaccumulative congeners, and fostering debromination pathways throughout a bioremediation application on a contaminated site in order to transform bioaccumulative congeners to less brominated and less bioaccumulative ones.

HBCDD degradation studies have been relatively scarce, and hence elucidation of their degradation under various conditions is necessary to understand the fate of this compound in the environment. Sediment microcosms, similarly with PBDE study, were prepared to observe the behavior of HBCDD when three different bioremediation strategies were applied. These strategies were biostimulation, bioaugmentation and natural attenuation, whose conditions were the same with those of the PBDE study. The target compound was chosen as γ-HBCDD, since it is the predominant congener in technical HBCDD mixture, and has been abundantly found in environmental media when compared to the other isomers (Covaci et al., 2006). As a result, biostimulation was found to yield the highest degradation rate in sediments. Natural attenuation and bioaugmentation were observed to demonstrate no statistically significant difference. Therefore, it can be concluded that DF-1 was not capable of degrading HBCDD in sediment microcosms. Degradation rates identified in this study were generally lower when compared to the limited previous studies. These findings shed light on the degradation potential of HBCDD in contaminated sediments undergoing bioremediation.

Abiotic degradation of PBDEs and HBCDD has been previously demonstrated via various processes. This study aimed at testing the applicability of a novel abiotic degradation mechanism, catalyzed hydrogen peroxide propagations (CHP), for the first time for BFR degradation. CHP is a modified Fenton process, in which high concentrations of hydrogen peroxide is used with Fe^{3+} as the catalyst. Previously, CHP was demonstrated for degrading PCBs and PAHs in soil systems (Manzano et al., 2004; Quiroga et al., 2009; Venny et al., 2012a). Initial experiments with single-fill microcosms revealed the optimum operating conditions, and optimum process as

fill-and-draw (F&D) operation. F&D means withdrawal of reaction solution and refilling the reactor with fresh solution at predetermined time intervals. Subsequently, CHP was applied as F&D treatment to sediments simultaneously spiked with BDE-209 and BDE-99. Despite the appearance of no net decreasing trend in BDE-209, a continuously decreasing trend was observed in BDE-99 throughout the treatment scheme, reaching an overall 83% reduction for BDE-99 after 5 treatments. Hence, it can be evaluated that CHP was more effective in BDE-99 removal in sediments when compared to BDE-209. The F&D application for BDE-209 needs further optimization to achieve higher percent degradation, if possible. CHP was also found to be effective in HBCDD degradation, reaching an overall 93.9% reduction after 4 F&D treatments. When compared with different abiotic degradation mechanisms, total percent reductions were found comparable with CHP.

The bioremediation strategy achieving highest degradation rate in PBDE sediment microcosms was operated in larger scale reactors, i.e mesocosms, to test its performance for field applications. Biostimulation was, therefore, investigated for BDE-209 and BDE-99 degradation in individual reactors. The reason to include BDE-99 in mesocosm sets was that it was one of the congeners highly found in sediments as well as in biological matrices (Hites, 2004). The amount of biostimulating agents added on sediments was altered in mesocosms to provide an acceptable chemical oxygen demand (COD) input to lake sediment media. The organic medium for biostimulation had a COD of 2500 mg/L, hence it was diluted to keep the COD load close to the regulatory COD levels for surface waters. This resulted in the carbon source and electron donor provided per gram of sediments to decrease to 6% of that in microcosm application. Correspondingly, the decrease in contaminants yielded lower degradation rates compared to the microcosm study. An interesting change, on the other hand was in the debromination pathways. The observed debromination products were not identical to those observed in microcosms, pointing to the effect of concentration of biostimulants on debromination mechanisms. Nona- and octa-BDE congeners were observed as products of BDE-209, and had continuously increasing trends during incubation.

BDE-99 products were tetra- and tri-BDEs. Investigation of biostimulation in mesocosms with a reduced carbon source and electron donor content provided valuable data on possible consequences of field applications since reduced organic content was believed to have a direct impact on rate and pathways of debromination.

Final objective of this study was to make an overall evaluation of the remediation strategies tested in terms of sustainability. Sustainable remediation is an emerging topic in remediation applications in the past decade. It is defined as the remediation activities having greater benefits than impacts in terms of environmental, social and economical aspects. This study revealed the fate of BFRs in aquatic sediments when various remediation alternatives were applied, and hence it can provide information for the assessment in terms of environmental aspect. Therefore, a hypothetical case for PBDE/HBCDD contaminated sediments was created, and Tier 1 approach was adapted for sustainability evaluation. As a methodology, the framework presented by Sustainable Remediation Forum – UK (2010, 2011) was followed with the proposed sustainability indicators. This preliminary attempt to assess the sustainability of four remediation techniques revealed biostimulation as the most sustainable option for the hypothetical BDE-209 and HBCDD contamination case. The evaluated strategies differentiate mainly in the environmental aspects of water quality, natural resources and waste, soil conditions, and emissions to air. Meanwhile, the differentiating factors for economic indicators were direct costs, employment, and lifespan, while that for social indicators were human health and uncertainty.

Further research is recommended for the elaboration of BFR contamination levels and identification of hot spots in Turkey. Especially, levels in other environmental matrices, as well as biota and humans, around the regions where the highest BDE-209 level was detected should be revealed.

To test the applicability of proposed remediation strategies for BFR contaminated sites, larger scale reactors and/or pilot scale applications may follow since in the present study mesocosm reactors yielded reduced degradation rates than microcosms. Use of BFR contaminated sediments in degradation reactors rather than freshly

spiked sediments is also recommended to extensively elucidate the consequences of remediation applications when aged environmental sediments is of concern. Additionally, collaborative studies may proceed to determine microbial populations responsible for biodegradation of BFRs in sediment reactors, and may result in enrichment of microbial cultures to be used in bioaugmentation efforts.

Furthermore, the present study provided valuable input for modeling efforts to identify the fate and behavior of BFRs in the environment, regarding the debromination pathways, and rates of degradation. Hence, the data gathered from degradation reactors can be used to estimate degradation rate constants for each debromination pathway, and further used in fate and transport models.

The abiotic degradation mechanism of the current study, namely CHP, needs further investigation in terms of its outcomes, i.e. production of any bioaccumulative or toxic compounds, or achievement of complete mineralization. Furthermore, tandem biotic and abiotic degradation can be evaluated for their effectiveness, especially in BDE-209 degradation, via producing lower brominated congeners initially, and further degradation of these congeners with CHP.

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APPENDIX A

PBDE NOMENCLATURE

#	Structure	#	Structure	#	Structure	#	Structure
Mono-BDE		31	25-4	63	235-4	95	236-25
1	2-	32	26-4	64	236-4	96	236-26
2	3-	33	34-2	65	2356-	97	245-23
3	4-	34	35-2	66	24-34	98	246-23
Di-BDE		35	34-3	67	245-3	99	245-24
4	2-2	36	35-3	68	24-35	100	246-24
5	23-	37	34-4	69	246-3	101	245-25
6	2-3	38	34-5	70	25-34	102	245-26
7	24-	39	35-4	71	26-34	103	246-25
8	2-4	Tetra-BDE		72	25-35	104	246-26
9	25-	40	23-23	73	26-35	105	234-34
10	26-	41	234-2	74	245-4	106	2345-3
11	3-3	42	23-24	75	246-4	107	235-34
12	34-	43	235-2	76	345-2	108	234-35
13	3-4	44	23-25	77	34-34	109	2346-3
14	35-	45	236-2	78	345-3	110	236-34
15	4-4	46	23-26	79	34-35	111	235-35
Tri-BDE		47	24-24	80	35-35	112	2356-3
16	23-2	48	245-2	81	345-4	113	236-35
17	24-2	49	24-25	Penta-BDE		114	2345-4
18	25-2	50	246-2	82	234-23	115	2346-4
19	26-2	51	24-26	83	235-23	116	23456-
20	23-3	52	25-25	84	236-23	117	2356-4
21	234-	53	25-26	85	234-24	118	245-34
22	23-4	54	26-26	86	2345-2	119	246-34
23	235-	55	234-3	87	234-25	120	245-35
24	236-	56	23-34	88	2346-2	121	246-35
25	24-3	57	235-3	89	234-26	122	345-23
26	25-3	58	23-35	90	235-24	123	345-24
27	26-3	59	236-3	91	236-24	124	345-25
28	24-4	60	234-4	92	235-25	125	345-26
29	245-	61	2345-	93	2356-2	126	345-34
30	246-	62	2346-	94	235-26	127	345-35

#	Structure	#	Structure	#	Structure	#	Structure
Hexa-BDE		151	2356-25	174	2345-236	197	2346-2346
128	234-234	152	2356-26	175	2346-235	198	23456-235
129	2345-23	153	245-245	176	2346-236	199	2345-2356
130	234-235	154	245-246	177	2356-234	200	23456-236
131	2346-23	155	246-246	178	2356-235	201	2346-2356
132	234-236	156	2345-34	179	2356-236	202	2356-2356
133	235-235	157	234-345	180	2345-245	203	23456-245
134	2356-23	158	2346-34	181	23456-24	204	23456-246
135	235-236	159	2345-35	182	2345-246	205	23456-345
136	236-236	160	23456-3	183	2346-245	Nona-BDE	
137	2345-24	161	2346-35	184	2346-246	206	23456-2345
138	234-245	162	235-345	185	23456-25	207	23456-2346
139	2346-24	163	2356-34	186	23456-26	208	23456-2356
140	234-246	164	236-345	187	2356-245	Deca-BDE	
141	2345-25	165	2356-35	188	2356-246	209	23456-23456
142	23456-2	166	23456-4	189	2345-345		
143	2345-26	167	245-345	190	23456-34		
144	2346-25	168	246-345	191	2346-345		
145	2346-26	169	345-345	192	23456-35		
146	235-245	Hepta-BDE		193	2356-345		
147	2356-24	170	2345-234	Octa-BDE			
148	235-246	171	2346-234	194	2345-2345		
149	236-245	172	2345-235	195	23456-234		
150	236-246	173	23456-23	196	2345-2346		

APPENDIX B

CONGENER CONCENTRATIONS OF COMMERCIAL PBDE MIXTURES

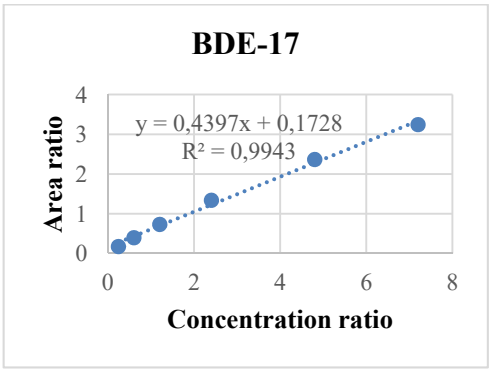
Concentrations of each homolog group in commercial mixtures (%, w/w) (Kim et al., 2017; La Guardia et al., 2006)

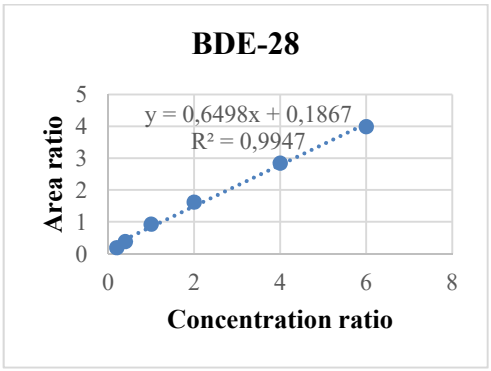
	Penta-BDE mixtures		Octa-BDE mixtures		Deca-BDE mixtures	
	DE-71	Bromkal 70-5DE	DE-79	DE-79B	Saytex 102E	Bromkal 82-0DE
Tri-BDEs	0.32	0.15				
Tetra-BDEs	39.5	43.37				
Penta-BDEs	64.9	54.9		0.5		
Hexa-BDEs	11.89	9.68	10.5	12		
Hepta-BDEs	0.1	0.33	45.6	45		
Octa-BDEs			37.9	33		0.56
Nona-BDEs			13.07	10	2.49	9.3
Deca-BDE			1.31	0.7	96.8	91.6

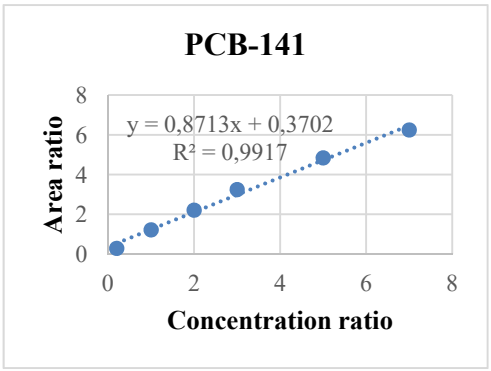
APPENDIX C

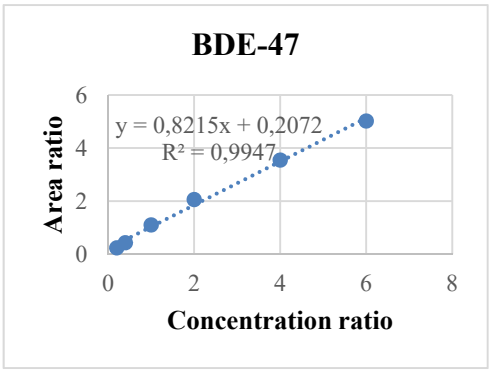
CALIBRATION CURVES FOR INSTRUMENTAL ANALYSIS

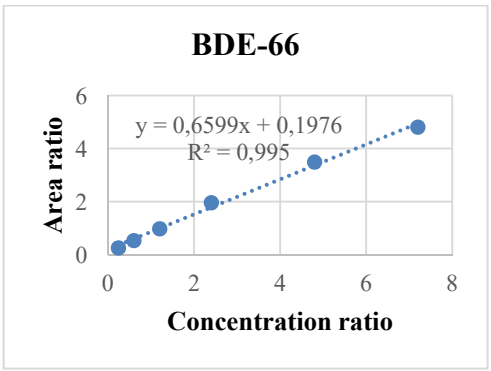
Calibration curves for PBDEs in Gas Chromatography – Micro-cell Electron Capture Detector

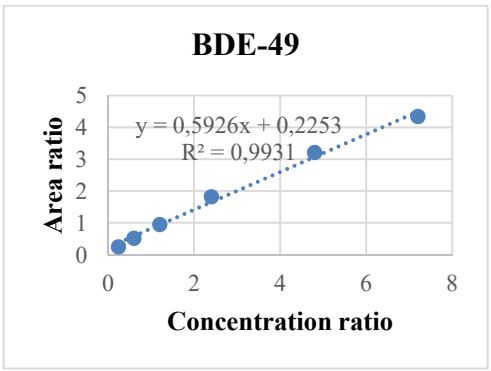


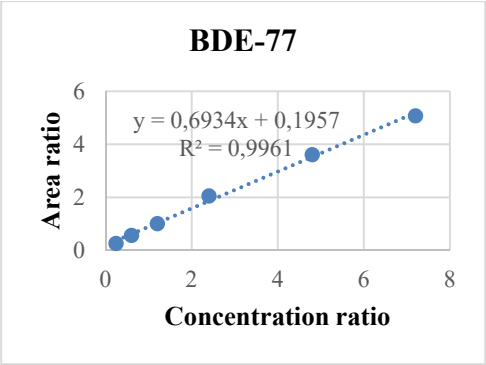


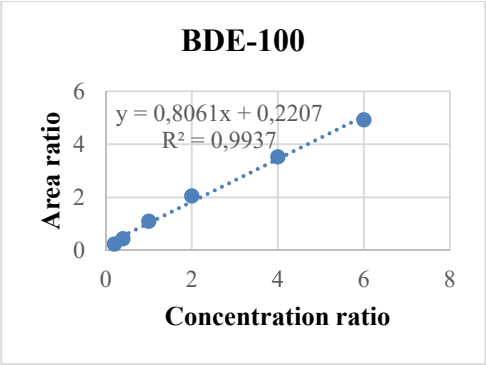


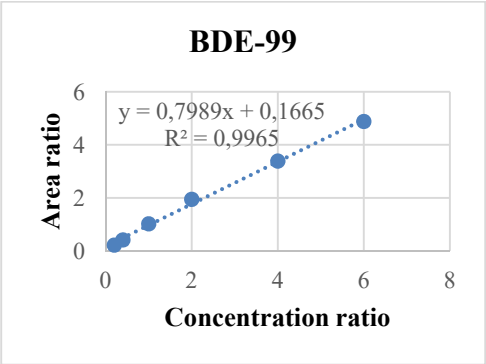


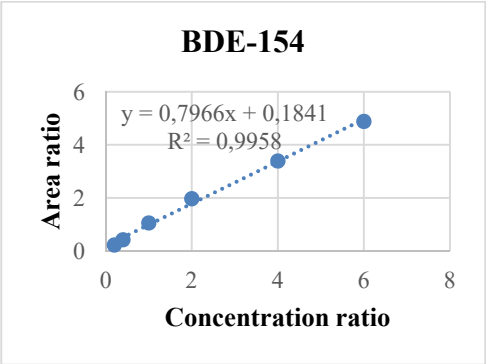


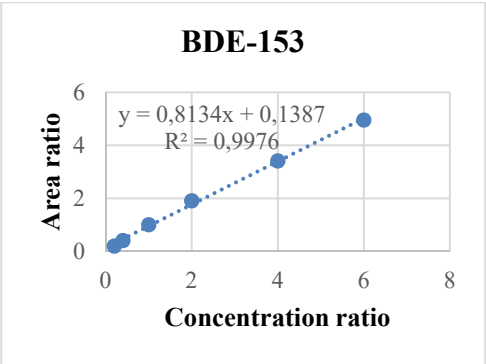


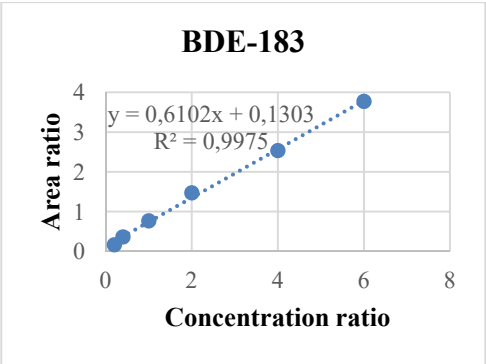

BDE-77
y = 0,6934x + 0,1957
R² = 0,9961
Area ratio
Concentration ratio

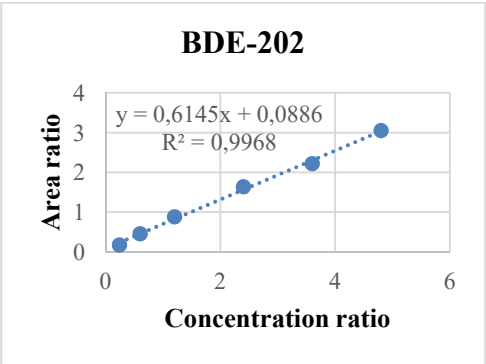

BDE-100
y = 0,8061x + 0,2207
R² = 0,9937
Area ratio
Concentration ratio

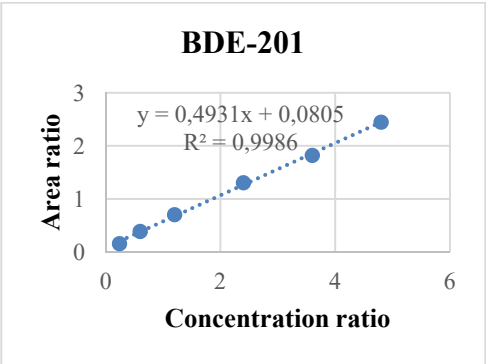

BDE-99
y = 0,7989x + 0,1665
R² = 0,9965
Area ratio
Concentration ratio

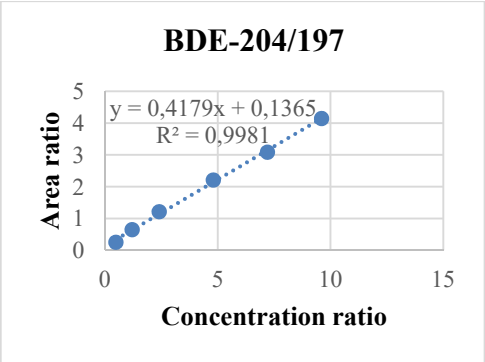

BDE-154
y = 0,7966x + 0,1841
R² = 0,9958
Area ratio
Concentration ratio

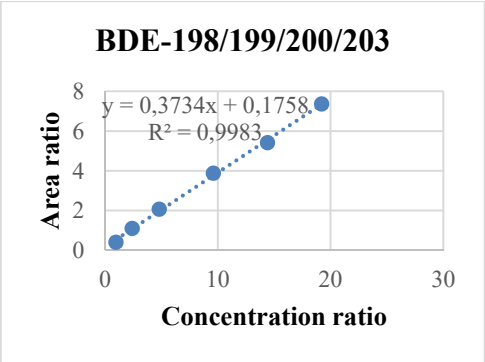

BDE-153
y = 0,8134x + 0,1387
R² = 0,9976
Area ratio
Concentration ratio

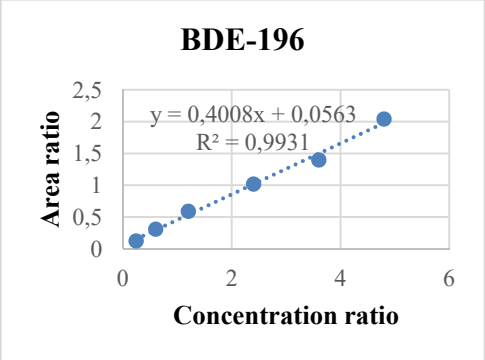

BDE-183
y = 0,6102x + 0,1303
R² = 0,9975
Area ratio
Concentration ratio

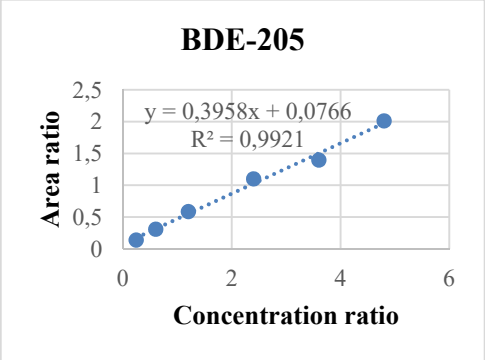

BDE-202
y = 0,6145x + 0,0886
R² = 0,9968
Area ratio
Concentration ratio

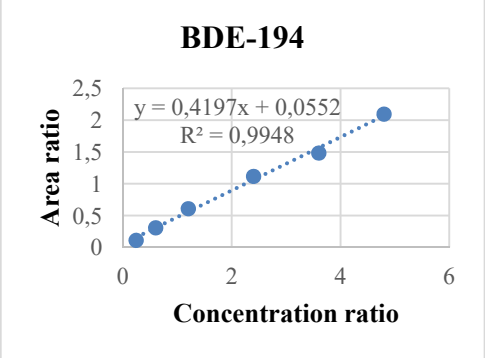

BDE-201
y = 0,4931x + 0,0805
R² = 0,9986
Area ratio
Concentration ratio

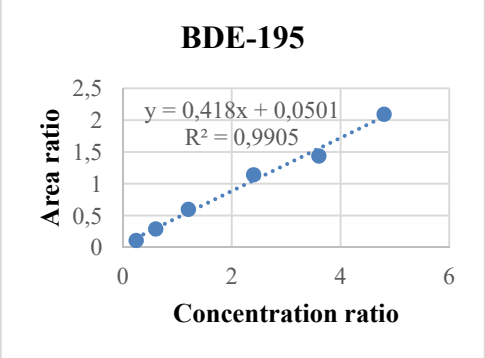

BDE-204/197
y = 0,4179x + 0,1365
R² = 0,9981
Area ratio
Concentration ratio

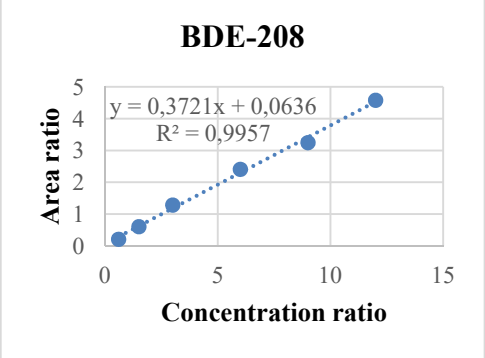

BDE-198/199/200/203
y = 0,3734x + 0,1758
R² = 0,9983
Area ratio
Concentration ratio

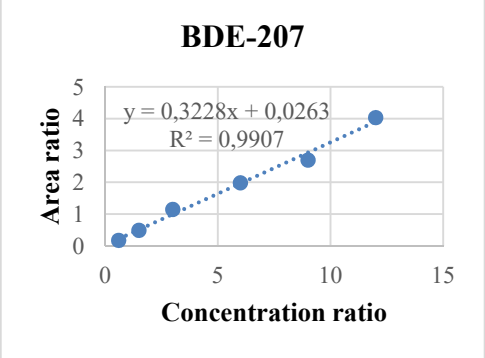

BDE-196
y = 0,4008x + 0,0563
R² = 0,9931
Area ratio
Concentration ratio

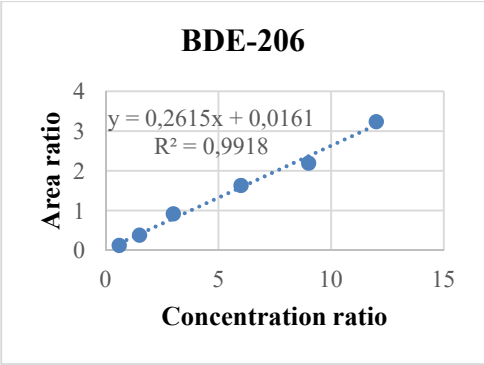

BDE-205
y = 0,3958x + 0,0766
R² = 0,9921
Area ratio
Concentration ratio

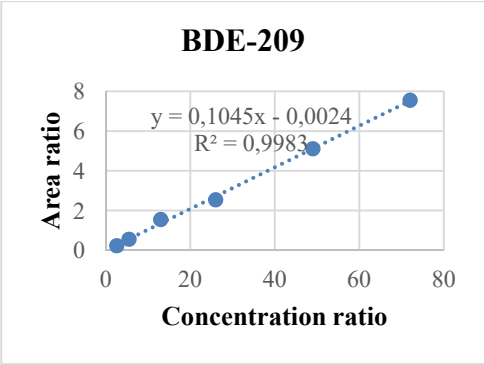

BDE-194
y = 0,4197x + 0,0552
R² = 0,9948
Area ratio
Concentration ratio


BDE-195
y = 0,418x + 0,0501
R² = 0,9905
Area ratio
Concentration ratio

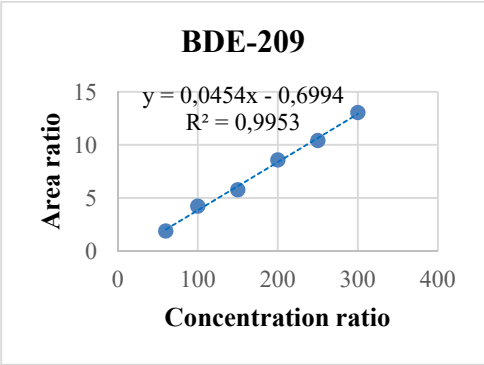

BDE-208
y = 0,3721x + 0,0636
R² = 0,9957
Area ratio
Concentration ratio

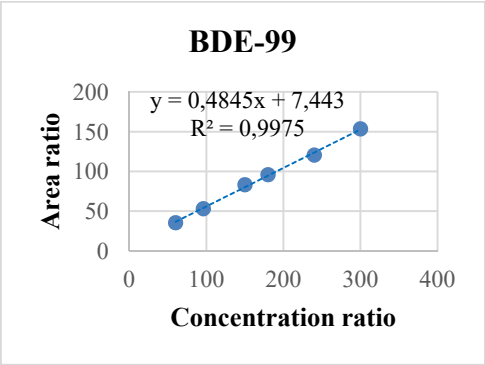

BDE-207
y = 0,3228x + 0,0263
R² = 0,9907
Area ratio
Concentration ratio



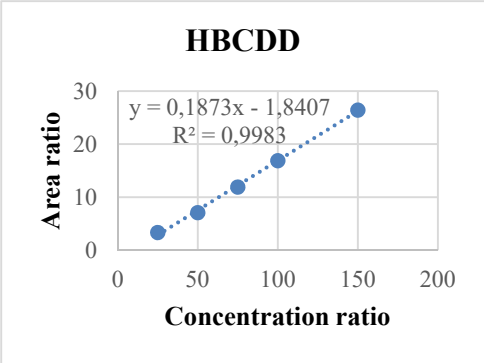


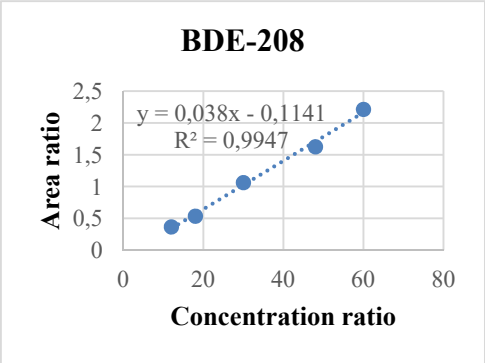
Calibration Curves for high concentration BDE-209 and BDE-99 in Gas Chromatography – Micro-cell Electron Capture Detector





Calibration Curves for HBCDD in Gas Chromatography – Mass Spectrometry





CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Demirtepe, Hale
Nationality: Turkish (TC)
Date and Place of Birth: 13 June 1985, Denizli
Marital Status: Single
Phone: +90 312 210 26 43
Fax: +90 312 210 26 46
email: haledemirtepe@yahoo.com

EDUCATION

Degree	Institution	Year of Graduation
MS	METU Environmental Engineering	2012
BS	METU Environmental Engineering	2008

WORK EXPERIENCE

Year	Place	Enrollment
2012-present	METU, Dept. Env. Eng.	Teaching Assistant
2009-2011	TÜBİTAK	Scientific Programs Assistant Expert
2008-2009	Mavi Engineering, Ankara	Environmental Engineer

FOREIGN LANGUAGES

Advanced English, elementary German

PUBLICATIONS

1. **Demirtepe H.**, Kjellerup, B. Sowers, K. and Imamoglu, I. Evaluation of PCB dechlorination pathways in anaerobic sediment microcosms using an anaerobic dechlorination model, *Journal of Hazardous Materials*, 296, 120-127, (2015).
2. **Demirtepe H.**, Imamoğlu I. Investigation of Bioremediation Alternatives for BDE-209 using Sediment Microcosms: Biostimulation and Bioaugmentation. Submitted for publication.