ABSTRACT

Title of Dissertation:	OCCURRENCE AND REMOVAL OF POLYCHLORINATED BIPHENYLS (PCBS) IN URBAN STORMWATER
	Siqi Cao, Doctor of Philosophy, 2020
Dissertation directed by:	Associate Professor Birthe Veno Kjellerup Professor Allen P. Davis Department of Civil and Environmental Engineering

Polychlorinated biphenyls (PCBs) are a group of chlorinated organic compounds. They are persistent in the environment and can threaten the health of humans and wildlife. Urban stormwater runoff is considered as an important source of PCBs to aquatic environments. The objective of this study is to provide information on the occurrence and removal of PCBs in stormwater; specifically, the occurrence, concentrations, and biological transformations of stormwater PCBs were studied together with their removal. Concentrations of 209 PCB congeners were determined in surface stormwater sediments collected from various roadway sites and bioretention media. The total PCB concentrations ranged from 8.3 to 57.4 ng/g dry weight (dw), with a mean value of 29.2 ng/g dw. Land use had an impact on the concentration of PCBs, where higher stormwater sediment PCB concentrations were found in dense urban areas (average: 39.8 ± 10.5 ng/g) compared to highways passing through greenspace (average: 18.0 ± 0.4 ng/g). PCB sorption tended to increase with the concentration of total organic carbon (TOC) and smaller particle size (< 75 μm) of stormwater particulate matter. In bioretention core

samples, PCB concentrations decreased with bioretention media depth (from 30.0 ± 2.0 ng/g at the surface to 21.2 ± 4.8 ng/g at 40 cm depth), and with distance from the stormwater entrance (from 38.4 ± 2.3 ng/g at the entrance to 33.2 ± 2.9 ng/g at 3 m distance). A non-Aroclor congener, PCB 11, was detected in all samples, likely originating from yellow road paint. Putative organohalide respiring bacteria within Chloroflexi and aerobic PCB degrading bacteria containing the functional genes encoding for biphenyl dioxygenase (*bph*A) and ring cleavage (*bph*C) were detected in some of the stormwater sediments and bioretention media. The presence of such bacteria and a higher level of *ortho*-chlorinated biphenyls indicated the potential of PCB biotransformation in these samples. The performance of an on-campus bioretention indicated that bioretention is effective in removing PCBs from stormwater, with 64–92% reduction of dissolved PCB concentrations. Overall, urban stormwater is an important environmental source of PCBs. Bioretention has the potential to remove PCBs from stormwater via adsorption and biotransformation.

OCCURRENCE AND REMOVAL OF POLYCHLORINATED BIPHENYLS (PCBS) IN URBAN STORMWATER

by

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List of Abbreviations

BMP	Best Management Practice
C&DW	Construction and Demolition Waste
DHB	*Dehalobacter*
DHC	*Dehalococcoides*
DHG	*Dehalogenimonas*
DLPCBs	Dioxin-like PCBs
DOC	Dissolved Organic Carbon
EPA	Environmental Protection Agency
GC/ECD	Gas Chromatography/Electron Capture Detector
IC	Inorganic Carbon
IHSC	Indiana Harbor and Ship Canal
ISQGs	Interim Sediment Quality Guidelines
LID	Low Impact Development
LOQ	Limit of Quantification
MAE	Microwave-assisted Extraction
OHR	Organohalide Respiring Bacteria
OTU	Operational Taxonomic Unit
PAHs	Polycylic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCE	Tetrachloroethene
PEL	Probable Effect Level
POPs	Persistent Organic Pollutants
PSD	Particle Size Distribution
SCM	Stormwater Control Measure
SPE	Solid Phase Extraction
TC	Total Carbon
TCDDs	2,3,7,8-tetrachlorodibenzo-*p*-dioxins
TCMX	Tetrachloro-m-xylene
TEC	Threshold Effect Concentration
TEFs	Toxic Equivalency Factors
TEQ	Toxic Equivalency
TMDLs	Total Maximum Daily Loads
TOC	Total Organic Carbon
TSS	Total Suspended Solids
WHO	World Health Organization
WQS	Water Quality Standards
U.S.	the United States

Chapter 1. Introduction

1.1 What are PCBs?

Polychlorinated biphenyls (PCBs) are a group of chlorinated organic chemicals with the formula $C_{12}H_{10-m}Cl_m$. The structure of PCBs is shown in Figure 1-1. With different numbers and positions of the chlorine atoms, PCBs include 209 different congeners. PCBs were commercially produced for the first time in 1929 (Silverstone et al., 2012). Their manufacturing was ceased in 1977 in the United States (U.S.) and later in other parts of the world because of their adverse effects on laboratory animals and humans (Kimbrough, 1995).

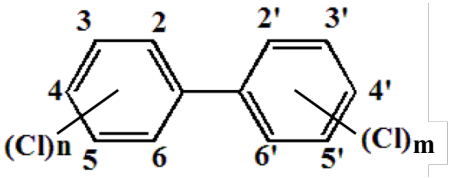

Figure 1-1. Structural formula of PCBs.

PCBs were widely manufactured and used in industrial processes because they are heat resistant and have anti-flammable characteristics (Kimbrough, 1995, ATSDR, 2000). The first major use of PCBs were as electrical fluids, with sealants as the second (Herrick et al., 2004, Kohler et al., 2005). Commercial PCB mixtures were manufactured in several countries with different trade names. These names include Aroclor in the U.S., Clophens in Germany, Phenoclors in France, Phenoclors and Pyralenes in France, Fenclors in Italy and Kanechlors in Japan (Safe, 1994). In the U.S., the Aroclors are identified by a four-digit code with the second two numbers indicating the percentage of chlorine by mass in the mixture (ATSDR, 2000). For example, Aroclor 1254 contains 54% chlorine. The most commonly manufactured Aroclors in the U.S. were A1016, A1232, A1242, A1248, A1254, A1260 (ATSDR, 2000).

1.2 What are the environmental challenges of PCBs?

1.2.1 PCBs are persistent

The octanol/water partition coefficients (log Kow) for PCBs range from 4.50 to 8.26 (Hermanson and Johnson, 2007). Thus, PCBs are considered to be hydrophobic and the solubility in water decreases with increased number of chlorines (ATSDR, 2000). In addition, PCBs are inert and resistant to both acids and alkalis. They have thermal stability as well (ATSDR, 2000).

Due to their high stability and the steric hindrance in their structure, PCBs are recalcitrant to biodegradation (Field and Sierra-Alvarez, 2008). PCBs are persistent in the environment and are classified as a group of persistent organic pollutants (POPs) (Jones and Voogt, 1999). POPs are a group of intentionally or inadvertently produced chemicals, which are resistant to photolytic, biological and chemical degradation (Ritter, 2007). Due to their long-life and harmful effects, PCBs and other POPs were listed in the Stockholm Convention (Stockholm Convention, 2008), which means that they are hazardous and persistent chemicals to be reduced or eliminated.

1.2.2 PCBs are toxic

Sharing a similar structure with 2,3,7,8-tetrachlorodibenzo-*p*-dioxins (TCDDs), the coplanar PCBs can bind to the cytosolic aryl hydrocarbon receptor (AhR) and act as an agonist in organisms to cause toxic and biological effects (Safe, 1994, Van den Berg et al., 2006). Among the 209 congeners, 12 dioxin-like PCBs (DLPCBs): PCBs 77, 81, 126, 169, 105, 114, 118, 123, 156, 167, 189 are of special concern (Van den Berg et al., 2006). The concept of toxic equivalency factors (TEFs) was developed and introduced to facilitate assessment and regulatory control of exposure to these dioxin-like and carcinogenic compounds (Van den Berg et al.,

1998). TEF expresses the toxicity of such compounds in terms of equivalent amount of 2,3,7,8-TCDD (Alcock et al., 1998). Toxic equivalency (TEQ) values are calculated based on the concentration and TEF of the DLPCBs. They are used by United States Environmental Protection Agency (EPA) to account for how dioxin and dioxin-like compounds vary in toxicity and to calculate the accumulated toxicity for mixed contaminant sites.

1.2.3 Many PCB sources are current and PCBs are continuously released

Even though the production of PCBs was banned in the United States in the late 1970s (Kimbrough, 1995), there are still many active PCBs sources (Andersson et al., 2015). This can cause (re)contamination of pristine or remediated sites (Diamond and Hodge, 2007). PCBs are continuously released from for instance old electrical or mining equipment and waste sites (Breivik et al., 2002, Li et al., 2010).

In addition, before they were banned, PCBs were frequently added into sealant materials in windows and other building materials to increase their flexibility (Herrick et al., 2004, Kohler et al., 2005). Diamond et al. (2010) estimated the mass of PCBs in sealants in Toronto to be 12 (0.14–231) tons. Since sealants were used in building construction, PCBs can be leached or washed from sealants and caulking on building exteriors. PCBs can also be released from these buildings over time and result in elevated PCB concentrations in indoor air (Diamond and Hodge, 2007). Increased air concentrations and subsequent atmospheric deposition of PCBs also contribute to PCBs in stormwater and surface waters (Diamond and Hodge, 2007). Besides, surface films, which are composed of biogenic compounds as the organic portion, accumulate on the surface of building materials (windows). The constituents of the surface films could be influenced by local sources like buildings themselves or plants. For example, of the 18 testing sites, the highest PCBs concentration was found in a building located right next to an electrical

plant that housed electrical transformers containing PCBs (Gingrich et al., 2001). As a result, buildings constructed before the 1970s might be important sources of PCBs released from building sealants and surface films (Diamond and Hodge, 2007).

Another potential PCB source is construction and demolition waste (C&DW). Utilization of C&DW is practiced in many European countries (Butera et al., 2014). The crushed materials possess favorable geotechnical properties for construction of roads (Wahlström et al., 2000). However, in Denmark, PCBs were detected in all collected C&DW aggregates from recycling facilities receiving, crushing and selling C&DW, with a mean total PCB concentration at 17 μg/kg total solid (range: 2–70 μg/kg total solid) (Butera et al., 2014). Most of the tested samples were used as road sub-base material. As a result, the roads and buildings using C&DW could also be sources of PCBs to the environment.

Furthermore, public transportation corridors such as highways are often locations containing PCBs (Diamond et al., 2010). PCBs were found in road paint (3520 mg kg^{-1}) and small capacitors (114000 mg kg^{-1}), which indicated that these two groups of items are among current sources of PCBs (Jartun et al., 2009). Since 8% of PCBs were used as a plasticizer before 1980, vehicle parts like the interiors from vehicles manufactured before 1980 could also be a source of PCBs (Scott and Snyder, 2015).

1.2.4 Occurrence of PCBs in stormwater

Since the 1960's, urban stormwater runoff has been identified as an important source of pollution (Granier et al., 1990). Cole et al. (1984) found that PCBs (Aroclor 1260) were detected in urban runoff from one of 19 cities in the United States, and the concentration was 30 ng/L. The reason for the low detection rate and low concentration might be that Aroclors were the target compounds in this study instead of individual PCB congeners. Marsalek and Ng (1989)

determined the concentration of PCBs in runoff at three sites in Canada, ranging from 27 ng/L to 179 ng/L. Granier et al. (1990) measured the concentration of PCBs at the outfall of a stormwater drain during three rain events in 1988 and 1989 in France. In this study, the mean concentrations of PCBs for each rain event were 130, 633 and 625 ng/L, with a range of 36–2600 ng/L. The mean concentration of PCBs in stormwater in Switzerland determined during the early 2000s ranged from values below the detection limit (Detection limit: 0.11–0.24 ng/L) to 403 ng/L (Rossi et al., 2004). The median concentration of seven selected PCBs in three urban areas in Paris and its suburb were 211, 259 and 468 ng/L, respectively (Zgheib et al., 2011). In a watershed in Hayward, California, PCBs concentrations in storm flow ranged from 3.98 to 109 ng/L (Gilbreath and McKee, 2015). PCBs were detected in stormwater around the world, at various concentrations.

Other stormwater studies also detected particulate matter and the particle-bound characteristics of PCBs. Jartun et al. (2008) found that the total concentration of seven PCBs in urban runoff sediment in the inner city of Bergen, Norway ranged between < 0.0004 and 0.704 ng/g. Hwang and Foster (2008) studied PCBs in runoff entering the tidal Anacostia River, Washington, DC and found that stormwater contained levels of PCBs that ranged from 10 to 211 ng/L. The concentrations of PCBs in the particulate matter in stormwater ranged from 31 to 755 ng/g, accounting for more than 90% of the total PCBs in storm flow (Hwang and Foster, 2008). In addition, mean concentrations of seven selected PCBs (PCB 28, 52, 101, 118, 138, 153, 180) in stormwater in the eastern suburb of Paris, France ranged between 27 and 42 ng/L, while the concentrations in the particulate phase ranged between 10–50 ng/g (Zgheib et al., 2011). A study in Norway suggested that stormwater each year carried 0.4 g of seven selected PCBs into Lille Lungegårdsvannet Lake in Bergen via particles in stormwater (Andersson et al., 2015). This

indicated that stormwater plays an important role in carrying PCBs from source to receiving areas. These studies indicate that the worldwide concentration of PCBs in stormwater ranged from several ng/L to hundreds of ng/L and some urban areas in Paris exhibited the highest concentrations of PCBs with 633 ng/L as the mean concentrations. Due to their high persistence, PCB concentrations do not decline as fast as that of some other organic pollutants, such as bisphenol analogues, in environmental matrixes like soil and water (Danzl et al., 2009). These concentrations indicated that PCBs are persistent and removal methods are needed to decrease PCB concentrations in the environment.

1.3 Current and potential solutions for PCBs in the environment

1.3.1 Removal pathways for PCBs

Several physical or chemical methods have been used to remediate PCB contaminated environments (ATSDR, 2000). For example, activated metal treatment can be used to remove PCBs from various materials, including PCB-containing caulks and paints (EPA, 2012). Laboratory testing results showed that the removal rates were over 80% for PCBs from paint and primer (EPA, 2012). However, the removal efficiency decreased for thicker sources. Thus, these methods may not be practical in many cases because they are expensive and energy-demanding, and may produce chlorine-containing products (ATSDR, 2000, Luo et al., 2008).

On the contrary, biotransformation is a sustainable, energy-saving and relatively inexpensive method to remove PCBs from the environment (Luo et al., 2008). Some microorganisms and enrichment cultures have been reported to utilize and metabolize PCBs as carbon and/or energy sources (Abraham et al., 2002) under both aerobic and anaerobic conditions. As a result, bioremediation may be a solution for many PCB-contaminated sites.

Phytoremediation can also be used to remove PCBs from soils. Two PCB phytoremediation mechanisms are phytodegradation and rhizoremediation (Javorská et al., 2009, Gomes et al., 2013). PCBs can be taken up from soil and accumulate in the stems and leaves of the plants (Zeeb et al., 2006, Whitfield Åslund et al., 2007). The amount of phytoextraction is limited due to the hydrophobicity of PCBs (Passatore et al., 2014). However, the role of plants in PCB remediation is still worth studying, since plants can enhance bacterial activity by diffusing oxygen and providing nutrients (Passatore et al., 2014).

1.3.2 Bioretention is an effective treatment for stormwater

Bioretention is an infiltration-based stormwater control measure (SCM) and an increasingly popular best management practice (BMP) (DiBlasi et al., 2009, Li and Davis, 2014). Bioretention cells usually consist of a layer of hardwood mulch and porous soil media (Li and Davis, 2008). Above that, a vegetation mix can be planted to promote biological activity, soil quality, pollutant removal and positive aesthetics (Davis and McCuen, 2005). Bioretention has shown to be effective in improving stormwater quality with regards to the removal of particulate matter, metals, nutrients and pathogens, and the hydrologic condition of the developed landscape (Davis, 2007, Hunt et al., 2008, Li and Davis, 2008, David et al., 2015, LeFevre Gregory et al., 2015).

1.4 Research goals

The hydrophobic nature of PCBs enables them to adsorb to particles and eventually sediments, especially sediments containing a high proportion of carbon (Choi and Al-Abed, 2009). Hwang and Foster (2008) found that stormwater PCBs were significantly enriched in the particulate matter of stormwater runoff and PCBs on particulate matter accounted for more than

90% of the total PCBs in storm flow. Zgheib et al. (2011b) also found that PCBs were only observed in the particulate phase and they were below detection limit (< 30 ng/L) in the dissolved phase. This indicated that particulate matter should be the focus of PCB removal studies in stormwater. PCBs are expected to be removed via particulate matter removal processes such as sedimentation and filtration in stormwater due to the high affinity for adsorption to particulate matter. In addition, excellent capture of particulate matter via filtration-based SCMs were observed for suspended solids (load reduction ≥ 89%) (Houng and Davis, 2009, Landsman and Davis, 2018). Thus, PCB load reduction could be expected in filtration-based SCMs via total suspended solids (TSS) removal.

Bioretention was reported to be a potential solution for the removal of polycyclic aromatic hydrocarbons (PAHs) from stormwater runoff with a reduction rate ranging from 31% to 99% (DiBlasi et al., 2009). Thus, PCBs are expected to be strongly retained in the bioretention media due to the similarity of the chemical characteristics for PAHs and PCBs primarily hydrophobicity.

Reduction of PCB loadings to surface waters requires an understanding of the sources of PCBs in the watersheds (Hwang and Foster, 2008). Elimination of the point sources and identification of potential non-point sources will enable establishment of control measures. To achieve that, information about congener patterns is important. The abundance of specific congeners may indicate certain sources such as PCB 11 (3,3′ -dichlorobiphenyl), which is an unintentional by-product from the manufacturing processes of diarylide yellow pigments often used for road paint (Grossman, 2013). Also, comparison of congener profiles in stormwater contaminated with PCB products may also help in identification of the source. Zhang et al (2011) compared congener profiles in indoor air with exterior building sealants and found that some

indoor air samples grouped with sealant samples as a cluster, indicating they shared similar congener profiles thus the sealant was the PCB source. In addition, a quantitative understanding of PCB behavior in stormwater is essential in developing and maintaining SCMs targeting at PCB removal.

PCBs can be biotransformed by microorganism. Under aerobic conditions, lower-chlorinated congeners (< 5 chlorines) are good substrates and they act primarily as electron donors (Abraham et al., 2002). During the process, hydroxylation on the benzene rings as well as ring cleavage can occur (Passatore et al., 2014). Under anaerobic conditions, PCBs serve as electron acceptor and organohalide respiration takes place. During this process, the number of chlorines attached to highly chlorinated congeners is reduced (Passatore et al., 2014). Anaerobic respiration in the natural environment is very slow because the microorganisms use compounds other than oxygen as electron acceptors and gain less energy (Madigan et al., 2014). The process of biotransformation is affected by congener distribution and many environmental site-specific variables (Sinkkonen and Paasivirta, 2000).

Potential for PCB transformation has been reported in bioretention media and river sediments. Flanagan and May (1993) detected metabolites of aerobic PCB biodegradation in upper Hudson River (NY) sediments and suggested PCB biodegradation occurs naturally in the environment. A study on sediments from Indiana Harbor and Ship Canal (IHSC) (IN) strongly suggested the presence of *in situ* dechlorination based on vertical PCB congener profile patterns (Liang et al., 2014). Both microorganisms capable of anaerobic PCB dechlorination and aerobic PCB degradation were reported from different PCB-contaminated sediment microcosms (Rodrigues et al., 2006, Kaya et al., 2016, Xu et al., 2016, Xu et al., 2019). *Dehalococcoides mccartyi* from Hackensack River (NJ) sediment were reported to be involved in the

dechlorination of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin based on DNA-stable isotope probing results (Dam et al., 2019). In addition, biostimulation in the rhizosphere and root zone of plants like Austrain Pine (*Pinus nigra*) and Goat Willow (*Salix caprea*) could enhance the microbial PCB degradation potential in soil (Leigh et al., 2006).

Bioremediation could also take place in bioretention cells (Davis et al., 2009). In an urban bioswale in New York, U.S., *bph*A genes have been detected (Gill et al., 2017). This indicated that PCB degrading bacteria were present and they had the potential to be active in the bioswale. In addition, the plants in the bioretention cell could potentially enhance the biotransformation of PCBs. Plants can diffuse oxygen in soil as well as release organic carbon or structural analogs of PCBs as degradation inducers from root exudates (Passatore et al., 2014). Thus, it is important to evaluate the potential of *in-situ* PCB biotransformation in stormwater particulate matter as well as bioretention system.

Stormwater appears to be an important source of PCBs to the environment, but little information is available about occurrence and removal of PCBs in stormwater. Therefore, the overall research objectives of this study include:

1) Provide background information about occurrence of PCBs in urban areas.
2) Study the affiliation of PCBs with land use pattern and the characteristics of stormwater particulate matter.
3) Evaluate the performance of bioretention regarding PCB removal.
4) Evaluate the potential of PCB biotransformation in bioretention media and stormwater particulate matter.
5) Use the above information to assist with SCM implementation and design recommendations for the effective PCB removal.

To achieve these objectives, both surface stormwater sediment samples and bioretention media core samples were collected at different urban areas in Maryland and the concentrations and congener distributions of PCBs were determined. The affiliation of PCB concentrations with land use pattern, particle size and total organic carbon (TOC) content were studied. In addition, a field study was conducted to investigate the removal of PCBs from urban stormwater through a bioretention system. The concentration of PCBs in both the dissolved phase and the particulate phase of stormwater was determined and the removal efficiency was calculated.

To study the potential of PCB biotransformation in bioretention media, the presence and abundance of bacteria and their activity were assessed using molecular approaches including both DNA and RNA communities. The results are expected to show if bacteria capable of PCB transformation, including aerobic and anaerobic pathways, are present in the bioretention cells.

Chapter 2. PCBs in stormwater sediments: Relationships with land use and particle characteristics

Abstract

Polychlorinated biphenyls (PCBs) are classified as persistent organic pollutants (POPs). Concentrations of 209 PCB congeners as well as profiles of the ten homologs were determined in stormwater sediments collected from various (primarily roadway) sites with different land use. The total PCB concentrations ranged from 8.3 to 57.4 ng/g dry weight (dw), with a mean value of 29.2 ng/g dw. PCB concentrations varied with nearby land use. Higher stormwater sediment PCB concentrations were found in dense urban areas (average: 39.8 ± 10.5 ng/g) and residential areas (average: 35.3 ± 6.2 ng/g) compared to highways passing through greenspace (average: 18.0 ± 0.4 ng/g). The number of chlorines per biphenyl ranged from 3.63 to 5.39 and the toxic equivalency (TEQs) of the PCBs were between 1.5 and 18.0 pg/g at all sites. A non-Aroclor congener, PCB 11, was detected in all samples and was dominant at two sites. PCBs were sorbed to smaller stormwater particulate matter (≤ 75 μm) at higher concentrations compared to larger particles (> 75 μm). PCB sorption tended to increase with the total organic carbon (TOC) of the particulate matter in the sediment samples. However, greater PCB mass (almost 80%) was present in the larger particles. Information on sediment PCB concentrations from different land uses, along with stormwater particulate matter data can allow the estimation of PCB loads and load reductions using stormwater control measures.

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2.1 Introduction

Polychlorinated biphenyls (PCBs) are a group of chlorinated organic compounds derived from biphenyl with 1 to 10 of the hydrogen atoms substituted by chlorine; thus 209 different congeners exist. PCBs were commercially produced from 1929 to 1977 in the United States (U.S.) and widely used in industrial processes as coolants, in transformer oils and as flame retardants (Kimbrough, 1995). Aroclor is the trade name for specific PCB mixtures that were manufactured in the U.S. In other countries names such as Clophens, Phenoclors, Pyralenes were used (Safe, 1994). The Aroclors are identified by a four-digit code such as Aroclor 1254. The second two numbers in Aroclors indicate the percentage of chlorine by mass in the mixture (ATSDR, 2000). The most commonly manufactured Aroclors in the U.S. were A1016, A1232, A1242, A1248, A1254, A1260 (ATSDRs, 2000).

Due to their high stability, PCBs are persistent in the environment and are classified as persistent organic pollutants (POPs) (Jones and Voogt, 1999, Stockholm Convention, 2008). In spite of the ban, PCBs are still being released into the urban environment (Andersson et al., 2015). Buildings constructed before the 1970s can be sources of PCBs from building sealants and caulking (Diamond and Hodge, 2007, Zhang et al., 2011), while roads constructed with recycled construction and demolition waste (C&DW) also can serve as PCB sources (Wahlström et al., 2000, Butera et al., 2014). Increased air concentrations and subsequent atmospheric deposition of PCBs contribute to the presence of PCBs in stormwater and surface waters (Diamond and Hodge, 2007). Thus, runoff from building roof and wall surfaces may contain PCBs.

Urban runoff/storm sewers are considered important sources of pollution for impaired rivers, streams, lakes, reservoirs and ponds, accounting for 13–19% of the total load (EPA,

2012). Other important probable sources include atmospheric deposition, agriculture, and contaminated sediments.

Most previous stormwater studies have primarily addressed the presence of dissolved PCBs and did not consider particulate matter in stormwater as a separate phase for PCB transport (Granier et al., 1990, Rossi et al., 2004, Gilbreath and McKee, 2015). A few studies evaluated particulate matter and the particle-bound characteristics of PCBs (Hwang and Foster, 2008, Jartun et al., 2008, Zgheib et al., 2011b). Hwang and Foster (2008) found that PCBs in stormwater particulate matter accounted for more than 90% of the total PCBs in storm flow. This indicates that particulate matter should be the focal point when evaluating PCBs in stormwater. Hwang and Foster (2008), however, studied only 85 of the 209 PCB congeners. Jartun et al. (2008) studied seven PCBs (PCB 28, 52, 101, 118, 138, 153 and 180) in urban runoff sediments. Similarly, Zgheib et al. (2011) also focused on these seven PCB congeners because they were listed as priority pollutants in urban stormwater (Zgheib et al., 2008). Thus, information about all 209 congeners in stormwater particulate matter is lacking.

In order to reduce PCB loadings to surface waters, an understanding of the sources of PCBs in the watersheds is required (Hwang and Foster, 2008). To achieve this goal, information about concentrations of all 209 congeners is important. The abundance of some specific congeners may indicate certain sources. Also, comparison of congener profiles in stormwater contaminated with PCB products may assist in source identification. One congeners of special concern is PCB 11 (3,3′-dichlorobiphenyl), which is an unintentional by-product from the manufacturing process of diarylide yellow pigments (Grossman, 2013).

Because PCBs are sorbed to particulate matter, they are expected to be removed from stormwater via particulate matter removal processes such as sedimentation and filtration. Thus, it

is important to study the affiliation of PCBs with particulate matter based on particle size. However, most relevant studies focused on the organic carbon fraction instead of particle size (Bucheli and Gustafsson, 2003, Choi and Al-Abed, 2009, Beless et al., 2014). Little research has been completed regarding PCB concentration and particle sizes (Ghosh et al., 2003), especially as related to stormwater (Marsalek and Ng, 1989, Andersson et al., 2015).

The objectives of the current study were (1) to evaluate PCB concentrations and congener distributions for stormwater particulates collected near urban areas, (2) to identify potential sources of stormwater PCBs in urban areas, and (3) to use the above information to assist with stormwater control measure (SCM) implementation and design recommendations for the effective removal of PCBs from urban stormwater runoff. To address these objectives, surface sediment/soil samples were collected from parking, highway, and residential areas in Maryland (U.S.), and tested for PCBs.

2.2 Materials and methods

2.2.1 Sampling sites

Samples were collected from September 2016 to July 2018. Seven sampling sites were included in this study to represent different land uses: 1) dense urban area: stormwater gutters along roadways in Bladensburg (BBG-U), and Baltimore (BTM-U), MD; 2) institutional area: an inlet to a bioretention cell at the University of Maryland (UMD-I) College Park campus; 3) commercial area: a SCM near a 4-lane state highway (BV-C); 4) greenspace: a stormwater channel adjacent to a highway (R1-G) and a stormwater gutter along roadways through wooded areas in Laurel, MD (R2-G); and 5) residential area: storm drains in a residential area in College Park, MD (CP-R). Figure S2-1 shows a map of sampling sites in this study. At BBG-U, two sample were collected from the gutter, one (BBG-U1) was at 1 m and the other (BBG-U2) was 2

m from the drainage point. The sample from the Baltimore roadway (BTM-U) was collected from the catch basin of a gutter by Dr. James Hunter from Morgan State University. At UMD-I, one sediment sample was collected at the entrance of the stormwater inlet to a bioretention cell. Three samples were collected at BV-C: at the entrance (BV-C1) and the discharge (BV-C2) of the rip-rap channel to the bioretention SCM as well as inside the SCM (BV-C3). At R1-G, two sediment samples were collected at two parallel locations in the stormwater channel (R1-G1 and R1-G2). One sediment sample was sampled from the gutter at R2-G. At CP-R, three samples were collected: from the gutter nearby (CP-R1) and near two different storm drains (CP-R2 and CP-R3). Site names with short descriptions, as well as sampling details are summarized in Table S2-1.

At each site, samples were collected once. Temperature difference in very different periods could affect the solubility and volatility of PCBs. In addition, the frequency of rain could affect the age of the sediments and PCBs accumulated in them. Thus, all samples were collected during May to September, with an antecedent dry period ranging from 1 to 7 days. The temperature ranged from 23°C to 30°C during sample collection. During the collection process, a clean and sterile stainless-steel soil scoop was used to collect surface sediments (0–10 cm deep) at different sites. To minimize biological and chemical transformations, all samples were stored at -20°C in the dark in glass containers with PTFE lids until analysis.

2.2.2 Sediment fractionation

Selected sediment samples were separated into three fractions, as done by Kim and Sansalone (2008). Briefly, wet sieving (No. 200 sieve, ELE INTERNATIONAL, Canada) was performed to separate the “sediment” fraction (> 75 μm) from the other two fractions. The filtrate was transferred to an Imhoff cone and settled for 2 h. The “settleable” fraction (~ 25 to 75

μm) was settled out in the Imhoff cone and the remaining fraction was defined as the "suspended" fraction (< 25 μm). The suspended fraction accounted for < 1% of the total sediment mass in all collected samples thus this fraction was not further studied. The > 75 μm fraction and the 25–75 μm fraction were air dried in the fume hood for two days until they appeared dry.

Clean sea sand (Merck, U.S.) was used as laboratory blank control (triplicate) and was exposed to the same treatments as the samples (triplicate).

2.2.3 Extraction of PCBs

Microwave-assisted extraction (MAE) (MARS 6, CEM, U.S.) was used to extract PCBs from the sediment samples. The extraction method was based on Lopez-Avila, et al. (1995) with minor modifications. Briefly, six grams of air dried sample was weighed and transferred to PTFE extraction vessels (100 mL, CEM, U.S.). Then 36 mL hexane (95% n-hexane for organic residue analysis)-acetone (HPLC grade, Honeywell) (1:1) was added into the vessels. Prior to extraction, 20 μL of the mixed solution of surrogates [0.5 μg mL^{-1} tetrachloro-m-xylene (TCMX), 2,4,6-trichlorobiphenyl (PCB 30) and 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB 204)] was added into each sample to calculate the recovery of the extraction procedure. Extractions were performed at 115°C for 10 min at 1000 W. After extraction, the vessels cooled to room temperature before they were opened. The extracts were settled by gravity for 30 min and the supernatants were transferred into 60 mL amber vials. The residues were washed with five mL of hexane and settled for 30 min. Five mL of hexane-acetone (1:1) and five mL of acetone were added separately to wash the residues as mentioned above. The supernatants were combined and then concentrated under nitrogen flow to less than 100 μL. Finally, 1 mL of hexane (HPLC grade) was added to dissolve the extracts. Extracts were stored at -20 °C until cleanup.

2.2.4 Cleanup of PCB extracts

The cleanup method was based on EPA method 3611B (EPA, 1996) and 3620C (EPA, 2014) with minor modifications. Briefly, alumina (Fisher Scientific, U.S.) was heated at 550°C for at least 24 h and cooled to room temperature in a desiccator. Deionized (DI) water was added (30 μL DI water/g alumina) to deactivate it to 3%. Columns for cleanup were made with six g of the prepared alumina, a layer of oven dried sodium sulfate (Fisher Scientific, U.S.) to remove water from the extracts, and glass wool (Acros Organics, Germany) at the bottom of a glass disposable pipet (Pyrex, U.S.). First, 20 mL hexane was added to equilibrate the column. Then the extracts were transferred into the column and the effluent was collected. The amber vials were rinsed with 15 mL of hexane and the rinsate was added into the column. The collected effluent was reduced in volume with nitrogen flow to concentrate the samples to less than 1 mL and spiked with 20 μL of the mixed solution of internal standards [0.5 μg mL^{-1} 4-bromobiphenyl (4-BB) and 2,2′,4 ,5,5′-pentabromobiphenyl (penta-BB)]. Hexane (HPLC grade) was added to the concentrated effluent to reach a final volume of 1 mL and the samples were vortexed for 10 s before being transferred into GC vials for further analysis.

2.2.5 PCB analysis

Samples were analyzed by gas chromatography/electron capture detector (GC-ECD) (7890B, Agilent Technologies, U.S.) equipped with an Agilent J&W HP-5ms column (60 m x 250 μm x 0.25 μm). The samples were autosampled (Autosampler 7693, Agilent Technologies, U.S.) with an injection volume of 1 μL. Helium was used as the carrier gas. The temperature program (Table S2-2) was developed based on a congener-specific PCB analysis method used for analysis of PCB 11 (Guo, 2013). Target compounds, surrogate standards, and internal standards were purchased from AccuStandard (U.S.) and Restek (U.S.).

2.2.6 Quality control

All laboratory materials were made either of glass or PTFE to avoid sample contamination. To avoid contamination during sample preparation, all glassware used were cleaned by detergent, rinsed with hexane, acetone, methanol and DI water, and baked in a muffle furnace at 550°C for 4 h. PTFE containers were cleaned by detergent, ultrasonicated with hexane and acetone, and rinsed with DI water. During each run, clean hexane vials were added at the beginning and the end of each run to avoid significant carry over from previous runs. The results from the laboratory blanks and clean hexane at the end of each run indicated that carry over did not occur between samples.

For each sample, an extra treatment group (triplicate) was prepared as a standard control. The standard control treatment was treated the same way as other treatments except for the addition of surrogate standards or internal standards. This treatment was carried out to confirm the standards were not present in the sediment samples.

All 209 PCB congeners were analyzed and 131 peaks were detected in the samples. All peaks were verified by mass spectrometry by m/z and retention time (5977A MSD, Agilent Technologies, U.S.). Detection limits for PCB congeners ranged from 0.0008 to 0.33 ng/g. The method detection limits were obtained by dividing the instrument detection limits by the sample masses. For statistical analysis, all values below the detection limits were substituted with half of the detection limits. Concentrations of the target compounds were compared to average concentrations in laboratory blanks and calculated based on Audy et al. (2018). If the blank average was < 10% of the amount in the sample (178–195 congeners), no correction was applied. If the blank average was 10–35% of the concentration in the samples (11–21 congeners), the average concentration in the blanks were subtracted from the sample. If the blank average was

above 35% (3–10 congeners), the compound was reported as below the detection limit in the sample.

The measured concentrations of mono- to tetra-CBs were corrected for the recovery of TCMX and the measured concentrations of penta- to deca-CBs were corrected for the recovery of PCB 204. Average surrogate recoveries were 73.0% for TCMX (range: 54.8–91.9%) and 83.0% for PCB 204 (range: 50.8–114.0%). All were within the acceptable range of 50–125% (Hermanson and Johnson, 2007). 4-BB was the internal standard for mono- to tetra-CBs and penta-BB was the internal standard for penta- to deca-CBs.

2.2.7 Total organic carbon (TOC) measurement

Total carbon (TC) and inorganic carbon (IC) were measured using a TOC analyzer (TOC-L, Shimadzu, Japan) with solid sampling module (SSM-5000A). All sediment samples were measured in triplicate. TOC was calculated by subtracting IC from TC.

2.2.8 Data analysis

Concentrations of the homologs were calculated based on data from GC analysis. Concentrations were equally distributed between congeners when there was co-elution. 12 dioxin-like PCBs (DLPCBs: PCBs 77, 81, 126, 169, 105, 114, 118, 123, 156, 167, 189) are of special concern due to their elevated toxicity and their individual concentrations were also calculated. The average number of chlorines per biphenyl was determined based on a molar average.

For the comparison of two groups of data, F-tests were performed to test the equality of two variances and Student *t*-tests were performed to examine differences among different sites

and sediment fractions. Statistical significance was set at $p < 0.05$. For comparison of three groups of data, pairwise t-tests was performed, and Holm's sequential Bonferroni method was applied to correct the results by reducing the possibility of type I error (Bunzel et al., 2013, Huang et al., 2018).

2.3 Results and discussion

2.3.1 PCB concentrations at different land uses

PCBs were detected in the samples from all the studied sites (Figure 2-1). The highest total PCB concentration (51.6 ± 5.6 ng/g) in this study was found in BBG-U1, 1 m from a stormwater inlet (Figure 2-1A). BBG-U2 had a total PCB concentration of 36.4 ± 1.4 ng/g. The second highest PCB concentration (41.4 ± 5.6 ng/g) was found in CP-R, from the residential area (Figure 2-1C), where a parking area and residential buildings are nearby. UMD-I, collected at the entrance of the bioretention cell, contained high concentrations of PCBs, at 37.3 ± 6.3 ng/g (Figure 2-1G). BTM-U contained PCBs at 31.5 ± 2.3 ng/g (Figure 2-1B). Total PCB concentrations varied at BV-C (Figure 2-1D). The lowest PCB concentrations in this study were found at R1-G (17.8 ± 3.9 ng/g) and R2-G (18.3 ± 1.1 ng/g) (Figure 2-1E and 2-1F).

The concentrations of total PCBs in stormwater particulate matter (9.8–51.6 ng/g) were in the range of concentrations observed in several previous studies (< 0.4–755 ng/g, Table S2-3). Zgheib et al. (2011b) measured concentrations of seven selected PCB congeners, with values ranging from below 10 ng/g to 60 ng/g in the particulate matter in stormwater, higher than those in this study (< 0.00167–1.92 ng/g). Potential reasons for the higher concentrations found in Zgheib et al. (2011b) include 1) the area in the Zgheib study was a watershed in a dense urban area with commercial centers, apartments and buildings, and 2) the study was carried out at least

six years ago in France, where PCBs were banned several years later than the U.S. (Zgheib et al., 2011).

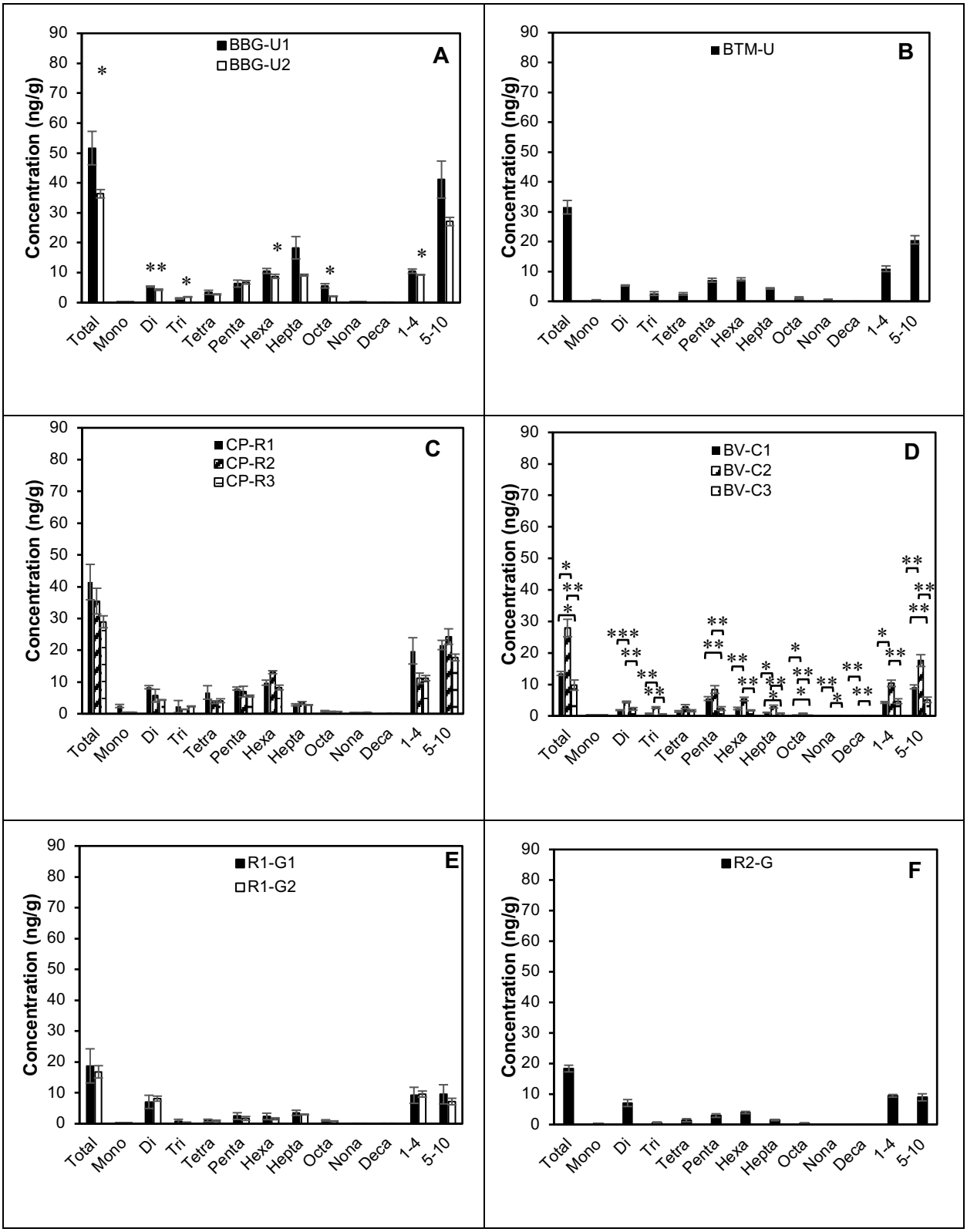
The consensus-based threshold effect concentration (TEC) based on various sediment quality guidelines for PCBs in freshwater ecosystems is 59.8 ng/g (MacDonald et al., 2000); for a total PCB concentration below 59.8 ng/g, harmful effects on sediment-dwelling organisms are unlikely to be observed. The interim sediment quality guidelines (ISQGs) and the probable effect levels (PEL) for PCBs in freshwater sediment are 34.1 ng/g and 277 ng/g, respectively (CCME, 2001). Both sediment samples from the BBG gutter, as well as sediment samples from CP-R1, CP-R2 and UMD-I bioretention cells were above the ISQGs for PCBs in freshwater sediment, suggesting adverse effects from PCBs exposure, but were below the consensus-based TEC and PEL. All the other samples were below the ISQG, indicating a low possibility of adverse effects at these sites.

Both BBG-U samples as well as UMD-I had similar PCB homolog patterns. Penta- to hepta-CBs were the most dominant among all the homologs. Di-CBs also had a high abundance at UMD-I, accounting for 25.1 ± 2.0% of total PCBs. BTM-U was also similar to BBG-U, where PCBs with two, five and six chlorines dominated. All three CP-R residential samples had similar homolog patterns. Di-CBs and hexa-CBs were dominant, followed by penta-CBs and tetra-CBs. At the BV-C SCM, the two channel locations (BV-C1 and BV-C2) showed similar homolog patterns; penta- and hexa-CBs were the most dominant. However, the homolog pattern inside the SCM (BV-C3) was different from that in the rip-rap channel. Tetra- to hexa-CBs were present at high concentrations (1.64 ± 0.31 ng/g, 2.33 ± 0.60 ng/g) compared to other homologs. At R1-G and R2-G, di-CBs was the most dominant homolog (7.61 ± 1.55 and 7.03 ± 1.13 ng/g, respectively). The amounts of mono- to tetra-CBs and penta- to deca-CBs were similar at these

two sites. This result was noticeably different from the other sites, where the concentrations of penta- to deca-CBs were higher than mono- to tetra-CBs.

The highest number of chlorines per biphenyl was found in BBG-U (1 m from the stormwater inlet, Table 2-1). Highly chlorinated PCBs ($Cl \geq 5$) mainly experience biotransformation through dechlorination; anaerobic conditions are essential for this process. The process however is slow compared to degradation of lower chlorinated PCBs ($Cl \leq 4$), which can be degraded aerobically (Payne et al., 2011, Passatore et al., 2014). Products of anaerobic dichlorination act as substrates for the aerobic biodegradation processes (Passatore et al., 2014). Except for CP-R1, R1-G, R2-G, and BV-C3, all the other samples had a chlorine number greater than four. The lowest numbers of chlorines were found at R1-G and R2-G, due to the dominance of di-CBs at these two sites.

The easier loss of chlorines in the *meta* and *para* positions results in an increase in the level of *ortho*-chlorinated PCBs during microbial dechlorination (EPA, 2004). Thus, the low levels of *ortho*-chlorinated PCBs in BBG-U, CP-R2, CP-R3 and R2-G suggest limited or no reductive dechlorination (Table 2-1). The level of *ortho*-chlorinated PCBs at the other sites were at least four-fold higher, which suggest impacts from microbial dechlorination. The level of *ortho*-chlorinated PCBs in BTM-U was high compared to BBG-U samples, indicating dechlorination taking place at this site. A lower chlorine number per biphenyl in BTM-U could also support the occurrence of dechlorination.


A
BBG-U1
BBG-U2
B
BTM-U
C
CP-R1
CP-R2
CP-R3
D
BV-C1
BV-C2
BV-C3
E
R1-G1
R1-G2
F
R2-G
Concentration (ng/g)
Total
Mono
Di
Tri
Tetra
Penta
Hexa
Hepta
Octa
Nona
Deca
1-4
5-10

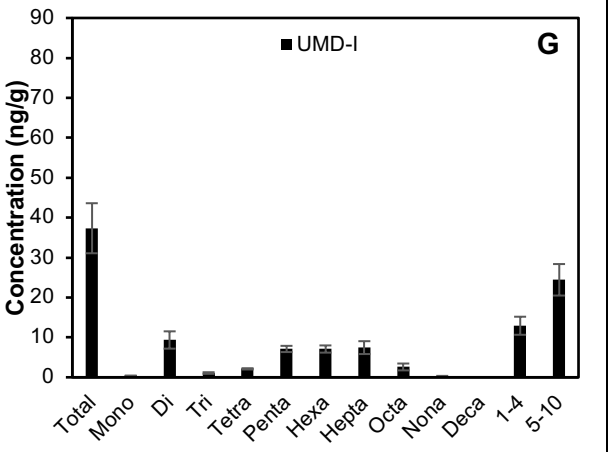


Figure 2-1. Concentrations of total PCBs, homologs, mono- to tetra-CBs, and penta- to deca-CBs in the surface sediment samples at different sites: A: BBG-U (gutter), B: BTM-U (catch basin in storm inlet) C: CP-R (Student t-test was not performed at this site because CP-R3 had only duplicates), D: BV-C (bioretention SCM), E: R1-G (stormwater channel), F: R2-G (gutter), G: UMD-I (bioretention). See text for more details on sampling locations (significance levels: * = 0.05; ** = 0.01; *** = 0.001).

Table 2-1. Number of chlorines per biphenyl, concentrations of Dioxin-like congeners, TEQ_{PCB} and level (mol%) of *ortho*-chlorinated PCBs at different sites; ranges and means of PCBs based on land use.

Site	Approximate Land use	Cl per biphenyl	Dioxin-like PCBs (ng/g)	TEQ_{PCB} (pg/g)	Mol% of *ortho*-chlorinated PCBs	PCB ranges* (ng/g)	PCB means* (ng/g)
BBG-U1	Dense urban	5.39 ± 0.14	2.36 ± 0.18	5.72 ± 1.88	0.41 ± 0.03	31.5–51.6	39.8
BBG-U2	Dense urban	4.99 ± 0.05	2.08 ± 0.21	6.34 ± 0.22	0.59 ± 0.13		
BBG-U1_> 75	Dense urban	5.64 ± 0.08	2.62 ± 0.04	7.48 ± 0.45	0.47 ± 0.05		
BBG-U1_25–75	Dense urban	5.65 ± 0.01	9.57 ± 0.26	40.3 ± 3.60	0.24 ± 0.03		
BTM-U	Dense urban	4.47 ± 0.05	1.88 ± 0.03	18.0 ± 1.30	4.85 ± 0.42		
BV-C1	Commercial	4.37 ± 0.07	0.91 ± 0.08	2.03 ± 0.28	1.50 ± 0.35	9.77–37.3	22.1
BV-C2	Commercial	4.33 ± 0.09	2.28 ± 0.27	2.94 ± 0.97	4.76 ± 0.05		
BV-C3	Commercial	3.95 ± 0.04	0.45 ± 0.04	1.48 ± 0.06	2.54 ± 1.33		
UMD-I_> 75	Institutional	4.42 ± 0.05	1.42 ± 0.10	4.45 ± 0.42	4.19 ± 0.43		
UMD-I_25–75	Institutional	5.06 ± 0.07	2.85 ± 0.44	7.34 ± 1.39	2.30 ± 0.71		
UMD-I_Total	Institutional	4.44 ± 0.11	1.75 ± 0.53	4.74 ± 1.24	3.31 ± 0.96		
R1-G	Greenspace	3.63 ± 0.20	0.55 ± 0.29	5.16 ± 6.81	1.69 ± 2.46	17.8–18.3	18.0
R2-G	Greenspace	3.63 ± 0.21	0.87 ± 0.02	5.93 ± 0.78	0.78 ± 0.014		
CP-R1	Residential	3.92 ± 0.05	2.95 ± 0.31	14.8 ± 3.10	4.10 ± 0.27	28.9–41.4	35.3
CP-R2	Residential	4.58 ± 0.14	1.72 ± 0.01	10.6 ± 4.70	0.53 ± 0.02		
CP-R3	Residential	4.42 ± 0.01	2.09 ± 0.08	9.04 ± 0.59	0.68 ± 0.06		

Note: * Ranges and means of PCBs based on land use were determined using complete samples only and do not include size-fractionated samples.

Comparison of sites

Results from this study suggest an influence of land use on the PCB concentrations and homolog patterns. The ranges and means of PCB concentrations based on different land uses are summarized in Table 2-1. Higher PCB concentrations were found in dense urban areas (average: 39.8 ng/g) and residential areas (average: 35.3 ng/g) compared to greenspace (average: 18.0 ng/g). A similar trend was also found in a study carried out in France (Zgheib et al., 2011). In the Zgheib et al. study, the median concentration of seven selected PCBs (PCB 28, 52, 101, 118, 138, 153, 180) in stormwater was greatest in a highly dense area, at 468 ng/L. The median concentrations in residential area and dense urban area were lower, at 211 ng/L and 259 ng/L, respectively.

The building fronting the BBG samples was brick, built in 1968. PCBs can be leached or washed from sealants and caulking on building exteriors. Also, elevated PCB concentrations in indoor air could vent into the outdoor air. The total emission of PCBs estimated from one intensively studied office in Toronto, Canada was 280–5879 ng/h, and up to 90% of total losses could be to the outdoors based on the air exchange rate (Zhang et al., 2011). Additionally, stormwater runoff could wash flakes of material containing PCBs from the surface of older buildings. This is supported by research which found that among 80 buildings constructed from 1945 to 1980 in Toronto, Canada, the mean concentration of PCBs was 4630 mg kg^{-1} (of sealants) (Diamond et al., 2010). Diamond et al. (2010) also estimated that concentrations of PCBs in sealants and caulking were geographically higher in residential areas within buildings constructed during 1950 to 1970.

Brick research/educational buildings dating from the 1970's to 1990's are 50 to 100 m away from UMD-I. The CP-R residential buildings, primarily built from wood, were constructed in 2011.

The SCM at BV-C is about 40 m from a low-rise commercial building built in 1986 and is surrounded by a small parking lot (approximately 40 spots) on the rip-rap channel side. On the other sides, it is surrounded by a 4-lane highway and local roads. The stormwater treatment media elevation was high at the end of the channel, which allowed sediments to deposit and not enter the SCM, possibly causing a higher PCB concentration at the end of the channel.

No buildings were nearby R1-G and R2-G, a major difference compared to the other sites. Instead these two highways traversed through forested areas. As a result, the lower levels of PCBs detected in the sediments at these locations could be a consequence of the absence of buildings surrounding the collection site. Therefore, the measured PCB concentrations at these two sites can be assumed to result only from highway sources.

Overall, the results of this analysis suggest that buildings, and especially older buildings, contribute significantly as PCB sources in urban areas. Also, roads themselves may play a lesser role in PCB contamination compared to buildings. Since CP-R had comparatively new buildings, the relatively high PCB concentrations at this site may result from the use of recycled building materials or PCB byproducts from paints (Anezaki and Nakano, 2014).

2.3.2 Comparison of congener profiles among different sites

None of the sediment samples had a similar congener pattern with any Aroclor mixture, indicating that Aroclors were not the direct sources, or the Aroclor mixtures were weathered due to biodegradation and/or mixing. However, some congeners detected at relatively high

abundance in the sediment samples were also present in A1254 or A1260, indicating that some congeners may have originated from these Aroclors. Relative abundance (% of mass) of the dominant congeners are summarized in Table 2-2. PCB 11 was only congener detected at all sites at a relatively high abundance. It is a non-Aroclor congener and is inadvertently produced during the manufacturing of paint pigments (Hu and Hornbuckle, 2010).

PCB 99, 110, 180, 193 and 194 were detected with high abundance in samples from BBG-U, BTM-U and UMD-I. PCB 99 and PCB 110 are important components of A1254. PCB 99 is also a dechlorination product from A1260 (Fagervold et al., 2007). PCB 194 accounted for 2.1% of the total PCBs in A1260 (Frame et al., 1996). PCB 194 showed no dechlorination after 500 days of incubation with Baltimore Harbor sediment microcosms (Fagervold et al., 2007), indicating its stability. PCB 180 accounted for 11.4% of A1260. Overall, the dominant congeners in these sediment samples were present, and in some cases abundant, in A1254 and A1260.

The CP-R congener fingerprints were different from the other sites, with PCBs 11, 14, 141 and 161 dominating. PCB 141 accounted for 2.6% of total PCBs in A1260 and 1.0% in A1254 (Frame et al., 1996). PCB 99 was dominant at BV-C (4.5–22.5%). PCB 8 was detected with high abundance at this site. It was one of the major congeners detected in polycyclic-type pigments (Anezaki and Nakano, 2014). Overall, sediments from dense urban and institutional sites were dominated by congeners present in A1254; residential sites had smaller signatures of A1254. A1254 has been reported to have been added into sealants and caulking compounds (ATSDR, 2000). This information supports the hypothesis that old buildings remain as important PCB sources to stormwater. PCB 11 and 14 dominated in the sediments from greenspace (R1-G and R2-G).

Table 2-2. Relative abundance (% of mass) of the dominant congeners at different sites and A1254.

	Sites							A1254
	BBG-U	CP-R	BTM-U	UMD-I	BV-C	R1-G	R2-G	
PCB 8					0.21-5.16			0.13
PCB 11	1.48-3.27	1.28-10.0	11.18	15.2	3.19-3.38	8.97	30.90	
PCB 14	6.15-8.86	5.77-11.6		4.62	5.82-13.3	28.6	5.86	
PCB 96			2.07	4.56	0.66-2.30	1.45		0.04
PCB 99	2.34-4.79	0.47-2.17		3.23	4.47-22.5	4.08	1.79	3.02
PCB 110	2.40-2.90	0.160-1.30		2.21	1.56-3.28	1.01		9.29
PCB 118		0.317-1.58			1.08-5.63			7.35
PCB 141		1.64-4.00	2.09	1.06				0.98
PCB 161		1.98-3.94					2.10	
PCB 180	3.17-4.09			3.21	1.88-2.71	1.97	1.15	0.67
PCB 193	9.99-23.2		1.12	7.03	1.20-1.90	10.2		0.03
PCB 194	4.11-9.19		1.24	2.96	0.517-1.38	3.90	1.19	0.01

2.3.3 Concentrations and potential sources of PCB 11

The concentration and relative abundance of PCB 11 at the different study sites showed that the highest concentration was found at UMD-I (Figure 2-2), ranging between 4.47 and 6.58 ng/g; it was the most dominant congener and accounted for 15% of the total PCB masses. R2-G also had a high concentration of PCB 11 (5.66 ± 1.07 ng/g), accounting for 30.9% of total PCBs at this site. At the other sites, PCB 11 was also detected, but at lower concentrations compared to

UMD-I and R2-G, ranging from 0.33 ± 0.08 to 3.52 ± 0.10 ng/g. The relative abundance of PCB 11 compared to total PCBs at these sites ranged from 1.3–11.2%. These results indicate that PCB 11 is an important component of the total PCB fingerprint in urban stormwater and the surrounding area.

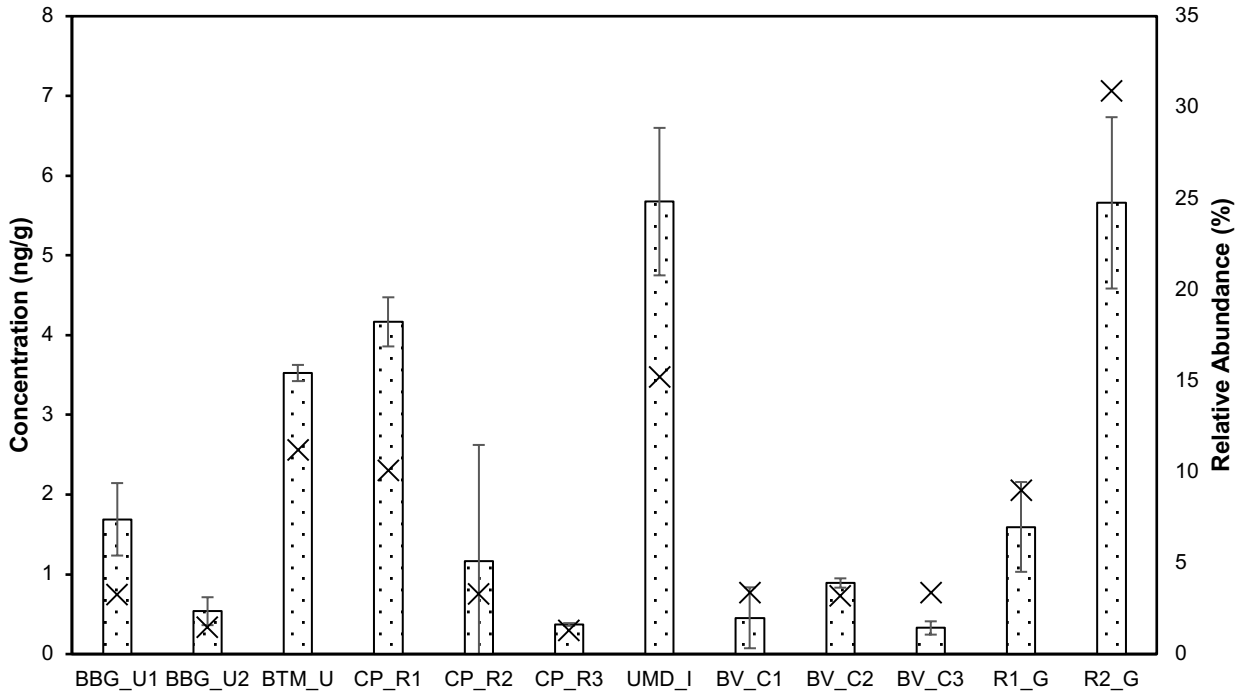


Figure 2-2. Concentration and relative abundance of PCB 11 at different sites (bar represents concentration (left axis), x represents relative abundance (right axis)).

Yellow flakes were noted in the UMD-I samples. The larger yellow flakes, 0.5–4 mm in diameter, were separated from the sediment by hand and both were analyzed separately for PCB concentrations. Without the large yellow flakes, the concentration of PCB 11 was 3.8 ± 0.7 ng/g

in the sediment, which was significantly different from the concentration (5.4 ± 0.1 ng/g) measured with the flakes included ($p = 0.02$). The separated yellow flakes were tested using the same procedure as the sediment and the concentration of PCB 11 was 182 ± 10 ng/g. The yellow flakes appear to be yellow traffic paint.

PCB 11 has been detected as the major congener present in azo-type pigments (Anezaki and Nakano, 2014). It has been frequently detected in urban air (Chicago) and pigments or dyes (Hu et al., 2008, Hu and Hornbuckle, 2010). Among all 209 congeners, PCB 11 was found in 13 of the 33 commercial paint pigments tested (Hu and Hornbuckle, 2010). Near New York/New Jersey Harbor, PCB 11 concentrations in the effluents of the two wastewater treatment plants which receive wastewater from pigment manufacturing plants ranged from 5 to 116 ng/L, while PCB 11 concentrations ranged from 0.0016 to 9.4 ng/L in the effluents from other wastewater treatment plants (Rodenburg et al., 2010). PCB 11 has been frequently detected in commercial goods, such as newspapers, magazines, and cardboard boxes (Rodenburg et al., 2010). Thus, PCB 11 is emerging as a marker of non-legacy PCB contamination.

2.3.4 PCB concentration dependencies on particle size and TOC

BBG-U1 and UMD-I samples were separated into > 75 μm and 25–75 μm size fractions. At BBG-U1, both fractions shared a similar homolog pattern and concentrations of all but mono- and deca-CBs were significantly higher in the 25–75 fraction compared to the > 75 fraction ($p < 0.001$, Figure 2-3A). In the 25–75 fraction, total PCB concentration was 180 ± 6 ng/g, which was approximately three times of the concentration in the > 75 fraction (56.3 ± 6.3 ng/g).

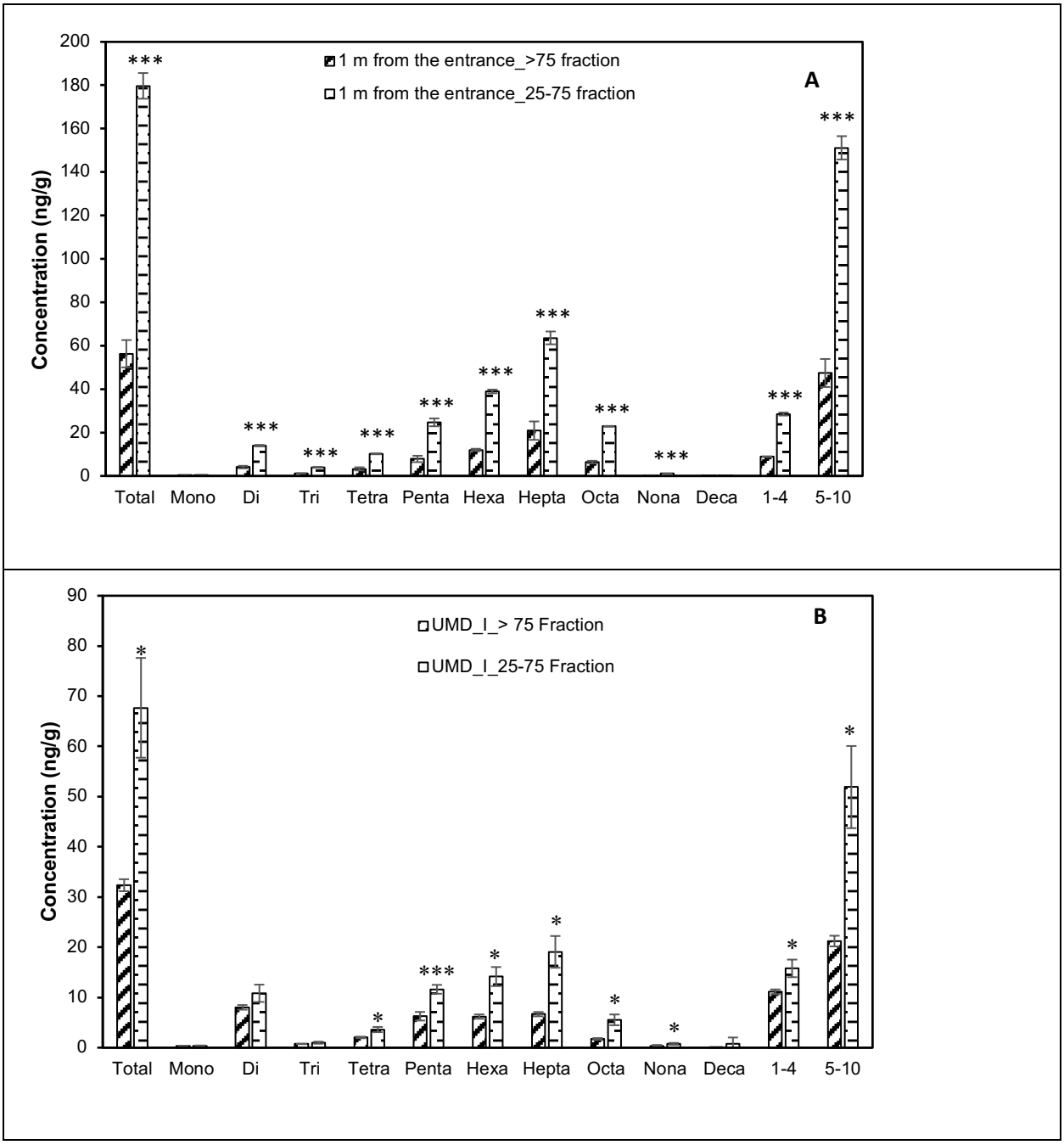


Figure 2-3. Concentrations of total PCBs, homologs, mono- to tetra-CBs, and penta- to deca-CBs in different fractions for (A) BBG-U1, 1 m from the entrance, (B) UMD-I (* reflects a significance level of 0.05; ** reflects a significance level of 0.01; *** reflects a significance level of 0.001).

For UMD-I, hexa-and hepta-CBs were the most dominant in the 25–75 μm fraction, while di-CBs dominated the > 75 μm fraction. Both fractions had mono- and deca-CBs below detection limit (Figure 2-3B). No significant difference was observed between the di-CBs in the two fractions ($p = 0.10$).

Both sites showed higher proportion of *ortho*-chlorinated PCBs in the > 75 μm fraction, suggesting greater degree of PCB dechlorination in larger particles (Table 2-1). Larger particles have lower PCB concentrations and as a result lower toxicity to microorganisms (Abraham et al., 2002).

The > 75 μm fraction accounted for 98.1% of the total mass of the UMD-I sediment sample. As a result, 94.9% of the total PCB mass, 96.5% of mono- to tetra-CBs and 94.1% of penta- to deca-CBs were sorbed to the > 75 μm fraction. Thus, the removal of the particles larger than 75 μm, which is relatively easy to perform in a SCM because they are easy to settle or be filtered (Kim and Sansalone, 2008), can remove approximately 94% of the PCB mass applied to a SCM. For BBG-U1, the > 75 μm fraction accounted for 93.3% of the total sediment mass. When considering the mass of different BBG-U1 fractions, nearly 80% of total PCBs mass were sorbed to larger particles.

PCB distribution based on particle size have been studied on sediment samples from Harbor Point, New York, where the fine fraction (< 63 μm) contributed approximately 80% of the PCB mass in the total sediment, attributed to their large surface area (Ghosh et al., 2003). This disagreement with the stormwater results may be attributed to the differences in the TOC of different particle size fractions and the particle mass distribution in the sediment samples, which were not mentioned. Carbonaceous particles contributed 60–90% of the PCBs in the sediment

samples even though they accounted for only 5–7% of the total mass (Ghosh et al., 2003). PCBs are generally associated with sediment or soil via two mechanisms: adsorbed to carbonaceous sorbents on the surfaces and absorbed within the sorbent matrix by solvation with amorphous organic carbons (Beckingham and Ghosh, 2016). In both processes, carbonaceous matter in sediments or soils plays an important role.

The TOC content of BBG-U1 was 1.30%, which was higher than the value at 2 m (1.02%). Correspondingly, the total PCB concentration in the total sediment at BBG-U1 (51.6 ± 5.6 ng/g) was significantly higher than that at BBG-U2 (36.4 ± 1.4 ng/g) ($p = 0.01$) (Figure 2-1A). Also, the 25–75 µm fraction at BBG-U1, with TOC of 4.70%, had a significantly higher PCB concentration than the > 75 µm fraction (TOC: 1.63%, $p < 0.01$, Figure 2-3A). The TOC content in the UMD_I 25–75 µm fraction (9.54%) was higher than that in the > 75 µm fraction (1.14%); higher PCB concentrations were found in the 25–75 µm fraction.

In both BBG-U and UMD-I, the total concentration of penta- to deca-CBs in the 25–75 µm fraction was more than two times of the concentration in the > 75 µm fraction, correlating with the TOC contents of the two fractions. PCBs with more chlorines are more hydrophobic, resulting in a stronger sorption of PCBs to the organic matter on the sediment (ATSDR, 2000). Relations between PCBs and TOC in stormwater sediments were also found in a study in Norway, which reported that the concentration of seven selected PCB congeners was strongly correlated with the TOC content of sediments (using PCA) (Jartun et al., 2008).

2.3.5 Toxic equivalency values

Of the 209 congeners, DLPCBs are of special concern due to their higher levels of health risks. DLPCBs are congeners which have been shown to exert toxic responses similar to those observed for 2,3,7,8-Tetrachlorodibenzodioxin (2,3,7,8-TCDD) (Van den Berg et al., 1998). Toxic equivalency factors (TEF) for DLPCBs were set by the World Health Organization (WHO) (Van den Berg et al., 1998, Van den Berg et al., 2006). Toxic Equivalency (TEQ) values are calculated by multiplying the mass concentrations of the 12 DLPCBs with the respective TEF (Table 2-1) and are used by the U.S. Environmental Protection Agency (EPA) to account for how dioxin and dioxin-like compounds vary in toxicity (EPA, 2018). The TEQ_{PCB} levels varied from 1.48 ± 0.06 pg/g to 14.8 ± 3.1 pg/g in the total sediment samples. All samples, except the 25–75 μm BBG-U1 fraction, were below the safe sediment value of 20 pg TEQ/g (Eljarrat et al., 2001). The TEQ_{PCB} levels fall within the range of 0.03 to 24.8 pg/g found in Northwest Mediterranean sediment (Eljarrat et al., 2001) and found in core samples in Indiana Harbor and Ship Canal (IHSC), Lake Michigan, U.S. (0.68–120 pg/g) and are similar to the TEQ value at the surface layer of one of the core samples (Martinez and Hornbuckle, 2011). The 25–75 μm fraction in BBG-U1 had the highest TEQ_{PCB} (40.3 ± 3.6 pg/g) due to the consistently high total PCB concentrations found in that fraction.

Except for BTM-U and CP-R, all other samples were close to the U.S. background level (2–5 ppb TEQ/g) (EPA, 2010). BTM-U and CP-R had values larger than 9 pg TEQ/g, indicating higher risk potentials at these sites. However, PCBs have been found to account for 1%–84% of the total TEQs in sediments (Eljarrat et al., 2001). More information about other dioxin or dioxin-like compounds is therefore needed to assess the risk of these stormwater sediment samples.

2.4 Conclusions

This study investigated the concentrations of all 209 PCB congeners as well as homolog distribution in stormwater sediments at seven sites with different land use patterns. Results from this study suggest that:

- Land use pattern has an impact on PCB concentrations and homolog patterns in stormwater sediments.
- Smaller stormwater particles had an increased tendency to sorb PCBs than larger particles. However, greater PCB mass (more than 80%) was present in larger particles.
- Targeting sediments from high density urban areas could reduce a large portion of PCB stormwater load via particulate matter capture and removal.
- PCB 11 was frequently detected in stormwater sediments and appears to be related to yellow pigments used in roadways. Additional studies are needed to clarify this relationship and to determine if pigments should be targeted as a nonpoint PCB source.

Appendix

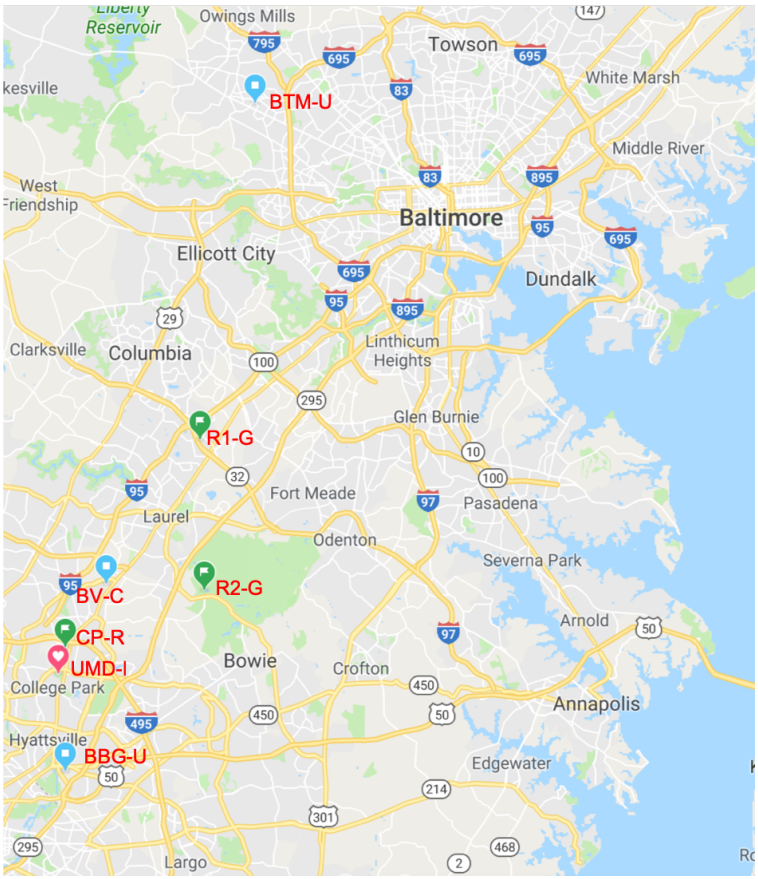


Figure S2-1. Map of sediment sampling sites in Maryland.

Table S2-1. Summary of the sampling sites.

Site Name	**Description**	**Location (GPS coordinates)**	**Drainage area**	**Sampling time**	**Sampling point**
UMD-I	Bioretention on campus	Along Regents Dr. (38.993593, -76.939007)	Road, sidewalk, parking lot,	Sep 2016	Inlet entrance

			teaching buildings		
R1-G	Runoff channel on highway	On MD Route 32 (39.144314, -76.820086)	Highway	June 2017	Two parallel locations in the channel
BV-C	SCM along Route 1	Along MD Route 1 (39.051953, -76.897692)	Roads, parking lot, commercial building	Sep 2017	BV-C1: Inlet entrance, BV-C2: Inlet discharge BV-C3: inside SCM
BBG-U	Roadside gutter	Along 52nd Ave. (38.933153, -76.930882)	Roads, buildings	Sep 2017	BBG-U1: 1 m from the drainage point BBG-U2: 2 m from the drainage point

BTM-U	Roadside gutter	Liberty Rd. (39.3595387-76.7739888)	Roads, buildings	May 2018	Catch basin of a gutter
R2-G	Roadside gutter	On MD Route 197 (39.047880, -76.816826)	Roads	July 2018	6 m from the gutter
CP-R	Storm drain	Along MD Route 1 (39.011177, -76.931005)	Buildings, parking lot	July 2018	CP-R1: 4 m from the gutter nearby
					CP-R2: around a storm drain receiving runoff from roofs and an electric box
					CP-R3: around a storm drain receiving runoff from roofs

Table S2-2. Temperature program for GC.

Run Time (min)	Rate (°C/min)	Hold Time (min)	Temperature (°C)
0		0	70
15.714	7	0	180
60.714	1	0	225
91.059	5.8	20	285
102.36	11.5	10	300

Table S2-3. Comparison of PCB concentrations in the particulate phase in stormwater (ng/g dw) analyzed in this study with concentrations reported from other locations around the world.

Location	Year	Compounds	Mean	Median	Range	ref
Anacostia River, U.S.	2002	Total PCBs			31.0–755	(Hwang and Foster, 2008)
Inner city of Bergen, Norway	2005	7 PCBs	80	29	< 0.4–704	(Jartun et al., 2008)
East suburb of Paris, France	2008	PCB28	20		20–30	(Zgheib et al., 2011b)
		PCB52	10		< 10–20	
		PCB101	20		< 10–40	
		PCB118	20		< 10–40	
		PCB138	50		30–60	
		PCB153	50		30–60	

		PCB180	30		20–40	
A bioretention cell near campus parking, U.S.	2016	Total PCBs	37.3		31.3–44.9	this study
		PCB28	0.0498		0.0303–0.0661	
		PCB52			< 0.0167	
		PCB101	0.188		0.173–0.204	
		PCB118	0.166		0.132–0.217	
		PCB138	0.640		0.538–0.860	
		PCB153	0.583		0.480–0.686	
		PCB180	1.198		1.096–1.425	
Route 32, U.S.	2017	Total PCBs	17.8		13.4–24.5	this study
		PCB28			< 0.0167–0.114	
		PCB52			< 0.0167	
		PCB101			< 0.00167–0.216	
		PCB118	0.0620		0.0422–0.0855	
		PCB138	0.142		0.110–0.164	
		PCB153	0.101		0.0859–0.121	
		PCB180	0.351		0.270–0.469	
A SCM near Route 1, U.S.	2017	Total PCBs	13.5		11.7–12.8	this study
			27.9		25.0–29.9	

			9.77		7.24–10.4	
		PCB28			< 0.0167	
		PCB52			< 0.0167	
		PCB101			< 0.00167–0.290	
		PCB118	0.626		0.0986–1.85	
		PCB138	0.189		0.110–0.298	
		PCB153	0.128		0.0774–0.209	
		PCB180	0.399		0.176–0.776	
A gutter in Bladensburg, U.S.	2017	Total PCBs	51.6		46.3–57.4	this study
			36.4		34.8–37.5	
		PCB28	0.267		0.167–0.394	
		PCB52			< 0.0167	
		PCB101	0.275		0.232–0.344	
		PCB118	0.297		0.261–0.348	
		PCB138	0.905		0.760–1.05	
		PCB153	0.801		0.677–0.947	
		PCB180	1.56		1.38–1.92	
Route 197, U.S.	2018	Total PCBs	17.8		17.3–19.5	this study
		PCB28			< 0.0167–0.440	

		PCB52			< 0.0167	
		PCB101			< 0.00167	
		PCB118	0.160		0.146–0.186	
		PCB138	0.300		0.282–0.322	
		PCB153	0.232		0.220–0.239	
		PCB180	0.210		0.207–0.214	
A residential area in College Park, U.S.	2018	Total PCBs	41.4		36.7–47.6	this study
			35.4		32.2–40.0	
			28.9		27.6–30.3	
		PCB28			< 0.0167–1.36	
		PCB52			< 0.0167–1.53	
		PCB101	0.0565		0.0413–0.0845	
		PCB118	0.328		0.0537–0.678	
		PCB138			< 0.0333–0.648	
		PCB153	0.418		0.0193–0.740	
		PCB180			< 0.00333–0.180	
A gutter in Baltimore, U.S.	2018	Total PCBs	31.5		28.9–33.0	this study
		PCB28	0.612		0.547–0.648	
		PCB52			< 0.0167	

		PCB101	0.0584		0.0541–0.626	
		PCB118	0.0211		0.0118–0.0302	
		PCB138			< 0.0333	
		PCB153			< 0.0167	
		PCB180	0.197		0.196–0.200	

Chapter 3. Evidence of organohalide respiration of PCBs in stormwater bioretention cells

Abstract

Core samples from bioretention cell media as well as surface stormwater sediment samples from seven urban areas were collected to assess the potential for biotransformation activity of polychlorinated biphenyls (PCBs). Based on DNA extracted from these samples, putative organohalide respiring bacteria within Chloroflexi were detected in all the samples. The putative organohalide respiring bacteria include *Dehalobacter*, *Dehalogenimonas*, and *Dehalococcoides*. Bacteria containing the functional genes encoding for biphenyl 2,3-dioxygenase (*bph*A) or 2,3-dihydroxybiphenyl 1,2-dioxygenase (*bph*C) were detected in 25 of the 27 samples. *Dehalococcoides mccartyi*, which can transform PCBs by organohalide respiration, was identified in one of the samples. Expressed bacterial genes from putative organohalide respiring bacteria as well as genes encoding for *bph*A and *bph*C were obtained from the microbial community thus showing that organohalide respiration of PCBs and aerobic PCB degradation under both aerobic conditions occurred in the surface samples collected at the bioretention site. These findings show that bacteria capable of transforming PCBs were present in the stormwater bioretention cell thus illustrating that in situ PCB removal can take place in this environment. Presence and concentrations of 209 PCB congeners in the bioretention media were also assessed. The total PCB concentration ranged from 38.4 ± 2.3 ng/g at the top layer of the inlet to 11.6 ± 1.2 ng/g at 20–30 cm at 3 m from the inlet. The decreasing PCB concentration with depth indicated that bioretention is effective in retaining PCBs, which could support organohalide respiration of PCBs in the deeper parts of the bioretention media. The average

number of chlorines per biphenyl in the core samples was 4.41 ± 0.24. The level of *ortho*-chlorinated PCBs ranged from 1.65–3.19% thus showing that *meta* and *para* organohalide respiration occurred. These results provide preliminary evidence that bacteria capable of PCB transformation, including both aerobic organohalide respiration and aerobic degradation, were present and active in the bioretention.

3.1 Introduction

Polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) (Stockholm Convention, 2008) that are resistant to physicochemical treatments (ATSDR, 2000), making it difficult to eliminate PCBs from the environment. Since the 1960s, urban stormwater runoff has been identified as an important source of water quality impairment pollution incl. for PCBs (Granier et al., 1990). To prevent continued PCB contamination of surface waters, measures must be taken to remove PCBs from stormwater. Chemical treatment methods may not be practical in most stormwater applications because chemical methods can be expensive and energy-demanding, and may produce more toxic chlorine-containing products than present in the initial contamination (ATSDR, 2000, Luo et al., 2008).

In contrast, biotransformation is a sustainable and potentially energy-saving and economical approach that can be used to remove PCBs from the environment (Luo et al., 2008). PCBs can be biologically transformed via two pathways: 1) aerobic degradation and 2) anaerobic organohalide respiration (Quensen et al., 1988, Flanagan and May, 1993). During aerobic degradation, lower-chlorinated congeners function as substrate and act as electron donors (Abraham et al., 2002). The two critical steps for this process are incorporation of two hydroxyl groups into the biphenyl ring structure (the ring fission reaction), which is catalyzed by the dioxygenase enzymes BphA (encoded by the *bphA* gene) and BphC (encoded by the *bphC* gene), respectively (Pieper, 2005, Petrić et al., 2011). The bottleneck process that is required prior to aerobic degradation of highly chlorinated PCBs is anaerobic organohalide respiration. Here, PCBs serve as electron acceptor and the chlorine substituent is replaced with hydrogen. Chlorines can be substituted at all three ring positions: *para*, *meta*, and *ortho* depending on the involved bacteria. Due to steric hindrance at the *ortho* position, chlorine substitution during

organohalide respiration is more likely to happen at flanked *para* and *meta* positions (Quensen et al., 1988).

Several studies have reported on the potential for PCB biotransformation taking place in stormwater drainage areas and a bioswale (Kjellerup et al., 2012, Gill et al., 2017). Organohalide respiration was observed in soil samples collected in a stormwater drainage ditch in Mechanicsburg, PA. Additionally, in the soil aggregates, putative organohalide respiring bacteria (OHR) and biphenyl dioxygenase genes were detected (Kjellerup et al., 2012). In an urban bioswale stormwater control measure (SCM) in New York, NY, *bph*A genes were detected (Gill et al., 2017).

Bioretention has become an increasingly popular SCM, consisting of a layer of engineered media, typically hardwood mulch, and a layer of vegetation (Davis, 2007). Various active biological processes have been reported in bioretention systems. Biodegradation of oil and grease was found in the mulch layer in a bench-scale infiltration study (Hong et al., 2006), while biodegradation of naphthalene was observed with 12–18% of mineralization taking place in laboratory bioretention columns (LeFevre et al., 2012). Increased quantities of naphthalene dioxygenase genes were detected in the bioretention media, indicating the capacity for hydrocarbon biodegradation (LeFevre et al., 2012). Research concerning biotransformation of PCBs in bioretention media is lacking and is needed to sustainably treat PCBs in stormwater.

Since PCBs have been detected in stormwater samples (Cole et al., 1984, Rossi et al., 2004, Hwang and Foster, 2008, Jartun et al., 2008, Zgheib et al., 2011, Gilbreath and McKee, 2015, Cao et al., 2019), it is important to study the potential of biotransformation in stormwater sediments and bioretention media. The objectives of this study were (1) to examine the presence of microorganisms capable of PCB transformation, including aerobic and anaerobic pathways in

stormwater sediments and bioretention media; (2) to evaluate the abundance of such microorganisms and the potential for PCB biotransformation using gene expression and bioinformatics approaches; (3) to relate the presence and abundance of microorganisms capable of PCB transformation with accumulated PCB concentrations in the bioretention media; and (4) to use the above information to suggest enhancements for sustainable PCB removal from stormwater.

3.2 Materials and methods

3.2.1 Sampling sites

Surface stormwater sediment samples and bioretention media core samples were collected from September 2016 to July 2018. Surface sediment samples were collected at seven different sites, including sites near roadways (BBG and BTM), at an institutional area (UMD), at a commercial area (BV), surrounded by greenspace (R1 and R2) and at a residential area (CP). At each site, one to three samples were collected at different locations. Descriptions about the sampling sites are summarized in Table 3-1. More details about sampling process and PCB concentrations can be found in Cao et al. (2019).

Table 3-1. Summary of the sampling sites.

Site Name	Description	Drainage Area	Sampling Time
BBG	Roadside gutter	Roads, buildings	Sep 2017
BTM	Roadside gutter	Roads, buildings	May 2018
UMD	Bioretention on campus	Road, sidewalk, parking lot	Sep 2016
BV	SCM along Route 1	Roads, parking lot, commercial building	Sep 2017
R1	Runoff channel on highway	Highway	June 2017
R2	Roadside gutter	Roads	July 2018
CP	Storm drain in a residential area	Buildings, parking lot	July 2018

Media core samples were collected from a bioretention cell located on the University of Maryland campus (College Park, MD). The drainage area of the bioretention included concrete sidewalks as well as asphalt parking lots and roads (Figure 3-1). More details about the bioretention facility can be found in DiBlasi et al. (2009). The core samples were taken from the surface to 18–30 cm deep and split into 2 to 4 segments. Additional bioretention media samples at the top media layer as well as surface sediment samples for RNA analysis were collected in February 2020. They were stored in an RNA stabilization solution (DNA/RNA Shield, ZYMO RESEARCH, U.S.) and extracted within 24 h.

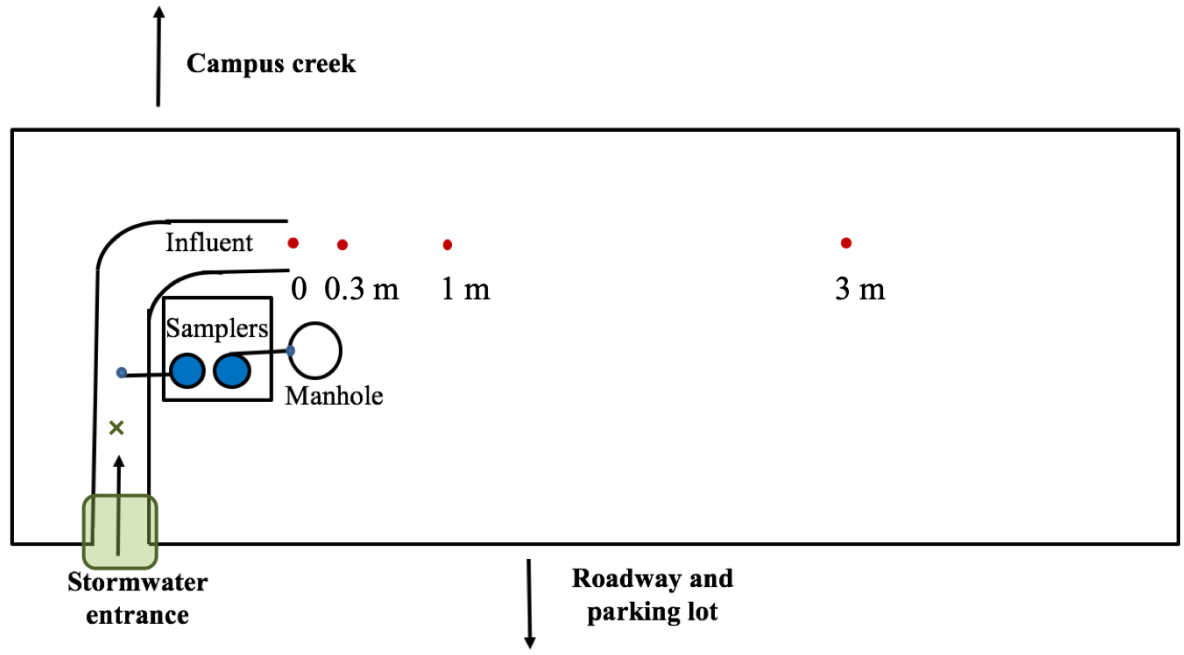


Figure 3-1. Overhead sketch of the bioretention cell at UMD [×: sampling site of the surface sediment samples (sample UMD); dots: sampling sites of the core samples (0, 0.3 m, 1 m and 3 m from the inlet; collected using a JMC environmentalist's subsoil probe [2 cm inner diameter, 71 cm length])].

3.3.2 PCB extraction and cleanup

The core samples were extracted using microwave-assisted extraction (MAE) and cleaned with alumina as described in Cao et al. (2019). Briefly, 6 g of core samples and 36 mL of hexane/acetone mixture (v/v=1:1) were extracted at 115°C for 10 min at 1000 W. Before extraction, 20 µL of the mixed solution of surrogates [0.5 µg mL^{-1} 2,4,6-trichlorobiphenyl (PCB 30) and 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB 204)] was added into each sample to calculate the recovery of the extraction and cleanup process. After MAE, the supernatant was collected

and combined with the solvents after washing the samples. The extracts were ready for cleanup after concentrating to less than 100 μL. The extracts were loaded to a pre-activated alumina column and eluted with hexane. The elution was collected and concentrated under N_2 flow. 20 μL of the mixed solution of internal standards [0.5 μg mL^{-1} 4-bromobiphenyl (4-BB) and 2,2′,4,4′,5,5′-hexabromobiphenyl (hexa-BB)] was added before GC analysis.

3.2.3 PCB analysis

Samples were analyzed using gas chromatography with electron capture detector (GC-ECD) (7890B, Agilent Technologies, U.S.). The samples were analyzed with an Agilent J&W HP-5ms column (60 m x 250 μm x 0.25 μm) with the following temperature program: initial temperature at 70°C, 7°C/min to 180°C, 1°C/min to 225°C, 5.8°C/min to 285°C, held at 285°C for 20 min, 11.5°C/min to 300°C and held at 300°C for 10 min. The injection volume was 1 μL and N_2 was used as the carrier gas.

3.2.4 Quality control

All laboratory materials for PCB analysis were made either of glass or PTFE to avoid sample contamination or loss. Detection limits for PCB congeners ranged from 0.0008 to 0.33 ng/g. All values below detection limits were substituted with half of the detection limits. All the measured concentrations were corrected based on the recoveries of surrogates (Mono- to tetra-CBs: using PCB 30; penta- to deca-CBs: using PCB 204). The acceptable range of recovery was 50–125% (Hermanson and Johnson, 2007). Samples with surrogate recoveries less than 50% were not included in the final data analysis. Average surrogate recoveries were 72.6% (range: 55.2–92.8%) for PCB 30 and 87.0% (range: 50.3–105%) for PCB 204.

3.2.5 Extraction of DNA/RNA from surface sediments and bioretention media

Sediment samples were freeze-dried (FreeZone 6 PLUS, LABCONCO, U.S.) before DNA extraction. Sediment genomic DNA (gDNA) were extracted from 0.25 g of the dry sediment samples using a DNeasy PowerSoil Kit (Qiagen, U.S.). Sediment RNA were extracted using a ZymoBIOMICS TM DNA/RNA Miniprep Kit (Zymo Research, U.S.). Both gDNA and RNA were extracted according to the manufacturer's protocols, with minor modifications. During the process of RNA extraction, gDNA was removed via DNase I treatment. A260/280 and A260/230 were measured using a nanoDrop spectrophotometer (Nanodrop 2000, Thermo Scientific, U.S.) to check the quality of extracted DNA and RNA.

cDNA was synthesized ProtoScript II Reverse Transcriptase (New England Biolabs, U.S.) and random primers. Briefly, reaction mixture I was prepared using 60 µM Random Hexamers (2 µL; Invitrogen, U.S.), 10 mM dNTP mix (1.25 µL; Invitrogen, U.S.) and RNase-free water (3.75 µL; Zymo Research, U.S.). 3 µL of RNA sample was added to reaction mixture I and denatured at 70°C for 5 min. Reaction mixture II was prepared using 5X ProtoScript II Buffer (5 µL; New England Biolabs, U.S.), 0.1 M dithiothreitol (2.5 µL; New England Biolabs, U.S.), 20 U/µL SUPERase·In RNase Inhibitor (0.5 µL; Invitrogen, U.S.), 200 U/µL ProtoScript II Reverse Transcriptase (1.25 µL) and RNase-free water (5.75 µL). Denatured mixture I with RNA was mixed with 15 µL of Reaction mixture II. The new mixture was incubated at 42°C for 1 hr and then inactivated at 70°C for 20 min.

3.2.6 Detection of bacteria capable of PCB transformation

Universal primers 27F and 1392R were used to amplify the 16S rRNA genes of total bacteria (Marchesi et al., 1998). *Escherichia coli* DNA was used as the positive control. Primers 348F and 884R (Fagervold et al., 2005) were used to detect the putative OHR within the Chloroflexi in the samples, since all known bacteria capable of organohalide respiration are

found in this group. Primers 634F/799R (Yan et al., 2009), 1200F/1271R (He et al., 2003) and 477F/647R (Grostern and Edwards, 2006) were used to detect the presence of *Dehalogenimonas (DHG)*, *Dehalococcoides (DHC)* and *Dehalobacter (DHB)*, respectively. DNA samples extracted from *Dehalococcoides*-containing microbial consortium (SDC-9) and the West Branch Canal Creek Consortium (WBC-2) culture were used as positive controls for anaerobic OHR. The *bph*A gene was amplified using primer sets 463F and 674R (Petrić et al., 2011), which targets the gene encoding for a dioxygenase catalyzing the first step of PCB transformation. P42U-F and P43D-R primers were used for the detection of *bph*C genes (Erb and Wagner-Dobler, 1993). *Burkholderia xenovorans* LB400 were used as positive control for *bphA* and *bphC* genes. PCR products of the correct length were confirmed by electrophoresis using 1.5% agarose gel. Details about the applied primers are shown in Table 3.2.

The abundance of total bacteria and *Dehalogenimonas* were estimated using qPCR targeting the bacterial 16S rRNA gene (341F/907R and 634F/799R, respectively) (Yan et al., 2009, Kjellerup et al., 2012). PCR conditions are shown in Table 3-1. For total bacteria, the standard DNA template was PCR products from *E. coli* with primers 341F/907R. For *Dehalogenimonas*, the standard DNA template was PCR products from WBC2 with 463F/799R. The qPCR reactions were performed with CFX Connect Real-Time System (Bio-Rad, U.S.). With each primer set, no amplification of genes was observed in the negative control.

Table 3-2. Primers used for amplifying targeted sequences and cycling conditions.

Primer	Nucleotide Sequence (5'-3')	PCR fragment size (bp)	Cycling Conditions	Reference
27F	AGAGTTTGATCMTGGCTCAG	1380	5 min at 94°C, 30 s at 94°C, 60 s at 55°C, 2 min at 72°C, 29 cycles, 7 min at 72°C	(Marchesi et al., 1998)
1392R	ACGGGCGGTGTGTRC			
348F	GAGGCAGCAGCAAGGAA	554	2 min at 95°C, 45 s at 95°C, 45 s at 58°C, 60 s at 72°C, 40 cycles, 30 s at 72°C	(Kjellerup et al., 2012)
884R	GGCGGGACACTTAAAGCG			
463F	CGCGTSGMVACCTACAARG	234	15 min at 95°C, 30 s at 95°C, 60s at 60°C, 30 s at 72°C, 40 cycles, 10 min at 72°C	(Petrić et al., 2011)
674R	GGTACATGTCRCTGCAGAA YTGC			
P42D	CGCGGATCCGCGGGGCGC CACACCAATGACCA	168	3 min at 95°C, 30 s at 95°C, 60 s at 60°C, 3 min at 72°C, 35 cycles, 10 min at 72°C	(Erb and Wagner-Dobler, 1993)
P43U	CCCAAGCTTGGGACTTGT GGCCCCACATG			
477F	GATTGACGGTACCTAACGA GG	191	10 min at 94°C, 45 s at 94°C, 30 s at 63°C, 30 s at 72°C, 45 cycles, 10 min at 72°C	(Grostern and Edwards, 2006)
647R	TACAGTTTCCAATGCTTTAC G			
634F	GGTCATCTGATACTGTTGG ACTTGAGTATG	194	2 min at 94°C, 30 s at 94°C, 45s at 57°C, 10 s at 72°C, 40 cycles	(Yan et al., 2009)
799R	ACCCAGTGTTTAGGGCGTG GACTACCAGG			
1200F	CTGGAGCTAATCCCCAAAG CT	89	2 min at 50°C and 10 min at 95°C, 15 s at 95°C, 60s at 60°C, 40 cycles, 10 min at 72°C	(He et al., 2003)
1271R	CAACTTCATGCAGGCGGG			
341F	CCTACGGGAGGCAGCAG	586	5 min at 94°C, 60 s at 94°C, 60s at 60.2°C, 10 s at 72°C, 40 cycles	(Kjellerup et al., 2012)
907R	CCGTCAATTCMTTTGAGTTT			
V3V4 primer pair	TCGTCGGCAGCGTCAGATG TGTATAAGAGACAGCCTAC GGGNGGCWGCAG	~550	3 min at 95°C, 30 s at 95°C, 30s at 55°C, 30 s at 72°C, 25 cycles, 5 min at 72°C	(Klindworth et al., 2013)
	GTCTCGTGGGCTCGGAGAT GTGTATAAGAGACAGGACT ACHVGGGTATCTAATCC			

3.2.7 Analysis of microbial community

Microbial communities in surface sediment and bioretention media samples were studied using Illumina Mi-seq. For Illumina sequencing, primer sets targeting the 16S V3 and V4 region and putative anaerobic OHR within Chloroflexi (348F and 884R) were used to amplify the specific region of 16S rRNA genes, respectively (Table 3-2).

3.2.8 Bioinformatic analysis

Sequencing data were processed in the open source statistical and graphing software RStudio (rstudio.com). Low-quality reads were trimmed using a maximum EE value of 2 and the reads were truncated using dada2 and phyloseq R packages (McMurdie and Holmes, 2013), after which, the average quality score was above 20 for all samples. The reads were truncated at position 290 for forward reads and at position 256 for reverse reads for the sequencing results targeting putative OHR. For sequencing results targeting V3–V4 region, the reads were truncated at position 250 and 230, respectively. Sequence abundances in each OTU table were normalized by rarefaction based on the minimum read count per sample using the vegan R package.

3.2.9 Data analysis

For the comparison of two groups of data, F-tests were performed to test the equality of two variances and Student *t*-tests were performed to examine differences among different samples. Pearson correlation coefficients were calculated to study the relationship between two variables. All tests were performed in Microsoft Excel 2016.

3.3 Results and discussion

3.3.1 Spatial distribution of PCBs in bioretention media

The total PCB concentrations in the core samples ranged from 38.4 ± 2.3 ng/g at the surface of the bioretention entrance to 11.6 ± 1.2 ng/g at 20-cm deep and 3-m from the entrance (Figure 3-2). In order to protect aquatic biota, different degrees to which adverse biological effects are likely to occur as a result of exposure to PCBs in sediments were calculated. These guidelines can be used for stormwater sediments because they are similar environments with freshwater and sediments. PCB concentrations in all the core samples were below the consensus-based threshold effect concentration (TEC: 59.8 ng/g) and the probable effect levels (PEL: 277 ng/g). Concentrations below the TEC indicate that adverse effects are not expected to occur on sediment-dwelling organisms (MacDonald et al., 2000). However, samples collected at the 0–20 cm section at the entrance were above the interim sediment quality guidelines (ISQGs) concentration (34.1 ng/g) thus adverse biological effects could happen as a result of exposure to PCBs in this sample. PCBs in other core samples were below ISQGs. Additionally, PCB concentrations higher than 1000 mg/kg may cause toxic effects on bacterial communities (Passatore et al., 2014). All the concentrations found in the bioretention media were below this level, indicating that the presence of PCBs would not cause toxicity nor reduction of activity in bacterial communities in the bioretention cell.

Total PCB concentrations decreased as the depth increased ($p < 0.05$). Additionally, the farther away from the entrance (horizontally), the lower the measured concentrations were. This can be explained by PCBs sorbing onto the bioretention media when they make contact with the media, or are being filtered/settled out. The surface media near the entrance of the inlet received runoff more frequently compared to farther locations, especially during small storm events (< 0.25 cm), which produced little runoff. As a result, the surface media near the entrance had a

higher potential for adsorption of PCBs in runoff. A similar media profile was found in a study on PAHs in this same bioretention facility (DiBlasi et al., 2009). PAH retention mainly took place in the surficial soil (0–10 cm) through sorption and particulate capture. Heavy metal accumulations in the bioretention cell media show a similar trend (Jones and Davis, 2013). Metal accumulation was governed by the distance from the inflow point and was greater toward the inflow point. A top-heavy accumulation pattern was found for most metals, like lead, zinc and copper (Li and Davis, 2008, Jones and Davis, 2013).

In all the core samples, the concentrations of penta- through deca-CBs were higher than that of mono- through tetra-CBs. In the inlet entrance core sample, the concentration of penta- to deca-CBs was 1.5 times that of mono- to tetra-CBs. The higher concentration of highly chlorinated PCBs compared to lowly chlorinated ones indicated that anaerobic organohalide respiration was more likely to impact PCB concentrations in the core samples than aerobic degradation if favorable conditions would be present, such as anaerobic and reduced media environment. Higher concentrations of more chlorinated PCBs were also found in a previous study on surface sediment samples (Cao et al., 2019). The finding of higher concentration of penta- to deca-CBs than that of mono- to tetra-CBs was in accordance with Gilbreath and McKee (2015), where results showed that the hexa- or higher chlorinated congeners were transported in larger proportions during storm flows due to higher K_{ow} and as a result stronger associations with particles.

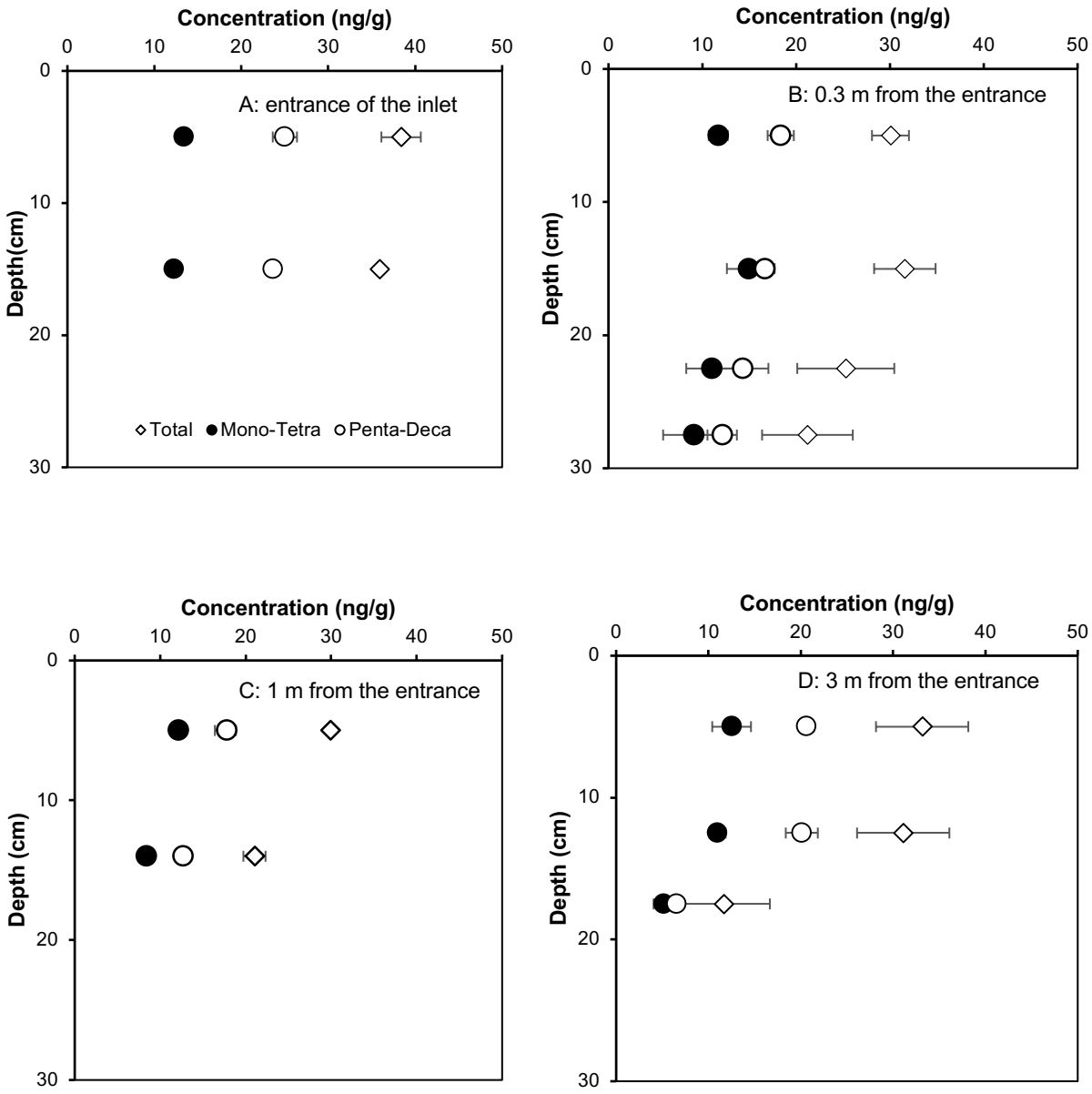


Figure 3-2. Concentrations of total PCBs, mono-to tetra-CBs, and penta-to deca-CBs in the bioretention media core samples at UMD [A: entrance of the inlet; B: 0.3 m from the entrance; C: 1 m from the entrance; D: 3 m from the entrance. Error bars represent the standard deviation from three samples (for samples from 0–10 cm at the entrance of inlet and 0–10 cm at 30 cm from the entrance, standard deviation was calculated from two samples)].

PCBs in all core samples followed a similar homolog distribution pattern, with di-, tetra-, penta-, and hexa-CBs as the predominant homolog groups (Figure 3-3). The high abundance of di-CBs was attributed to the presence of PCB 11 (3,3'-PCB). PCB 11 was detected in all the core samples with the highest concentration for individual congeners. The concentration of PCB 11 ranged from 0.49 to 5.29 ng/g. The relative abundance of PCB 11 in the core samples ranged from 4.2% to 15.0% of the total PCB concentrations. Prior study indicated the yellow roadway paints could be an active PCB 11 source (Cao et al., 2019). With vapor pressure higher than 10^{-4} mm Hg, PCB 11 can evaporate into the atmosphere (ATSDR, 2000). Thus, it is unlikely that PCB11 will persist in the environment at high concentrations.

Other dominant congeners found in surface stormwater sediments, including PCB 14, PCB 180, PCB 193, and PCB 194 (Cao et al., 2019), were also detected in the bioretention media at high concentrations. The relative abundance of these congeners ranged from 1.5–5.7%, 1.6–11.9%, 1.3–4.5%, and 1.9–3.7%, respectively. PCB 180 and PCB 194 accounted for 11.4% and 2.1% of the total PCBs in A1260 (Frame et al., 1996), respectively, indicating that A1260 could be one of the sources of PCBs in stormwater at this location. Additionally, PCB 70, which was present in A1242, A1248 and A1254, was also detected in the bioretention media (1.2–3.6%). The congener profile in the bioretention media suggested that PCB contamination in stormwater runoff came from a mixture of different sources.

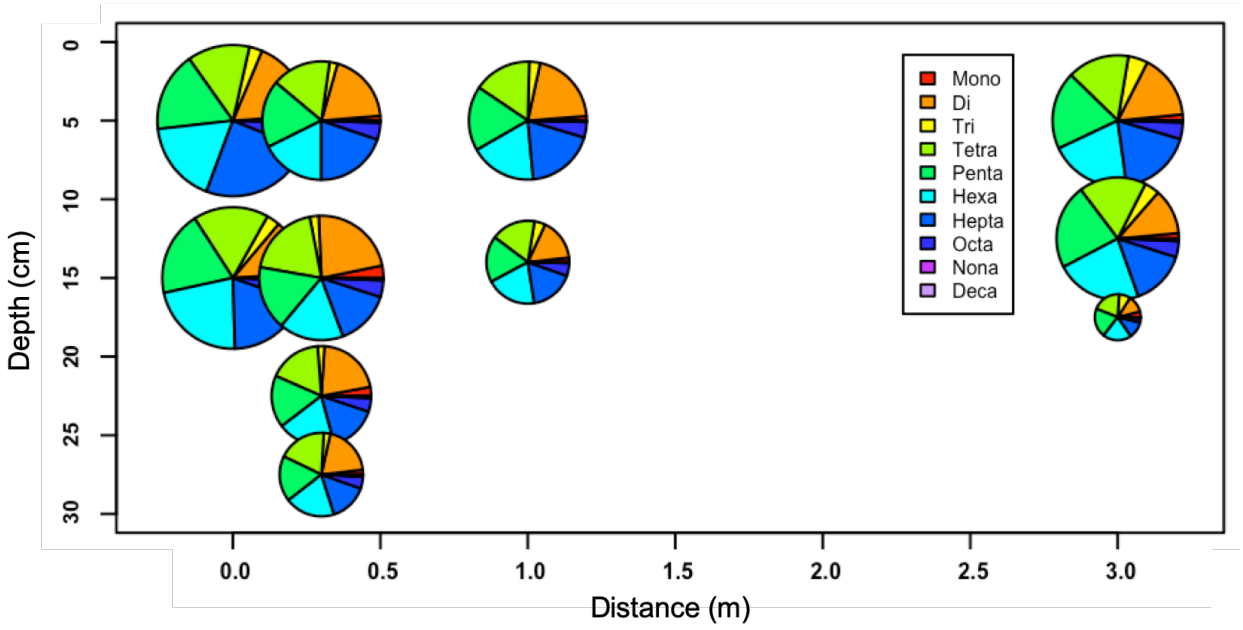


Figure 3-3. Relative abundance of homolog groups in the core samples from the bioretention cell. The size of the pies is proportional to the concentration in each sample (11.6–38.4 ng/g).

The number of chlorines per biphenyl in the core samples were similar, ranging from 4.13 to 4.75 with an average of 4.41 ± 0.24 (Table 3-3). No trend could be found between chlorine numbers and depth or distance from the entrance. This indicated that the mobility of PCBs in the bioretention media was not related to the number of chlorines.

The level of unflanked *ortho*-chlorinated PCBs, which is an indicator of organohalide respiration, ranged from 1.7 ± 0.03% to 2.7 ± 0.34%. The preferred loss of chlorines in the *meta* and *para* positions result in an increase in the level of unflanked *ortho*-chlorinated PCBs (EPA, 2004). As a result, a high level of unflanked *ortho*-chlorinated PCBs indicates the presence of anaerobic organohalide respiration. The level of *ortho*-chlorinated PCBs found in the core samples were significantly higher ($p < 0.001$) than the values found in accumulated stormwater

sediment samples at a dense urban area, where the level was 0.50% (Cao et al., 2019). The fraction of unflanked *ortho*-chlorinated PCBs in Aroclor mixtures ranged from 0.2% to 2.4% (ATSDR, 2000). Five of the eleven core samples had a higher level of *ortho*-chlorinated congeners than the maximum value found in Aroclor mixtures (2.4% in A1016). The fraction of chlorines at different positions was also calculated and the results showed an increase of chlorine at the *ortho*-position (Figure 3-4). Both of these measures suggest that some amount of organohalide respiration took place in the bioretention media.

Table 3-3. Number of chlorines per biphenyl, concentrations of dioxin-like congeners, TEQ_{PCB} and level (mol%) of *ortho*-chlorinated PCBs in the bioretention core samples. *Standard deviation is from three (a) or two (b) samples.

	Cl per biphenyl	**Dioxin-like PCBs (ng/g)**	**TEQ_{PCB} (pg/g)**	**Mol% of *ortho*-chlorinated PCBs**
Entrance 0–10 cm deep	4.65 ± 0.02 [b]	1.92 ± 0.05 [b]	6.03 ± 0.73 [b]	2.23 ± 0.18 [b]
Entrance 10–20 cm deep	4.75	1.75	7.12	2.03
0.3 m from Entrance 0–10 cm deep	4.47 ± 0.10 [a]	1.49 ± 0.10 [a]	4.85 ± 0.47 [a]	2.51 ± 0.20 [a]
0.3 m from Entrance 10–20 cm deep	4.13 ± 0.18 [a]	1.29 ± 0.09 [a]	5.55 ± 0.36 [a]	3.19 ± 2.71 [a]
0.3 m from Entrance 20–35 cm deep	4.25 ± 0.13 [a]	1.15 ± 0.27 [a]	4.87 ± 1.20 [a]	2.21 ± 0.10 [a]
0.3 m from Entrance 25–30 cm deep	4.37 ± 0.23 [a]	0.90 ± 0.14 [a]	4.16 ± 1.22 [a]	2.73 ± 0.34 [a]
1 m from Entrance 0–10 cm deep	4.39 ± 0.19 [b]	1.48 ± 0.08 [b]	4.90 ± 0.71 [b]	2.60 ± 0.07 [b]
1 m from Entrance 10–18 cm deep	4.48 ± 0.24 [a]	1.01 ± 0.04 [a]	4.64 ± 0.22 [a]	2.17 ± 0.34 [a]
3 m from Entrance 0–10 cm deep	4.52 ± 0.10 [a]	1.79 ± 0.10 [a]	5.84 ± 0.20 [a]	2.38 ± 0.56 [a]
3 m from Entrance 10–15 cm deep	4.64 ± 0.07 [a]	1.78 ± 0.19 [a]	6.57 ± 0.58 [a]	1.70 ± 0.07 [a]
3 m from Entrance 15–20 cm deep	4.30 ± 0.18 [a]	0.52 ± 0.03 [a]	2.23 ± 0.54 [a]	2.28 ± 0.33 [a]

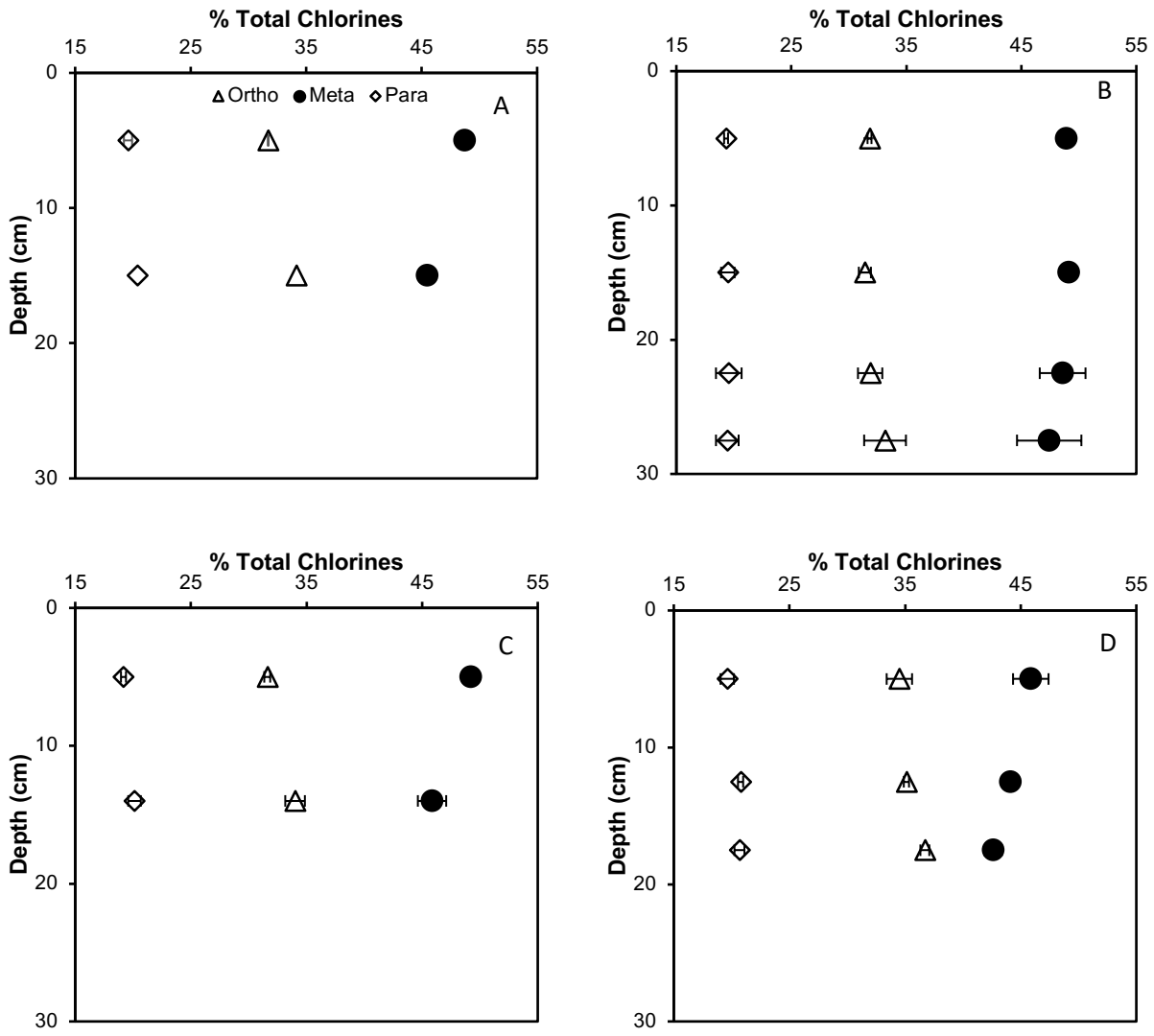


Figure 3-4. Fraction of *ortho*-, *meta*-, *para*-chlorines in PCBs present in core samples from the UMD bioretention cell. [A: entrance of the inlet; B: 30 cm from the entrance; C: 1 m from the entrance; D: 3 m from the entrance. Error bars represent the standard deviation from three samples (for samples from 0–10 cm at the entrance of inlet and 0–10 cm at 30 cm from the entrance, standard deviation was calculated from two samples)].

Dioxin-like PCBs (PCBs 77, 81, 126, 169,105,114, 118, 123, 156, 189) can exert toxic responses similar to those caused by 2,3,7,8-tetrachlorobenzodioxin (Van den Berg et al., 1998).

Due to their toxicity, the concentration of dioxin-like PCBs is of special concern. Toxic equivalency values (TEQs) were calculated to evaluate the toxicity of dioxin-like PCBs in the bioretention media. The TEQs ranged from 2.23 ± 0.54 to 6.57 ± 0.58 pg/g TEQ_{PCB} (Table 3-3), which were in the range of the TEQs found in Northwest Mediterranean sediment (0.03–24.8 pg TEQ/g) (Eljarrat et al., 2001). The lowest TEQ was found in the 20–30 cm deep sample at 3 m from the entrance. A Pearson correlation coefficient was calculated at -0.47, indicating no linear relationship could be found in TEQs regarding the depth and distance of the core samples. All the core samples were below the safe sediment value of 20 pg TEQ/g (Eljarrat et al., 2001). This finding indicated that the bioretention media samples at different locations of the bioretention were safe when only considering the dioxin-like congeners.

3.3.2 Presence and abundance of bacteria capable of PCB transformation

Quantitative PCR (Q-PCR) results showed that the gene copy number of total bacteria ranged from $3.23 \times 10^6 \pm 1.71 \times 10^5$ to $2.92 \times 10^9 \pm 1.78 \times 10^8$ copies g^{-1} sediment in the bioretention core samples (Figure 3-5). The gene copy number is approximate to the number of bacteria because the number of gene copies for each bacterium varies (Větrovský and Baldrian, 2013). The gene copy number decreased when the soil depth increased, especially for core samples at 2 m and 3 m from the inlet entrance. This might be related to the higher abundance of nutrients and oxygen level in the top layer. The total carbon content decreased from 4.5% to 1.5% in the top 30 cm and was stable at 1.5% at deeper layers. A similar trend was observed for total nitrogen in a previous study of the bioretention cell (Li and Davis, 2014). More bacteria as well as microbial activity found at the surface layer may be due to the higher abundance of nutrients like oxygen, nitrogen and phosphorus in the upper layers of the media.

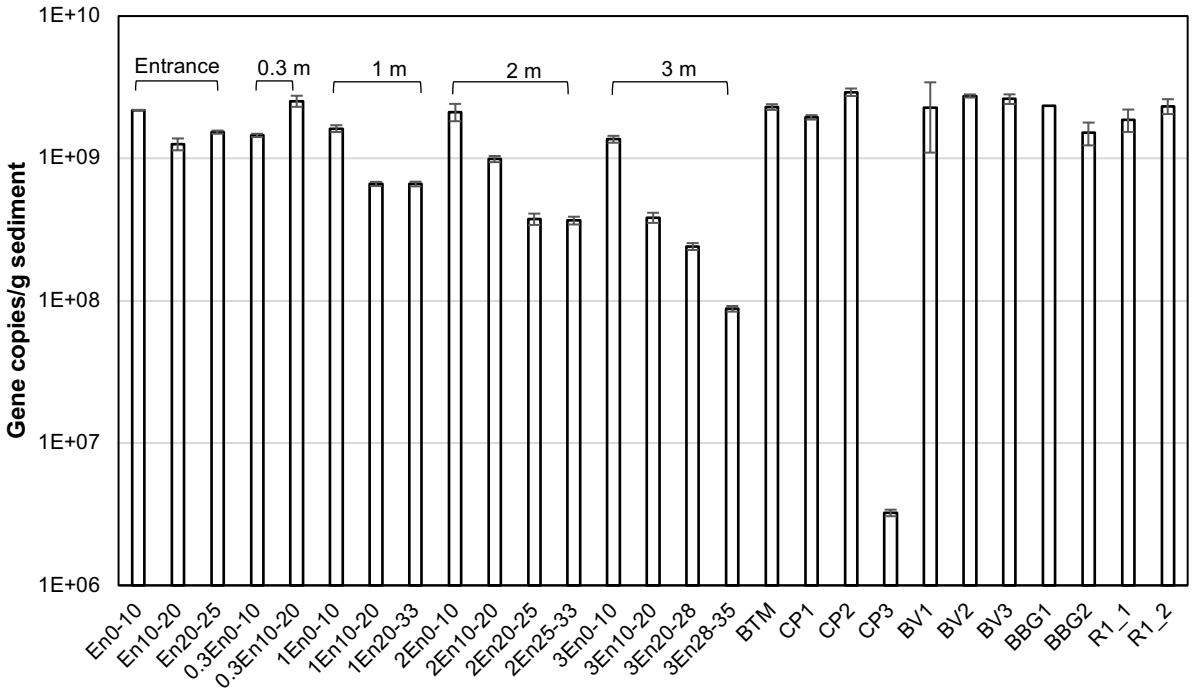


Figure 3-5. Quantitative assessment of the abundance of total bacterial 16S rRNA genes in the core samples as well as surface sediment samples. Error bars represent standard deviation (n=3).

PCR results showed that bacteria were present in all the sediment samples and bioretention core samples (Table 3-4). OHRs and sub-group of OHRs like *Dehalococcoides, Dehalobacter* and *Dehalogenimonas* were also detected. The functional genes encoding *bph*A and *bph*C also showed positive results in some of the stormwater sediments and bioretention cell.

Table 3-4. Presence of aerobic PCB degrading bacteria, putative OHR, and sub-group of OHR. (+ means presence of the target bacteria; - means absence of the target bacteria; blank means not analyzed).

Sites		**PCB-degrading bacteria (*bph*A gene)**	**PCB-degrading bacteria (*bph*C gene)**	**Putative organohalide respiring bacteria**	**DHG**	**DHB**	**DHC**
BBG1		+	+	+	+	-	-
BBG2		+	+	+	+	-	+
BTM		+	+		+	-	-
BV1		+	(+)		+	-	-
BV2		+	+		+	-	+
BV3		+	+		+	+	+
CP1		+	+		+	-	(-)
CP2		+	-		+	-	+
CP3		+	+		+	-	-
R1		+	+	+	+	-	+
R2		+	+		+	+	+
Distance/ m	**Depth/ cm**						
0	0–10	-	+	+	+	+	-
	10–20	+	+	+	+	-	-
	20–25	+	+	+	+	+	-
0.3	0–10	+	+	+	+	-	+
	10–20	-	+	+	+	-	-
1	0–10	+	+	+	+	-	+
	10–20	-	(-)	+	+	-	+
	20–33	-	+	+	+	-	-
2	0–10	-	+	+	+	-	-
	10–20	-	(-)	+	+	-	+
	20–28	+	+	+	+	+	+
	28–33		+	+	+	+	+
3	0–10	+	-	+	+	+	+
	10–20	+	-	+	+	-	+
	20–28	-	+	+	+	+	+
	28–35	-	+	+	+	-	+

Putative OHR within Chloroflexi were present in all the bioretention core samples and three of the surface stormwater sediment samples, indicating the potential for organohalide respiration in these samples. The high detection frequency in the bioretention media samples could be related to anaerobic microsites inside soil particles and/or brief inundation periods following each storm event. Saturation could reduce the redox potential in bioretention cells to -100 mv as it was observed in two raingardens in Haddam, CT (Dietz and Clausen, 2006). Anaerobic activities like denitrification were shown to occur during such conditions (Waller et al., 2018). Additionally, higher abundance of denitrifying bacteria was observed in the top media than deeper layers in SCMs, indicating the presence of anoxic microenvironment in the upper layers (Willard et al., 2017, Chen et al., 2019). These findings suggest that a favorable condition for organohalide respiration in bioretention cells could be present. Putative OHR were also detected in ten of the nineteen soil samples collected in a stormwater drainage ditch in 2012 in Mechanicsburg, PA, at 5×10^3 to 5×10^6 bacteria g^{-1} soil (Kjellerup et al., 2012). The presence of putative OHR within Chloroflexi in different environments indicated the potential of organohalide respiration in stormwater drainage and river sediments.

For aerobic degradation, PCR using the *bph*A genes as a target showed positive results in all the surface sediment samples and seven of the bioretention core samples (Table 3-4). The detection rate of *bph*C (81%) was lower compared to *bph*A (100%) for the surface sediment samples. However, *bph*C (75%) was more frequently detected in the core samples than *bph*A (44%). Aerobic PCB degraders were also present in half of the soil samples from the stormwater drainage ditch mentioned above (Kjellerup et al., 2012), which means such bacteria are widely distributed in natural environments. *bph*A was also found in the sediment samples from Indiana Harbor and Ship Canal (IHSC) and a correlation between *bph*A gene abundance and PCB

concentration was observed (Liang et al., 2014). Therefore, the occurrence of *bph*A in different natural environments could be a microbial response to PCB contamination and indicate the presence of potential of *in situ* hydroxylation on the biphenyl rings. *bph*C genes were also detected in PCB-contaminated soil from a long-term electronic waste recycling area in Taizhou, China (Hu et al., 2016). The presence of both *bph*A and *bph*C genes suggested that aerobic PCB degradation occurred in these samples, which could lead to complete mineralization.

Eight of 27 samples contained both putative OHR Chloroflexi and *bph*A/*bph*C. The co-occurrence in these samples indicated that bacteria capable of simultaneous aerobic and anaerobic PCB transformation were present. For example, during a storm event, the media is saturated and an anaerobic environment exists. In this condition, anaerobic organohalide respiration could take place and the number of chlorines on the PCB molecules would be reduced. When the water is drained and the environment subsequently becomes aerobic, the presence of *bph*A/*bph*C genes indicated a potential of biphenyl ring deoxygenation and/or ring cleavage. The presence of bacteria capable of PCB transformation indicates that a microbial response to PCB contamination would be a solution for bioretention cells in the long term, including organohalide respiration of highly chlorinated congeners and subsequent aerobic degradation of low chlorinated congeners.

The samples were further tested for OHR at the genus level with selected targets capable of organohalide respiration. *Dehalobacter, Dehalogenimona* and *Dehalococcoides* have been reported to each contain 10–36 reductive dehalogenase (rdh) genes (McMurdie et al., 2009, Maphosa et al., 2012, Siddaramappa et al., 2012) and are all capable of organohalide respiration. *Dehalococcoides* spp. are abundantly involved in organohalide respiration (Yoshida et al., 2007) and it was reported that they altogether can respire with 64 tetra- to nona-PCBs in A1260

(Bedard et al., 2007). *Dehalococcoides* strains were detected in six of the eleven surface samples and ten of the sixteen core samples. Yan et al. (2009) also detected *Dehalococcoides* in two of the eight contaminated groundwater samples from the PetroProcessors of Louisiana, Inc. Superfund site. The presence of this genus is crucial in the organohalide respiration of PCBs in various environments.

Dehalobacter strains have been detected in one of five soil samples contaminated with the aliphatic organohalide tetrachloroethene (PCE) (Yoshida et al., 2007). *Dehalobacter* spp. could be responsible for PCB dechlorination (Wang and He, 2013) even though *Dehalobacter* had the lowest detection frequency in this study (two surface sediment and six bioretention core samples, Table 3-4). *Dehalogenimonas* were detected in all the collected surface sediment and core samples. A high detection frequency was also observed in Yan et al. (2009), where *Dehalogenimonas* strains were detected in all the eight groundwater samples tested in this study. *Dehalogenimonas* can respire using a variety of polychlorinated alkanes (Moe et al., 2009). The presence of such genera in different environments is a likely result of microbial response to PCB contamination and suggest that organohalide respiration is occurring in stormwater sediments and bioretention media.

The abundance of *Dehalogenimonas* from different samples showed that higher levels were found in surface sediment than the core samples (Figure 3-6). Overall, *Dehalogenimonas* abundance was approximately six orders of magnitude lower than the presence of total bacteria, indicating the low abundance of *Dehalogenimonas* among all the bacteria in the bioretention media. Low relative abundance of *Dehalogenimonas* is commonly found in natural environments. In groundwater samples collected at the PetroPerocessors of Louisiana, Inc. Superfund site, abundance of *Dehalogenimonas* represented 0.0014–9.2% of total bacteria (Yan

et al., 2009). However, relative abundance of *Dehalogenimonas* of total bacteria could reach 16–30% in sediment mesocosms (Qiao et al., 2018). Additionally, the relative abundance of *Dehalogenimonas* was not related to PCB concentration in the cores (Pearson correlation: -0.16). These results indicate that *Dehalogenimonas* were not the major bacteria performing organohalide respiration in either surface sediments or bioretention media or the PCB concentrations were higher than what *Dehalogenimonas* need for metabolism. The presence of all these genes indicate a potential of both organohalide respiration and aerobic degradation on PCBs in stormwater sediments as well as bioretention cells.

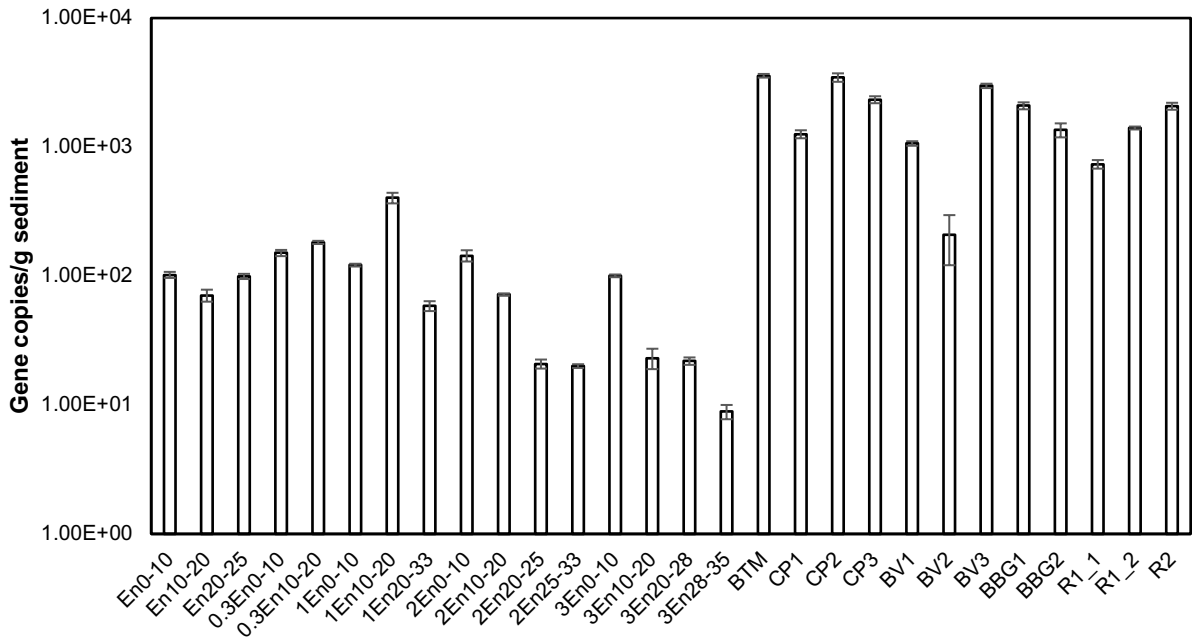


Figure 3-6. Abundance of *Dehalogenimonas* in the bioretention core and surface sediment samples. All data points are averaged triplicate tests. Error bars represent standard deviation from three samples.

3.3.3 Microbial communities in surface and sediment samples

A total of 6448 bacterial operational taxonomic units (OTUs) and 526 bacterial OTUs within Chloroflexi were distributed across all the samples. A way to estimate the number of species based on sampling effort is rarefaction analysis (Dodds and Whiles, 2020). The plateaus in all the rarefaction curves indicate that the majority of the resident phylotypes were collected in these samples (Figure 3-7).

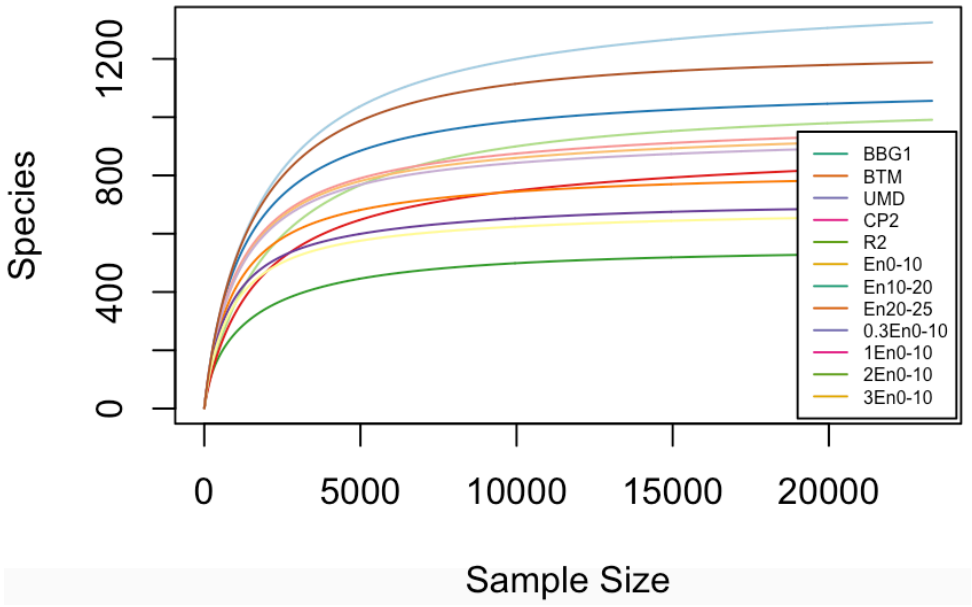


Figure 3-7. Rarefaction curves for sediment samples from different sites.

Among the 40 phylogenetic groups, the most dominant bacterial phyla were Actinobacteria (20.2–34.6%), Proteobacteria (13.6–47.9%), Chloroflexi (0.3–18.4%), Cyanobacteria (0.3–38.3%) and Acidobacteria (1.2–15.6%) (Figure 3-8). The predominance of Chloroflexi and Proteobacteria was observed in the acclimated raw sludge during the anaerobic

degradation of 2,4,6-trichlorophenol (Song et al., 2019). Some bacteria belonging to Chloroflexi are known to be obligate OHR (Dam et al., 2019).

Looking at the relative abundance of OTUs based on primers for putative OHRs at class level in the bioretention cores and surface sediment samples, Chloroflexia (0–63.9%) and Ktedonobacteria (0–70.5%) were the most dominant classes (Figure 3-9). The class Dehalococcoidia, to which several genera capable of organohalide respiration belong, was detected in three of the six core samples and three of the eight surface sediment samples. The relative abundance of Dehalococcoidia ranged from 3.78–27.9% in these samples. The presence of Dehalococcoidia further demonstrated the potential of organohalide respiration in these samples.

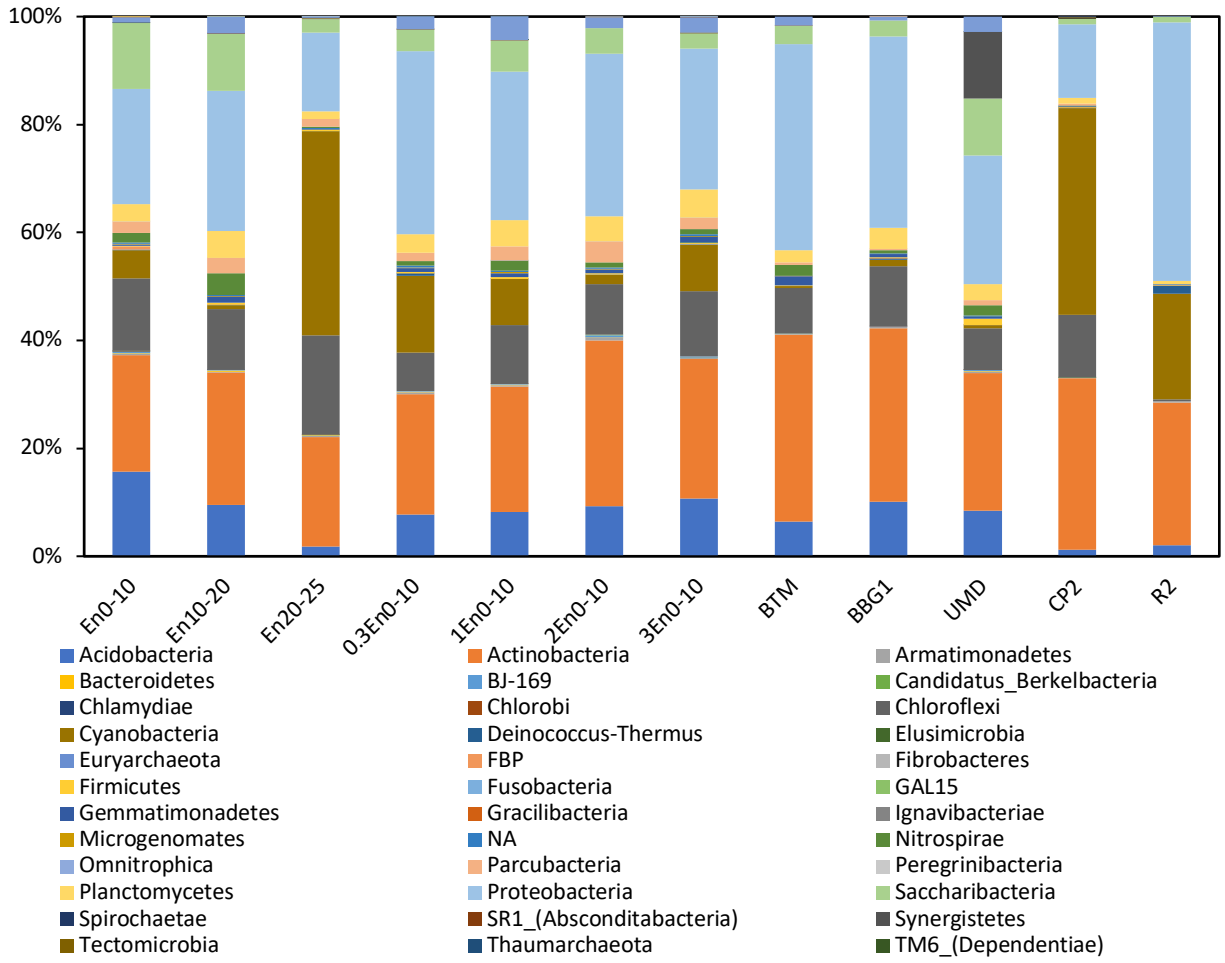


Figure 3-8. Relative abundance of bacterial OTUs at the phylum level in core samples and surface samples.

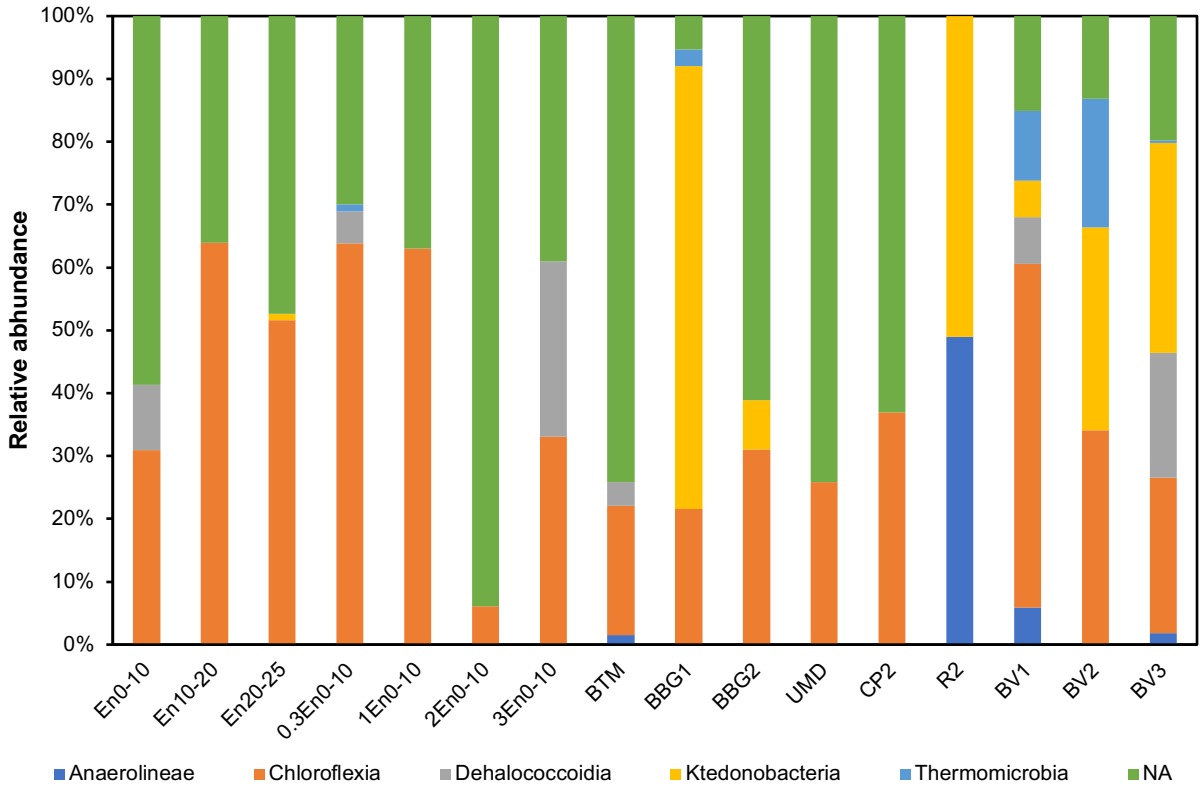


Figure 3-9. Relative abundance of bacterial OTUs at class level of a monophyletic group within Chloroflexi at different sites.

At genus level, *Dehalococcoides* was found at BV1, BV3 and 0–10 cm at the bioretention entrance. *Dehalococcoides mccartyi* was detected at BV3. This species performs organohalide respiration of Aroclor 1248, Aroclor 1254 and Aroclor 1260 (Adrian et al., 2009, Kaya et al., 2016). Top layer core samples at the inlet, 0.3 m and 3 m from the entrance, and surface sediment sample at BTM contained *Dehalobium* species. *Dehalobium chlorocoercia* DF-1 was isolated from a tidal estuary of Charleston Harbor and is utilizing 2,3,4,5-tetrachlorinated biphenyl (PCB 61) as the sole electron acceptor (Wu et al., 2000). *Dehalogenimonas* was detected at site BV3. *Dehalobacter* was not detected in any of the samples. The detection rates of

Dehalococcoides, *Dehalogenimonas* and *Dehalobacter* were lower compared to PCR results, suggesting the low abundance of these anaerobic bacteria. The presence of different OHRs are prerequisite for organohalide respiration and more evidence on the activity of such OHRs are needed.

In addition to the anaerobic OHR, bacteria potentially capable of aerobic degradation of PCBs were also explored. *Rhodococcus* was present in all the samples with a relative abundance ranging from 0.19% to 1.36% of total OTUs (Figure 3-10). *Rhodococcus* is an important genus because some strains were reported to degrade 45 PCB congeners from PCB mixture with mono- to octa-CBs (Seto et al., 1995). The presence of *Rhodococcus* in the samples suggested a potential for PCB degradation via aerobic pathways.

Sequencing data indicated that Chloroflexi was an important component of the microbial communities in both surface sediment samples and bioretention core samples. Within the phylum Chloroflexi, bacteria capable of organohalide respiration such as *Dehalogenimonas*, *Dehalococcoides*, and *Dehalobium* exist. Bacteria capable of PCB degradation under aerobic conditions such as *Rhodococcus* were also detected in the stormwater sediments and bioretention cores. The presence of such microorganisms capable of PCB transformation is a prerequisite for PCB transformation. However, it is still not clear if the microorganisms are actually biotransforming PCBs. Examination RNA communities to test the activity of these microorganisms is still needed.

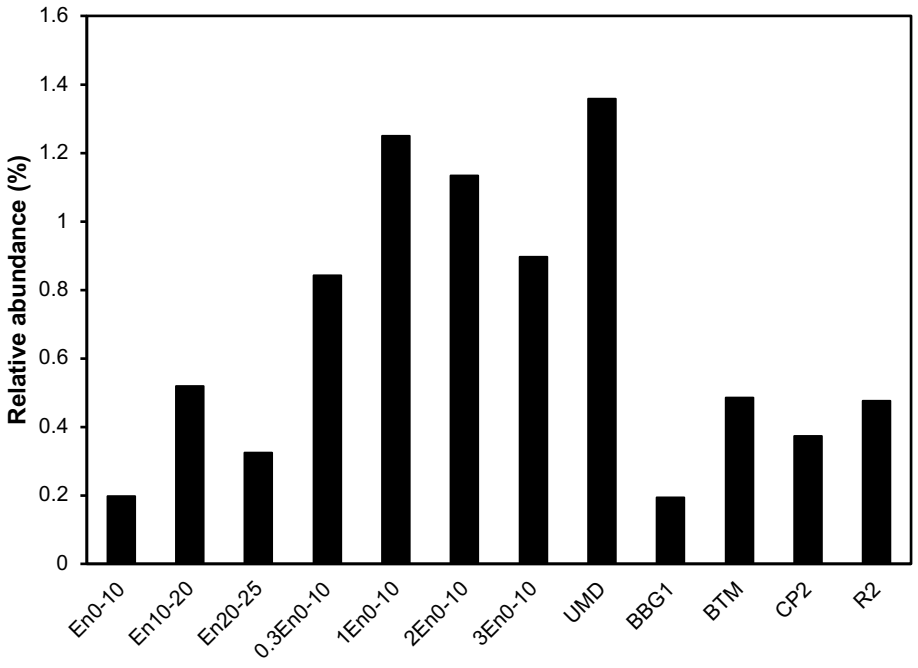


Figure 3-10. Relative abundance of bacterial OTUs belong to *Rhodococcus* genus.

3.3.4 Transcription of PCB degrading bacteria

Transcriptomic analyses of *bph*A and *bph*C functional genes, *Dehalogenimonas* as well as putative OHR were performed (Table 3-5). This was evaluation (the first of its kind to our knowledge) was performed to verify that expression of genes involved in PCB transformation occurred. Different from PCR results based on DNA, only one of the five samples was detected with *bph*A genes and three with *bph*C genes. All the samples were tested positive for putative OHR and subgroup *Dehalogenimonas*. Difference in microbial community as well as functional gene abundance between DNA and RNA were also observed in urban green infrastructure bioswale soils in New York City (Gill et al., 2017). The difference between DNA and RNA based results indicated that the presence of target sequence in DNA does not denote activity of the microorganisms. On the contrary, a positive result from RNA indicates that such genes were expressed and target microbial activity could be expected. Thus, the presence of transcripts is

more direct evidence than DNA when studying the activities in the microbial community (Gill et al., 2017). Based on the transcriptomic results, the presence of putative OHR and *bph*A/*bph*C genes showed that these bacteria were active at different locations in the bioretention cell at the time of sample collection. Thus, organohalide respiration and aerobic degradation were taking place at different locations in the bioretention.

Table 3-5. Transcripts confirmed from PCB transforming bacteria.

Sites	**PCB-degrading bacteria (*bph*A gene)**	**PCB-degrading bacteria (*bph*C gene)**	**Putative anaerobic dechlorinating bacteria**	***Dehalogenimonas***
UMD	-	-	+	+
En0–10	-	-	+	+
0.3En0–10	-	+	+	+
1En0–10	+	+	+	+
3En0–10	-	+	+	+

3.3.5 Potential of *in situ* biotransformation

Although the presence and activity of organohalide respiration and aerobic PCB degradation were found in the stormwater sediments, the feasibility of *in situ* biotransformation is still unknown. The study on the *in situ* biotransformation of PCBs was scarce. Loss of about 25% of the PCB mass was observed after 360 days in the nonbioaugmented treatment with PCB impacted sediment from Baltimore Harbor (Payne et al., 2013). The loss could be attributed to biostimulation of native microorganism by medium and/or abiotic factors such as volatilization. This indicated that with presence and activity of microorganisms capable of PCB transformation, PCB mass loss is expected in the long term. An 80% decrease by mass of PCBs was observed in

the mesocosm bioaugmented with both anaerobic *Dehalobium chlorocoercia* DF1 and aerobic *Burkholderia xenovorans* LB 400 (Payne et al., 2013). In this study, microorganisms capable of PCB transformation under both aerobic and anaerobic conditions were found in stormwater sediments and bioretention cells. The co-existence of both microorganisms could be an effective strategy to reduce PCB levels.

Organohalide respiring rate of PCBs is positively related to cell density of the OHR before it reaches the limiting point (Needham et al., 2019). However, the primary rate-limiting factor for PCB transformation in the environment is the low native abundance of microorganisms capable of PCB transformation in sediments (Needham et al., 2019). Thus, an *in situ* treatment of bioaugmentation is needed to enhance PCB transformation in natural environments such as bioretention cells (Payne et al., 2011).

3.3.6 Bioretention design and maintenance recommendations

The decreasing trend in PCB concentration found in the bioretention core suggests that bioretention is efficient in retaining PCBs from stormwater. Higher PCB concentrations found in the surface layers indicates that a shallow cell design might be enough for PCB removal. In addition, in order to enhance performance and reduce costs, only the top layer of the existing bioretention media needs to be replaced. Brown and Hunt (2012) repaired two sets of bioretention cells by excavating the top 75 mm of media and both surface storage volume and infiltration rate were increased significantly. PCB concentration in the bioretention media as well as effluent samples should be inspected at least once a year to decide whether maintenance is needed for the bioretention. The vertical profile of PCBs in the media core could also suggest the depth of media needs to be excavated.

3.4 Conclusions

PCBs were detected in the bioretention media core samples. Total PCB concentrations tended to decrease with increasing depth. Lower concentrations of total PCBs were found in media more distant along the flow path, indicating that PCBs were retained in the bioretention media when entering the bioretention cell. Bioretention is efficient in retaining PCBs from stormwater and a shallow cell design might be enough for PCB removal. Besides, when maintaining the existing bioretention, only the top layer of bioretention media needs to be replaced. The higher level of *ortho*-chlorinated biphenyls found in some of the bioretention core samples compared to Aroclors indicated the presence of organohalide respiration.

Bacteria capable of PCB transformation via both aerobic and anaerobic pathways were detected in the DNA extracted from the surface sediment samples as well as core samples. Core samples had a higher detection rate of putative OHR than the surface sediment samples. This might be due to the potential anaerobic conditions in the bioretention cell when it was saturated. In some samples, both the putative OHR and functional genes encoding enzymes for biphenyl degradation were found, which indicated the co-occurrence of simultaneous aerobic and anaerobic PCB respiration. Anaerobic respiration usually takes longer time because of the lower activity of anaerobic bacteria. The lag phase for organohalide respiration ranged from less than 10 days to 200 days (Adrian et al., 2009, Kjellerup et al., 2012). The benefit of using bioretention to treat PCBs in stormwater is that the bioretention media allows the cell to hold as much water as possible, including occasional ponding of water (Davis et al., 2009). This saturation can create anaerobic conditions and longer retention for anaerobic respiration to take place. During the process, some more-chlorinated PCBs become less-chlorinated PCBs ($Cl \leq 4$) and they are ready to be degraded when the media becomes aerobic. The higher level of unflanked *ortho*-

chlorinated congeners found in some bioretention media samples as well as vertical profile of different chlorines showed evidences of organohalide respiration.

Similar to natural soil samples, Acidobacteria, Proteobacteria, and Chloroflexi were the most abundant phyla in the stormwater sediment and bioretention media. Within Chloroflexi, genera capable of organohalide respiration like *Dehalococcoides*, *Dehalogenimonas* and *Dehalobium* were detected. The genus of *Rhodococcus*, which is capable of PCB degradation, was also detected in all the tested samples. RNA based PCR results also showed positive signal for putative OHRs and PCB degraders. For PCB degraders, the detection frequency from RNA based results was lower than DNA based results. This indicates that OHRs and PCB degraders are active in the media. DNA results show information about total bacterial gene while RNA results represent expressed genes. RNA is probably more indicative when information about bacterial activity is needed. Both the DNA and RNA based results indicate potential of PCB biotransformation in stormwater sediments and bioretention cell. However, due to the low abundance of bacteria capable of PCB transformation, the organohalide respiring rate and degradation rate could be very low in these environments. Thus, *in situ* bioaugmentation is needed to enhance the treatment of PCB impacted bioretention media.

Chapter 4. PCBs in dissolved and particulate phase of urban stormwater before and after bioretention treatment

Abstract

Despite their ban more than four decades ago, PCBs are still present in the environment and urban stormwater runoff is considered an important source of PCBs to aquatic environments. In this study, the presence of PCBs in the dissolved phase and associated with particles, respectively, in stormwater influent and effluent samples from a bioretention cell was studied. The stormwater quality varied between events depending on various factors like dry period, season and amount of rainfall. In the influent samples, total PCB concentrations ranged from 67 ± 17 to 755 ± 23 ng/L and the concentration decreased by 64–92% after bioretention treatment to 18.0 ± 4.8–57.8 ± 9.1 ng/L. The particulate phase in stormwater influent contained PCB concentrations at an average of 321 ng/g. A non-Aroclor PCB congener, 3, 3′-dichlorobiphenyl (PCB 11), was detected in all the stormwater influent samples. Particle-bound PCBs contributed to 2.6–17.1% of PCB mass from both the aqueous phase and solid phase. This indicates that PCBs in liquid phase are also important and cannot be overlooked. The results of this study showed that bioretention cells can be efficient in removing total suspended solids and PCBs from stormwater runoff. The estimated PCB load reduction ranged from 91.5% to 98.4% via bioretention treatment. This study indicates that bioretention cells are important infrastructures in order to meet PCB total maximum daily loads in different watersheds by removing PCBs from stormwater.

4.1 Introduction

Polychlorinated biphenyls (PCBs) are a group of chlorinated organic compounds widely produced in the U.S. during 1920s and 1970s. PCBs are toxic and can cause cancer and adverse skin and liver effects in humans (ATSDR, 2000). In 1979, the U.S. Environmental Protection Agency (EPA) issued final regulations banning the manufacture of PCBs and "non-closed" (open to the environment) uses of PCBs were prohibited (EPA, 1979). Aroclor is the trade name for commercial PCB mixtures that were manufactured in the U.S and in total 16 commercial Aroclors were manufactured with different physico-chemical properties (EPA, 2003).

PCB burdens in water bodies can be problematic to human and aquatic life. In the U.S. total maximum daily loads (TMDLs) are of specific importance in implementing state water quality standards. A TMDL establishes the maximum amount of an impairing substance or stressor that a waterbody can assimilate and still meet Water Quality Standards (WQS), and allocates that load among pollution contributors (MDE). PCB TMDLs have been approved in some watersheds in Maryland (MDE, 2020). Urban runoff or stormwater drains are important sources of pollutants, including PCBs, to impaired rivers, streams, lakes, reservoirs and ponds, accounting for 13%–19% of impaired waters (EPA, 2012). PCBs exist in urban stormwater as dissolved and affiliated with particulate phases (Granier et al., 1990, Hwang and Foster, 2008, Zgheib et al., 2011b, Cao et al., 2019). To address PCB contamination of stormwater, it is important to understand and minimize urban sources of PCBs and treat PCBs that are present in urban stormwater.

Bioretention, also called rain gardens, is a low impact development (LID) technology for treatment of stormwater (Davis, 2007). Bioretention cells often consist of a layer of hardwood

mulch and porous soil-based media with vegetation on top (Li and Davis, 2008, Li and Davis, 2014). Runoff from impervious areas flow into the bioretention cells and filter through the media. During the treatment process, hydrophobic pollutants like PCBs are retained in the bioretention media. PCBs are removed via particulate matter removal processes such as sedimentation and filtration in stormwater due to the high affinity of PCBs for adsorption to particulate matter (log K_{ow}: 4.50–8.26) (Hermanson and Johnson, 2007). Bioretention systems in Daly City, California were effective in reducing PCB concentrations by 44% (from 730 pg/L to 410 pg/L) (David et al., 2015). Additionally, removal of polycyclic aromatic hydrocarbons (PAHs) from stormwater has been documented with reduction rates ranging from 31% to 99% (DiBlasi et al., 2009). Due to similarities between the chemical characteristics of some PCBs and PAHs, PCBs with K_{ow} values in the same range as PAHs are also expected to be retained in the bioretention media.

In addition to infiltration and retention, biotransformation reactions can occur in bioretention cells (Davis et al., 2009). In an urban bioswale in New York, U.S., potential of aerobic biodegradation of PCBs was observed (Grill et al., 2017). Here, *bph*A genes, which catalyze the incorporation of two hydroxyl groups into the biphenyl ring, were detected in extracted DNA, and additionally expression of these genes was detected indicating active PCB biodegradation (Gill et al., 2017). Evidence of biotransformation of PAHs in bioretention was reported in both laboratory studies and field studies. In a column study mimicking a bioretention cell, naphthalene removal efficiency reached 78–93%, with adsorption, mineralization and plant uptake as the major removal pathways (LeFevre et al., 2012). Microbial communities present in soil samples collected from six raingardens in Minneapolis, MN mineralized naphthalene at an initial concentration of 10 mg/L (LeFevre et al., 2012).

In this study, the performance of an established bioretention system was evaluated with emphasis on PCB removal processes. The objectives of this study were 1) to determine PCB concentrations and congener distributions in the influent and effluent samples in a bioretention facility; 2) to characterize the partitioning of PCBs between dissolved and particulate phases in urban stormwater; 3) to estimate the annual PCB load reduction via adsorption and total suspended solids removal during bioretention treatment.

4.2 Materials and methods

4.2.1 Site description

The evaluated bioretention cell was located on the University of Maryland Campus in College Park, MD (38°59'36.8"N 76°56'20.4"W). It had an area of 181 m^2 (length = 50.3 m, width = 2.4–4.8 m) serving a drainage area of approximately 2800 m^2, including asphalt parking lots, roads, and concrete sidewalks (DiBlasi et al., 2009). It had an underdrain system and was constructed in 2004. The bioretention was fully covered by mixed vegetation year-round. This bioretention cell has been part of several water quality research studies (e.g., Li et al., 2009, Li and Davis, 2014, Liu and Davis, 2014).

4.2.2 Stormwater sample collection and hydrologic monitoring

Stormwater was collected via grab samples and automated composite samples (ISCO 6712FR, U.S.). Grab samples were collected from influent and the outlet of the bioretention cell during rain events (07/23/2019–12/13/2019). After installation of the autosampler (January 2020) volume-weighted composite samples were collected. A cutthroat flume (91 cm × 20 cm, Tracom, U.S.) was used to measure the influent flow rate and for stormwater sampling. The underdrain was equipped with a 20-cm plug-in weir (Thel-Mar Company, U.S.) to measure the

effluent flow rate. A bubble flow meter (ISCO 730, U.S.) was installed at the flume and weir to measure the depth of the flow. Rainfall was recorded using a factory-calibrated tipping bucket rain gauge (ISCO 674, U.S.) connected with the influent autosampler.

Twelve storms were collected (seven with autosamplers, five grab samples) and analyzed for pH, conductivity, TSS, particle size distribution (PSD), total organic carbon (TOC), dissolved organic carbon (DOC) and PCBs. The samples were stored on ice in glass jars with aluminum-lined caps. After each rain event, the stormwater samples were stored in a cooler and transported to the laboratory within 24 h of collection. Stormwater pH and TSS were measured within 24 h after sample collection. For TSS, 1 μm glass microfiber filter paper (GF/B, Whatman) was used. For some events (Events I & III, see below), 0.7 μm glass microfiber filter paper (GF/F, Whatman) was used. The procedure for TSS measurement followed Standard Method 2540D (American Public Health Association et al., 1915). The limit of quantification (LOQ) for TSS was 0.1 mg/L. PSD was measured using a SALD-2300 particle size analyzer (Shimadzu, Japan). The detection limit of the PSD measurement was 0.1 ppm. TOC and DOC were analyzed using a TOC analyzer (TOC-L, Shimadzu, Japan). The calibration curve was made with glycine with a range of 1–100 mg/L. The method quantification limit for TOC and DOC was 1 mg/L. Conductivity was measured using a conductivity meter (B40PCID, symPHony, VWR, U.S.).

Separation of the dissolved and the particulate phases for PCB analysis was performed by filtering samples through a 0.7 μm (the smallest pore size available) glass microfiber filter (GF/F, Whatman). PCBs in the filtered water were operationally designated as dissolved phase; the particles collected on the filters represented the particulate phase.

4.2.3 Extraction of PCBs

4.2.3.1 Quantification of dissolved PCBs

PCBs in the dissolved phase were concentrated using solid phase extraction (SPE), according to Liu et al. (2002) with modifications. Prior to concentration, 10 µL of a mixed solution of surrogates [0.5 µg mL^{-1} 2,4,6-trichlorobiphenyl (PCB 30) and 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB 204)] was added into each sample and sonicated for two hours to mix thoroughly. Biotage DVB (ISOLUTE 101, 500 mg/6 mL) SPE cartridges were conditioned with 6 mL of methanol followed by 6 mL of deionized (DI) water, all at a flow rate of ~150 µL/s. One to two L of stormwater samples spiked with surrogate standards were percolated through the cartridges via Cole-Parmer PTFE tubing. Afterward, the cartridges were dried under vacuum for at least three hours. 12 mL of hexane (HPLC grade) was used to elute the PCBs from the cartridges. The elutes were collected and concentrated under nitrogen flow to less than 1 mL. Finally, the concentrated elutes were spiked with 10 µL of the mixed solution of internal standards [0.5 µg mL^{-1} tetrachloro-m-xylene (TCMX) and 2,2′,4 ,5,5′-pentabromobiphenyl (penta-BB)]. Hexane (HPLC grade) was added to the concentrated effluent to reach a final volume of 1.0 mL and the samples were vortexed for 10 s before being transferred into GC vials for further analysis. Controlled experiments showed that the recoveries ranged from 56.8% to 120% for 17 selected di-to nona-CBs.

4.2.3.2 Quantification of particle bound PCBs

PCBs adsorbed to particulate matter were extracted using a microwave assisted extraction (MAE) (MARS 6, CEM, U.S.). Briefly, filters containing particle-bound PCBs were cut into pieces (5-mm rectangles) and transferred into PTFE extraction vessels (100 mL, CEM, U.S.). Clean filter paper was also cut into pieces and used as laboratory blank controls. The PCBs were

extracted using 20 mL hexane (95% n-hexane for organic residue analysis)-acetone (HPLC grade, Honeywell) (1:1) and cleaned up according to Cao et al. (2019) using alumina.

4.2.4 PCB analysis

Samples were analyzed by gas chromatography/electron capture detector (GC-ECD) (7890B, Agilent Technologies, U.S.) equipped with an Agilent J&W HP-5ms column (60 m × 250 μm × 0.25 μm). The injection volume was 1 μL with helium as the carrier gas. The temperature program was: initial temperature at 70 °C, 7 °C/min to 180 °C, 1°C/min to 225 °C, 5.8 °C/min to 285 °C, held at 285 °C for 20 min, 11.5 °C/min to 300 °C and held at 300 °C for 10 min.

4.2.5 Quality control

All laboratory materials were made of glass or PTFE to avoid sample contamination and adsorption of PCBs. All glassware was cleaned by detergent, solvents and DI water and baked in a muffle furnace at 550°C for at least four h. All the PTFE containers were cleaned with detergent, ultrasonicated three times with hexane and acetone, and rinsed with deionized water. To avoid traces of organic contamination from the filters, the filters were baked at 550°C for 4 h. For each sample, an extra treatment group (triplicate) was prepared as a standard control to confirm the standards were not present in the original samples.

The applied GC-ECD method could identify 209 PCB congeners in 131 peaks. The detection limits for PCB congeners ranged from 0.005 to 2 ng/L for dissolved PCBs, and 0.02 to 8 ng/g for particulate bound PCBs. The method detection limits were obtained by dividing the instrument detection limits by sample volumes or masses. In the liquid samples, 4–41 peaks were detected, whereas 21–43 peaks were found in particle samples. For liquid samples, all values

below the detection limits were substituted with half of the detection limits. Due to the low amounts of particles in the samples, all values below detection limits in solid samples were substituted with zero. Concentrations of the targets were corrected based on concentrations in laboratory blanks according to Cao et al. (2019). Average surrogate recoveries were 79% for PCB 30 (range: 51–113%) and 100% for PCB 204 (range: 52–119%). All were within the acceptable range of 50–125% (Hermanson and Johnson, 2007). The reported concentrations were adjusted based on the recoveries.

4.2.8 Data analysis.

F-test was used to test the equality of two variances and two tailed two-sample *t*-test was used to compare the means between events as well as between influent and effluent samples. Statistical analysis was performed using Microsoft Excel 2019.

Data of annual mass load per unit drainage area (L in g/ha-yr) from dissolved phase PCBs was calculated as:

$$L = (PC_F R_V C)/10^5$$

Where P is the average annual precipitation [1067 mm/yr for the State of Maryland (DiBlasi et al., 2009)]; C_F is the correction factor for events that do not produce runoff (0.9); R_V is the runoff coefficient for the drainage area (0.9); the values of C_F and R_V were chosen according to DiBlasi et al., 2009; C is the influent dissolved PCB concentration in ng/L

Particulate PCB load was calculated by multiplying PCB concentrations in the particulate phase with TSS annual loads found in previous studies (Houng and Davis, 2009). Load reduction was calculated by subtracting the estimated annual PCB load out from annual PCB load in.

4.3 Results and discussion

4.3.1 Hydrologic monitoring

In this study, the hydrology data for seven precipitation events were recorded, where four resulted in bioretention underdrain outflow. The precipitation ranged from 4.3 to 28.7 mm per event with five events below 10 mm (Table 4-1). The shortest storm was Event VIII (2.33 h), while the longest precipitation event was 9.07 h (Event VII). Due to the low precipitation amounts (< 3 cm), the flow rate could not be calculated for the influent samples for all the events.

Table 4-1. Precipitation characterization for the seven monitored events.

Event No.	Date	Precipitation depth (mm)	Precipitation Duration (h)	With outflow
VI	Jan 4, 2020	4.3	2.87	X
VII	Jan 24, 2020	28.7	9.07	X
VIII	Feb 27, 2020	5.1	2.33	X
IX	Mar 13, 2020	5.1	2.40	
X	Mar 15, 2020	4.6	8.93	
XI	Mar 19, 2020	13.7	5.80	X
XII	Mar 23, 2020	8.9	3.67	X

*Events I–V were not monitored for hydrology because the autosampler was not setup.

4.3.2 Properties of stormwater influent and effluent

For the collected stormwater samples, conductivity had a wide range for both the influents (79.0–334 µS/cm) and the effluents (82.4–569 µS/cm). This was in the conductivity range measured in 20 stormwater samples from Paris and its suburbs, which varied between 166

and 1316 µS/cm with 350 µS/cm as the median (Zgheib et al., 2012). Increased ionic strength was reported to decrease natural organic matter adsorption (Bjelopavlic et al., 1999). Thus, higher conductivity could have negative impact on the sorption of PCBs onto particulate matter.

The pH values of the influent samples ranged from 7.66 to 8.44, indicating mild alkaline characteristics in these samples. This could be due to alkaline compounds washed off from the drainage area such as particles from cementitious pavement materials that can increase the pH of runoff (Kuang and Sansalone, 2011). The effluent pH varied from 6.70 to 7.85. For all events, the effluent samples had lower pH values than influent samples. This was also observed in a pilot scale bioretention box in Norway, with influent pH ranging 8.1–8.2, while the effluent pH ranged from 7.1–7.3 (Muthanna et al., 2007). The pH of the bioretention media will contribute to the decreased pH in the effluent. A batch experiment found that the adsorption of PCBs onto soil was affected by pH and the maximum adsorption occurred between pH 6.5 and 7.5 (Adeyinka and Moodley, 2019). Pardue et al. (1988) also found that mineralization rates of PCBs were highest at pH 6.5 and decreased at pH 5.5 and 8.0. Thus, since pH values were lower in the effluents than influents, the bioretention treatment could enhance the adsorption and mineralization of PCBs.

Table 4-2. Stormwater quality parameters of the influent and effluent samples. [a] The TSS concentration was calculated based on particles larger than 0.7 µm. [b] The TSS concentration was calculated based on particles larger than 0.22 µm. [c] Below quantification limit. [d] Below detection limit. [e] Data not available.

Event No.		**pH**	**TSS (mg/L)**	**Particle Size (µm)** d_{10}, d_{50}, d_{80}	**Conductivity (µS/cm)**	**TOC (mg/L)**	**DOC (mg/L)**
I	In	7.82	66.3 ± 5.5[a]	19.4, 65.7, 123	79.0	8.25 ± 0.21	7.77 ± 0.11
	Out	6.70	4.6 ± 0.5[a]	< LOD[d]	82.4	8.60 ± 0.35	8.48 ± 0.03
II	In	7.72	42.2[b]	136, 425, 1391	NA[e]	NA	NA
	Out	6.77	20.2[b]	< LOD	NA	NA	NA
III	In	7.66	184 ± 45.8[a]	< LOD	334.0	64.0 ± 5.17	34.0 ± 0.52
IV	In	7.79	48.3 ± 1.8	129, 840, 1339	94.5	13.4 ± 0.12	7.92 ± 0.21
	Out	6.94	< LOQ[c]	< LOD	88.5	28.0 ± 0.31	12.7 ± 0.28
V	In	8.44	110 ± 19.3	178, 893, 1412	181	25.9 ± 0.71	6.97 ± 0.16
	Out	6.85	< LOQ	< LOD	240	10.9 ± 0.34	10.7 ± 0.32
VI	In	7.79	6.7	< LOD	122	18.2 ± 0.86	25.2 ± 0.50
VII	In	8.22	37.6	< LOD	182	8.76 ± 0.17	6.06 ± 0.02
	Out	7.06	4.4	< LOD	569	16.1 ± 0.08	15.0 ± 0.22
VIII	In	8.28	28.0 ± 1.37	< LOD	95.5	12.77 ± 0.23	5.76 ± 0.06
	Out	7.85	0.987	< LOD	245	NA	NA
IX	In	8.01	35.1 ± 2.1	< LOD	122	14.7 ± 0.28	12.8 ± 0.04
X	In	8.11	11.0 ± 3.8	< LOD	93.5	9.48 ± 0.13	8.21 ± 0.10
XI	In	7.92	22.3 ± 0.8	< LOD	157	15.7 ± 0.53	10.6 ± 0.12
	Out	6.94	0.411	< LOD	170	NA	14.6 ± 0.11
XII	In	7.94	14.3 ± 0.8	< LOD	98.8	7.46 ± 0.14	7.40 ± 0.12
	Out	6.97	< LOQ	< LOD	175	14.0 ± 0.07	13.8 ± 0.03

For samples from Event I and III, suspended solids were filtered through 0.7 µm filters to obtain a sufficient amount to analyze. A step filtration approach showed that suspended solids ranging from 0.7 to 1.0 µm accounted for < 3.5% of suspended solids > 0.7 µm. Thus, the concentrations of suspended solids > 0.7 µm is mostly representative of TSS. Compared to influent samples, TSS concentrations in effluent samples had lower variability with a range < 0.1 (limit of quantification) to 4.6 mg/L. These results showed that bioretention is effective in removing TSS from stormwater runoff, which has been found by many others. Earlier study on this bioretention cell found discharge TSS concentrations of < 1 to 37 mg/L (Houng and Davis, 2009) demonstrating consistency and continued excellent performance for more than 15 years operation.

Particle sizes can impact the affiliation of pollutants, including PCBs, with particulate matter in stormwater (Ghosh et al., 2003, Cao et al., 2019). PSD was measured for all the samples but for all effluent and 8 of 12 influent samples, PSD could not be determined (below detection limit) due to the low particulate matter content. The particles in Event I influent had a range of 5–400 µm (Figure 4-1), with a median particle size (d_{50}) of 65.7 µm. The PSD from Event I differed significantly from that of Event II (p =0.01). The stormwater influent samples collected during Event II had a wider range (36–2346 µm) than those collected during Event I. In addition, the d_{50} in the influent sample of Event II was 425 µm, higher than the influent d_{50} from Event I (65.7 µm). Selbig and Bannerman (2011) studied d_{50} in runoff from urban source areas in Madison, WI, using wet sieve (> 32 µm) and particle analyzer (< 32 µm). They found d_{50} ranged from 42 to 200 µm. Only d_{50} from Event I fell in this range. Sansalone et al. (1998) found that the particles in urban stormwater from paved surfaces ranged from < 1 µm to > 10 mm, with d_{50}

at 570 µm. This variation indicated the variability between each rainfall events and differences in analytical techniques.

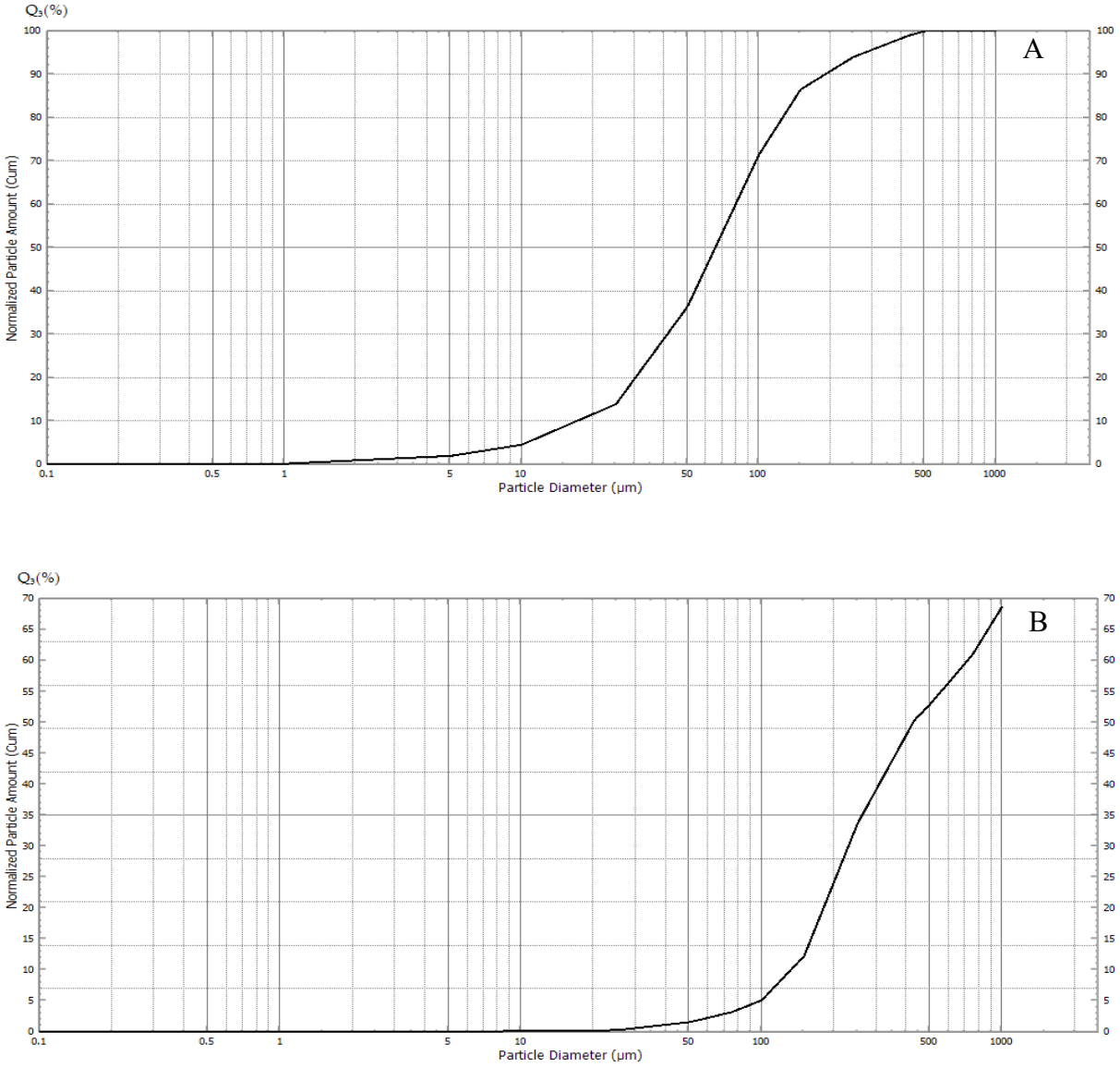


Figure 4-1. Particle size distribution (diameter of cumulative%) of the influent sample at the on-campus bioretention system collected from Event I (A) and Event II (B).

TOC in both influent and effluent samples were measured for all the collected samples, ranging from 8.25 ± 0.21 and 64.0 ± 5.71 mg/L (Table 4-2). The highest TOC concentration was found in the influent samples from Event III, which also had the highest TSS concentration. In influent stormwater, TOC increased with increasing TSS in the sample (Pearson correlation: 0.88 – data not shown). Except for Event VI, all influent samples had a significantly lower TOC concentration after filtration through 1 μm ($p < 0.05$). TOC in influent samples decreased by 13.1–73.1% after filtration, indicating that this portion of TOC was made up by organic carbon from suspended solids. This relationship was also noted for effluent samples. For Events I and V, low TSS content was present in the effluent samples and the TOC decrease in filtered effluent samples was not significant.

As a group of compounds with high hydrophobicity and octanol-water partitioning coefficient (log K_{ow}: 4.50–8.26), PCB sorption by soils and sediments is dominated by physico-chemical entrapment or covalent bonds with organic matter in the matrix (Yu et al., 2006, Kästner et al., 2014). The affiliation of particulate PCBs with TOC in stormwater sediments was also observed in our previous study (Cao et al., 2019). Thus, TOC in stormwater is expected to be positively related to the concentrations of PCBs, both in the liquid phase and particulate phase. TOC in different phases could also impact the partitioning of PCBs in the two phases.

4.3.3 Dissolved PCBs in stormwater samples

Dissolved PCBs were measured from sample collected in the bioretention discharge manhole on July 23, 2019 after the rain event (Figure 4-2). This sample was a mix of previous discharge, direct rainfall and effluent from the bioretention. The water sample from the discharge manhole contained a total PCB concentration of 159 ± 14.7 ng/L. The dissolved concentration of mono- to tetra- CBs (103 ± 9.80 ng/L) were higher than penta- to deca- CBs (56.4 ± 10.9 ng/L).

This is contrary to stormwater sediment samples (Cao et al., 2019) and could be explained by the fact that PCBs are hydrophobic and their solubility in water decreases with increased number of chlorines (ATSDR, 2000). The highly chlorinated congeners were likely sorbed onto the bioretention media when passing the bioretention cell, leaving a higher concentration of low-chlorinated PCBs in the discharge. Among all the homologs, di-, tetra- and penta-CBs dominated. The average number of chlorines was 3.35 ± 0.23. The high abundance of di-CBs (36.5%) contributed a great portion to this. A non-Aroclor congener, PCB 11 (3, 3′-dichlorobiphenyl), was detected as one of the most abundant congeners (24.5 ± 3.29 ng/L), accounting for around 15.4% of total PCBs.

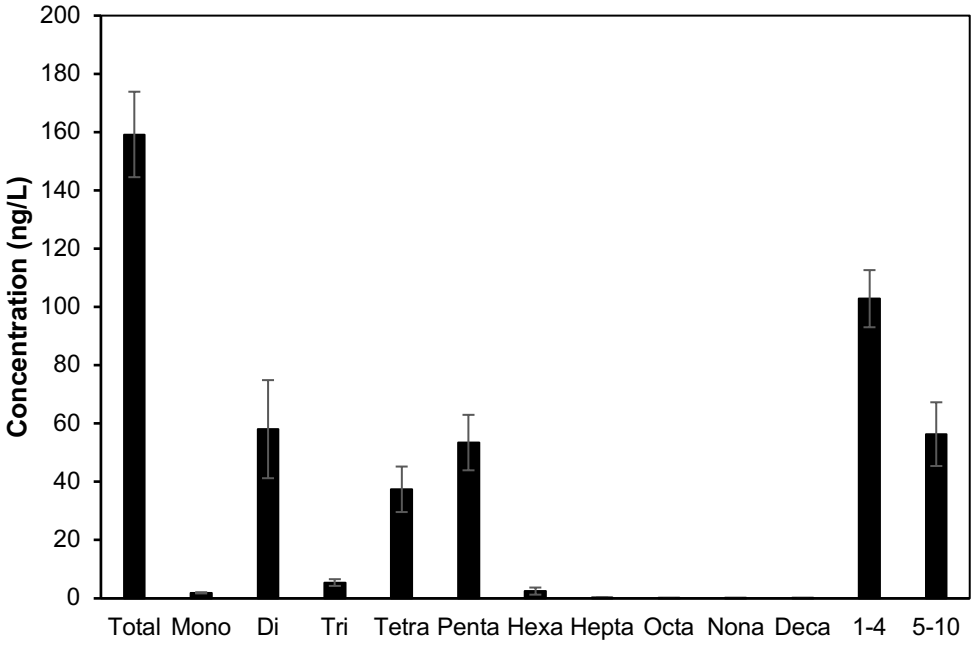


Figure 4-2. Concentrations of total dissolved PCBs, homologs, mono- to tetra-CBs, and penta- to deca-CBs in the bioretention discharge manhole after a rain event on July 23, 2019.

Table 4-3. Concentrations of dissolved PCBs (ng/L), particulate PCBs (ng/g) and number of Cl per biphenyl in stormwater samples (Blank cell means data not available).

Event No.		Dissolved PCBs (ng/L)	Particulate PCBs (ng/g)	Cl per biphenyl (dissolved)	Cl per biphenyl (particulate)
I	In	755 ± 23		3.37 ± 0.07	
	Out	57.8 ± 9.1		2.53 ± 0.03	
II	In	164 ± 8		2.86 ± 0.08	
	Out	18.0 ± 4.8		3.34 ± 0.12	
III	In		169 ± 16		3.80 ± 0.05
III	In	67.5 ± 17.2	156.8	3.05 ± 0.23	3.75
XI	In	83.2 ± 10.6	638	2.90 ± 0.10	2.63
	Out	29.4 ± 5.2	92.5	3.00 ± 0.13	3.96

Influent

Concentrations of total dissolved PCBs from Events I, II, X, and XI varied significantly between the events, also indicating the variability of stormwater (Table 4-3). The highest total PCB concentration was measured in the influent sample from Event I (755 ± 23 ng/L). Total dissolved PCB concentrations in the influent samples from the other three events were lower, ranging from 67.5 ± 17.2 to 164 ± 8 ng/L. The lowest total PCB concentration was detected in the influent sample from Event X. Possible reasons for the variation in total PCB concentrations could be the difference in antecedent dry period length. Longer dry period provides more time for drainage areas to receive and accumulate PCBs before they were washed off. Event I experienced a dry period of 52 prior days, while that for the other events were fewer than four

days. Other reasons like DOC, TSS and PSD could also impact the concentration of PCBs in dissolved phase.

Hwang and Foster (2008) reported 9.82 to 211 ng/L as the range of concentrations of 85 PCBs (total + dissolved) in stormwater runoff entering the tidal Anacostia River, Washington, DC. The dissolved concentrations were below 30 ng/L for each of the seven PCBs (PCBs 28, 52, 101, 118, 138, 153, 180) in a dense urban area in Paris suburb, France (Zgheib et al., 2011b). The concentration for Event I was higher than most of these reported concentrations. The dissolved PCBs from other events were in the range found by Hwang and Forster (2008). The use of analytical methods (extraction, GC detectors, etc.) with different sensitivities as well as various local PCBs sources and different PCBs selected could cause the variation in reported PCB concentrations in U.S. and France.

The influent samples from Events I and II shared similar homolog distributions for dissolved PCBs, with di-, and tri-CBs as the most dominant homologs (di-CBs: 40.3–42.8%, tri-CBs: 12.4–17.5%; Figure 4-3). Di-CBs were the most dominant homolog in both Events X and XI. Similar to samples from the discharge manhole, all of the samples except the ones from Event X had a significantly higher concentration of mono- to tetra-CBs than that of penta- to deca-CBs ($p < 0.001$). This was likely related to the lower K_{ow} values of lower chlorinated PCBs. The average number of chlorines per biphenyl in the influent samples ranged from 2.86 ± 0.08 to 3.37 ± 0.07. The highest number was detected in Event I. This was due to high amounts of hexa- and hepta-CBs in Event I influent samples compared to the other three events. The average number of chlorines per biphenyl found in the aqueous phase were lower than that in the sediment sample near this site, which was 4.44 ± 0.11 (Cao et al., 2019).

In the influent sample from Event I, the concentration of dissolved PCB 11 was 154 ± 16.8 ng/L, accounting for 20.8% of total dissolved PCBs. As for Event II, the dissolved concentration of PCB 11 was at 32.8 ± 1.38 ng/L in the influent sample, accounting for 20.2% of total dissolved PCBs. PCB 11 was also detected in the surface sediment sample collected near the same site. The relative abundance of PCB 11 in the liquid phase of stormwater from the two events was higher than that in the surface sediment sample (15%) (Cao et al., 2019). PCB 11 was also detected in influent samples from Events X and XI, but at lower concentrations (3.06 ± 1.20 ng/L and 4.47 ± 0.62 ng/L, respectively).

PCB 11 can be synthesized from 3,3′-dichlorobendizine in the manufacturing processes of azo-type pigments used in commercial paints (Anezaki and Nakano, 2014). It has been detected in various matrixes such as commercial paint pigments and consumer goods, urban air and wastewater treatment plants (Hu et al., 2008, Hu and Hornbuckle, 2010, Rodenburg et al., 2010). In addition to PCB 11, PCB 4 (0.7–12.6%) and PCB 22 (1.4–20.9%) were also detected at elevated concentrations in the dissolved phase in influent samples. These congeners are found in Aroclors 1016 and 1242 (ATSDR, 2000) indicating that A1016 and 1242 could contribute to dissolved PCBs in stormwater. Additionally, PCB 4 is an *ortho*-chlorinated congener, which could be a product from organohalide respiration. The relative abundance of PCB 8 (0.6–20.0%) and PCB 14 (0.2–14.9%) was high in influent samples. PCB 8 was also detected in polycylic-type pigments (Anezaki and Nakano, 2014). These two congeners, in addition to PCB 11, were dominant congeners in stormwater sediments (Cao et al., 2019), indicating the correlation between stormwater aqueous phase and stormwater sediments. The congener profile of dissolved PCBs indicated that PCBs in stormwater could come from legacy sources like Aroclors. Lower chlorinated Aroclor mixture like A1016 and 1242 were more related to stormwater dissolved

PCBs. Nonlegacy PCB sources could also contribute to particulate PCBs in stormwater and paint pigment is the major nonlegacy source.

Effluent

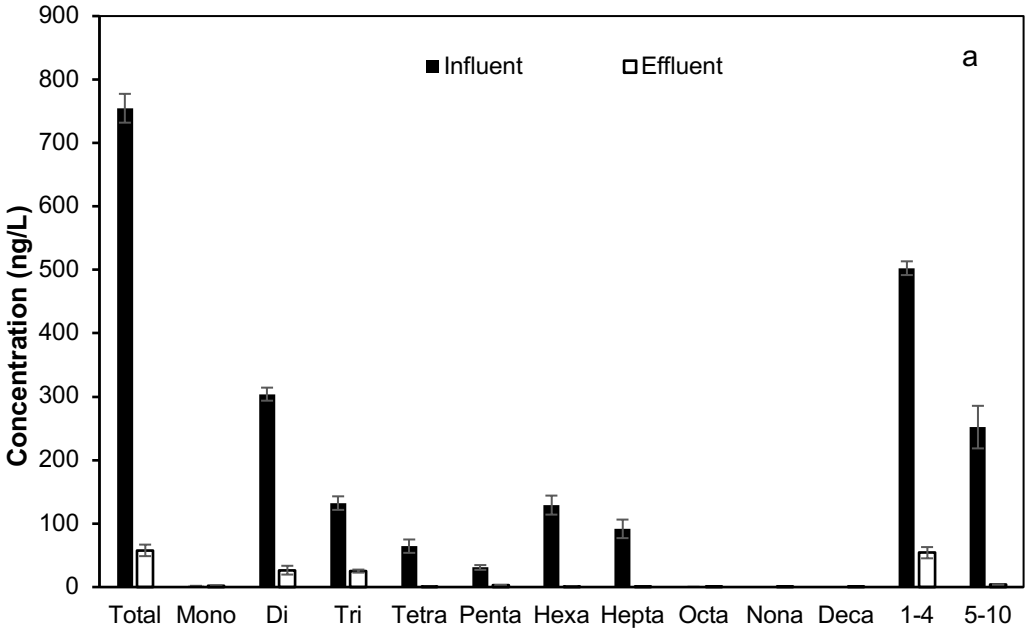
For Events I, II, and XI, the total dissolved PCB concentration was lower in the effluent samples than in the influent samples (Figure 4-3). This indicated that this bioretention was effective in removing dissolved PCBs from stormwater. The removal efficiency of the bioretention for dissolved PCBs ranged from 65–92.3% based on concentration in influent and effluent samples. Bioretention can remove PCBs from stormwater via sorption of PCBs to bioretention media. PCB removal (43.8% of dissolved + particulate PCBs, assumed) was also observed in a bioretention system in Daly City, California (David et al., 2015). Removal of other hydrophobic compounds like PAH (dissolved + particulate) was also observed in the same bioretention cell (31–99%) that was evaluated in this study (DiBlasi et al., 2009). The reported performance of bioretention in removing hydrophobic compounds including PCBs, show that bioretention is a promising treatment for dissolved stormwater PCBs.

In the dissolved phase of the effluent sample from Event I, di- and tri-CBs had the highest abundance. Concentrations of congeners with four or more chlorines were close to detection limits of 0.005–0.1 ng/L. Thus, the average number of chlorines per biphenyl in this sample (2.53 ± 0.03) was lower than detected in the influent sample. This indicated that treatment of the bioretention system adsorbed some highly chlorinated PCBs during Event I. However, during Events II and XI, the opposite occurred, where the average number of chlorines per biphenyl in the effluent sample (3.34 ± 0.12 and 3.00 ± 0.13) was higher than the influent sample. This was due to the high abundance of tetra-CBs in the effluent sample. When

comparing the homolog distribution of the influent and effluent samples, the concentrations of di- to hexa-CBs (except penta in Events II and X) decreased significantly in the effluent sample ($p < 0.05$). This means that, this bioretention system removed significant amounts of most PCB homologs present in the stormwater. The other homologs were not removed significantly due to their concentration close to detection limits in both influent and effluent samples. During Event II, the removal fractions for tetra- and penta-CBs were 70.7% and 30.8%, respectively, while the removal of di- and tri-CBs were > 92%. During Event III, the highest removal efficiency was achieved for di-CBs (93.4%).

The concentration of dissolved PCB 11 was lower in the effluent samples, at 8.15 ± 2.92 ng/L in Event I and 1.23 ± 0.17 ng/L in Event XI. PCB 11 was below quantification limit (< 0.5 ng/L) in Event II effluent.

For PCBs, the criterion to protect freshwater aquatic life is 14 ng/L as a 24-hour average (EPA, 1980). The low level is due to bioconcentration factors taken into consideration. Dissolved phase in all the measured effluent samples were above this value, and could cause possible toxicity to freshwater aquatic life. The PCB concentration to cause acute toxicity to freshwater aquatic life is > 2 µg/L (EPA, 1980). All the measured effluent samples from this study were below this value, meaning acute toxicity to freshwater aquatic life was not expected. The maximum contaminant level for PCBs in public drinking water supplies established by United States Environmental Protection Agency (EPA) is 0.5 ppb (EPA, 2009). Except for the influent sample from Event I, the other stormwater samples were below this level. The results from this study showed that after bioretention treatment, PCB levels in the effluent samples were much lower than the maximum PCB level allowed in public drinking water supplies and were not likely to cause acute toxicity to organisms when they are exposed to these samples.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Influent
Effluent
Concentration (ng/L)
900
800
700
600
500
400
300
200
100
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Total
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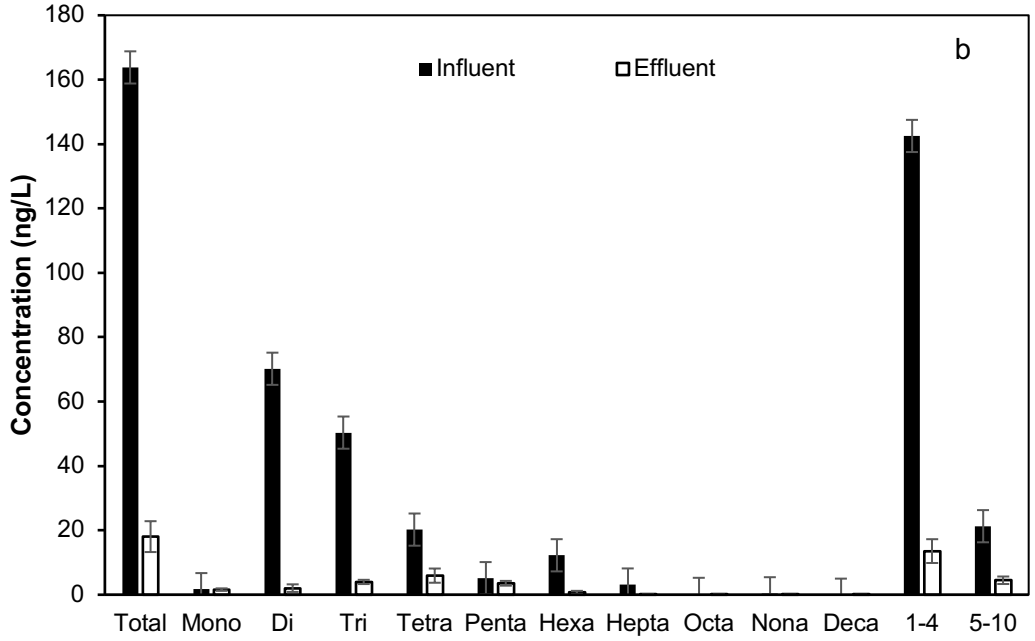
Tetra
Penta
Hexa
Hepta
Octa
Nona
Deca
1-4
5-10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Concentration (ng/L)
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160
140
120
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80
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40
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Total
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Octa
Nona
Deca
1-4
5-10

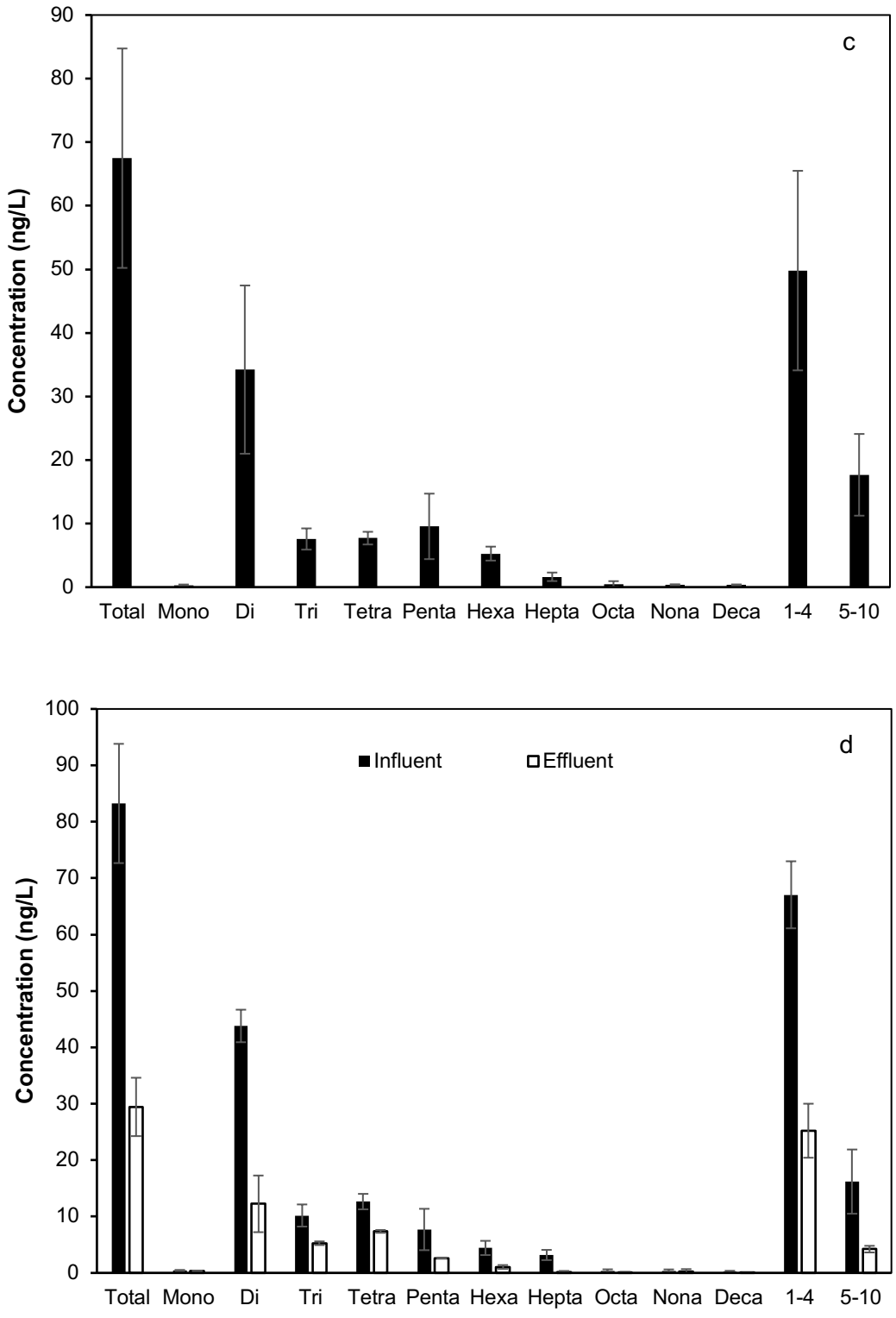


Figure 4-3. Concentrations of dissolved total PCBs, homologs, mono- to tetra-CBs, and penta- to deca-CBs in stormwater influent and effluent samples at the -campus bioretention system from

Events I (a), II (b), X (no effluent) (c), and XI (d). Error bars represent the standard deviation for three samples.

4.3.4 PCBs in the particulate phase in stormwater influent samples

The total PCB concentrations found in the particulate phase ranged from 157 to 638 ng/g. These concentrations were higher than the concentrations found in the stormwater sediment samples in our previous study (mean: 29.2 ng/g, max: 57.4 ng/g) (Cao et al., 2019). The possible reason could be that the particles in stormwater are smaller than the surface sediment samples (which were deposited at the entrance of the bioretention). Smaller particles have higher tendency to sorb PCBs due to larger specific area and higher TOC content (Ghosh et al., 2003, Cao et al., 2019). The smaller stormwater sediment particles (25–75 μm), contained PCBs at 180 ± 6 ng/g from a dense urban area and at 66.0 ± 8.5 ng/g from an institutional area. Particles > 75 μm accounted for 98.1% of the total sediments left at the entrance of the same bioretention cell. PSD was not available for Events III, X and XI, but the d50 for particles from Event I was 65.7 μm, indicating that the size stormwater TSS could be smaller than stormwater sediments.

In the solid phase, the concentration of mono- to tetra-CBs was higher than the concentration of penta- to deca-CBs. For Event III, the dominant homolog was penta-CBs, followed by di- and tri-CBs. Hwang and Foster (2008) also found a high abundance of penta-CBs in the stormwater entering the tidal Anacostia River, Washington, DC (only one di-CB was measured). For Events X and XI, di-CBs were the most dominant, followed by tri-CBs. A low average chlorine number (2.63) was the result of high abundance of di-CBs (51.9%) in Event III influent. The average numbers of chlorines per biphenyl in other samples ranged from 3.75 to 3.96. These values are higher than all the average numbers detected for dissolved PCBs in this

study. This is related to stronger affiliation between more chlorinated PCBs and particles due to their higher Kow values (ATSDR, 2000).

In the solid phase of the influent samples, PCB 11 was detected at 34.7–193 ng/g, accounting for 20.4-30.2% of the total PCB concentration. Except for PCB 11, the dominant PCB congeners in the particulate phase was different from that in surface stormwater sediments (Cao et al., 2019), indicating different PCB sources. PCB 28 was the second dominant congener in the particulate phase, accounting for 8.3% of total PCB concentration. PCB 28 was present in Aroclors 1016, 1242 and 1248 (ATSDR, 2000). In addition, PCB 28 was reported to be one of the predominant end products during the organohalide respiration of Aroclor 1254 (Kaya et al., 2016). The congeners PCB 12 (11.3%), PCB13 (11.3%), and PCB 19 (17.9%) were found at high abundance in the particulate phase of Event XI and were all present in Aroclor 1016 and 1242 (ATSDR, 2000). In addition, PCB 12 and PCB 13 were detected at high concentration (PCB 12+13: 18.2 ng/g) in commercial paint pigments. This indicated that Aroclor 1016 and 1242 and/or other nonlegacy sources may contribute as sources to PCBs to stormwater, while Aroclor 1254 and 1260 were probable sources to urban stormwater sediments (Cao et al., 2019). However, the predominance of PCB 28 indicated potential organohalide respiration of Aroclor 1254 in the watershed. The possible reason for the difference between stormwater sediments and particulate phase is that stormwater sediments were settled before they were washed into SCMs like bioretention while TSS were more mobile.

The total PCB concentration in the particulate phase was higher than the consensus-based threshold effect concentration for PCBs in freshwater ecosystems, which is 59.8 ng/g (MacDonald et al., 2000). This means PCBs in the particles could cause harmful effects on

organisms dwelling in these samples due to the partitioning effect of PCBs between particles and lipid phase of organisms.

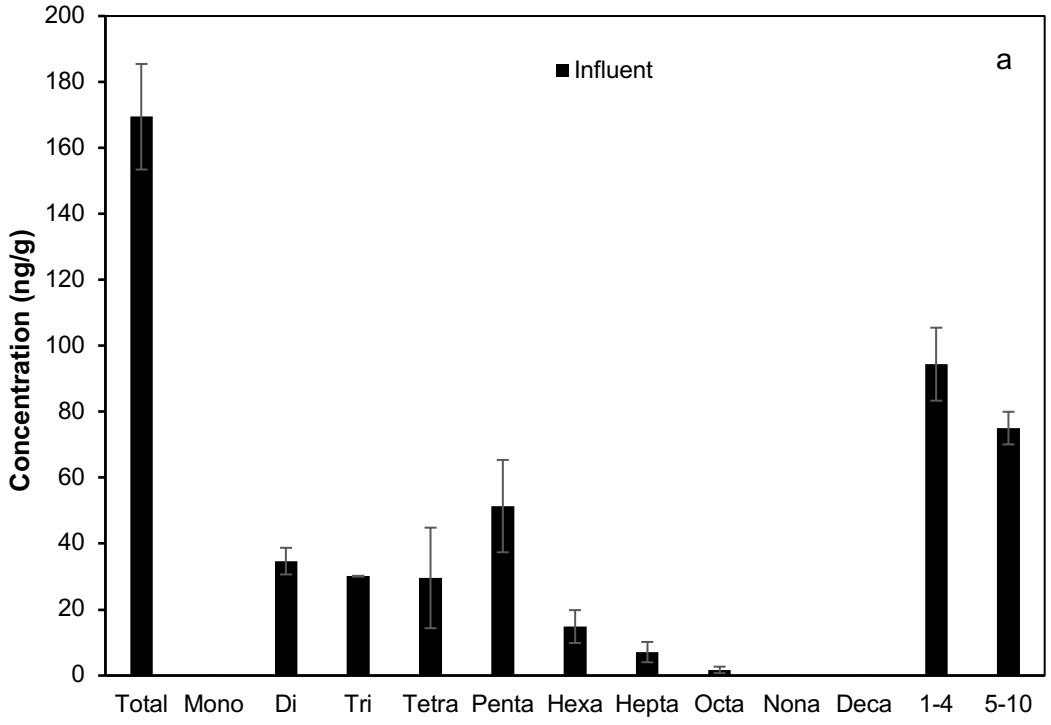
Using TSS concentrations to determine particulate-bound stormwater PCB concentrations, Event III influent sample contained 34.4 ng/L of PCBs in the particulate phase. The concentration of dissolved PCBs was not measured thus the mass of PCBs in the particulate phase could not be compared to the corresponding liquid phase.

Previous studies reported that more than 90% of the total mass of PCBs in storm flow runoff were particle bound (Hwang and Foster, 2008, Zgheib et al., 2011b). However, particle-bound PCBs appeared to play a less important role in total PCB contribution in this study. If using 152 ng/L from Event II as the total PCB concentration in the liquid phase for Event I, particle-bound PCBs only accounted for 18.4%. For Events X and XI, particle-bound PCBs only accounted for 2.6–17.1%. All these values suggested a less important role for particulate phase in the contribution of PCB mass in stormwater.

Zgheib et al. (2011b) emphasized the importance of particular phase on PCB mass contribution because all the seven selected PCBs were below their quantification limit (30 ng/L) for each congener. However, in this study, the quantification limit for PCBs were < 30 ng/L and 27 congeners were quantified in the liquid phase. Thus, concentrations of dissolved PCBs could be quantified and compared to the concentration found in particulate phase in this study. In addition, Hwang and Foster (2008) only reported PCB concentrations in the particulate phase as well as total PCBs in dissolved and particulate phase. Information about dissolved PCBs and particle concentration in the runoff samples were not reported. According to the information provided, the approximate range of particle-bound concentrations by Hwang and Foster was 11.7–6125 ng/L. It is possible that runoff samples from Hwang and Foster's study had a higher

particle concentration than this study. Additionally, Hwang and Foster's study focused on one di-CB (PCB 15) and congeners with three or more chlorines. This may result in underestimation on the total PCB concentration, since lowly chlorinated congeners (such as PCB 11) accounted for a significant portion in the dissolved phase. Similarly, the seven PCBs selected by Zgheib et al. (2011b) were PCBs with three to seven chlorines. As a result, in contrast to previous results, the dissolved phase cannot be overlooked when calculating the total PCB concentrations in urban stormwater.

After bioretention treatment, PCB amounts adsorbed onto the particulate matter decreased. During Event XI, the total PCB concentration for effluent particles decreased to 92.5 ng/g from 638 ng/g with 85.5% removal efficiency of PCBs. If taking the TSS concentration in both influent and effluent into consideration, the PCB concentration decreased from 14.2 ng/L to 0.04 ng/L. The removal efficiency via bioretention was 99.7%.


Influent
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Concentration (ng/g)
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180
160
140
120
100
80
60
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Total
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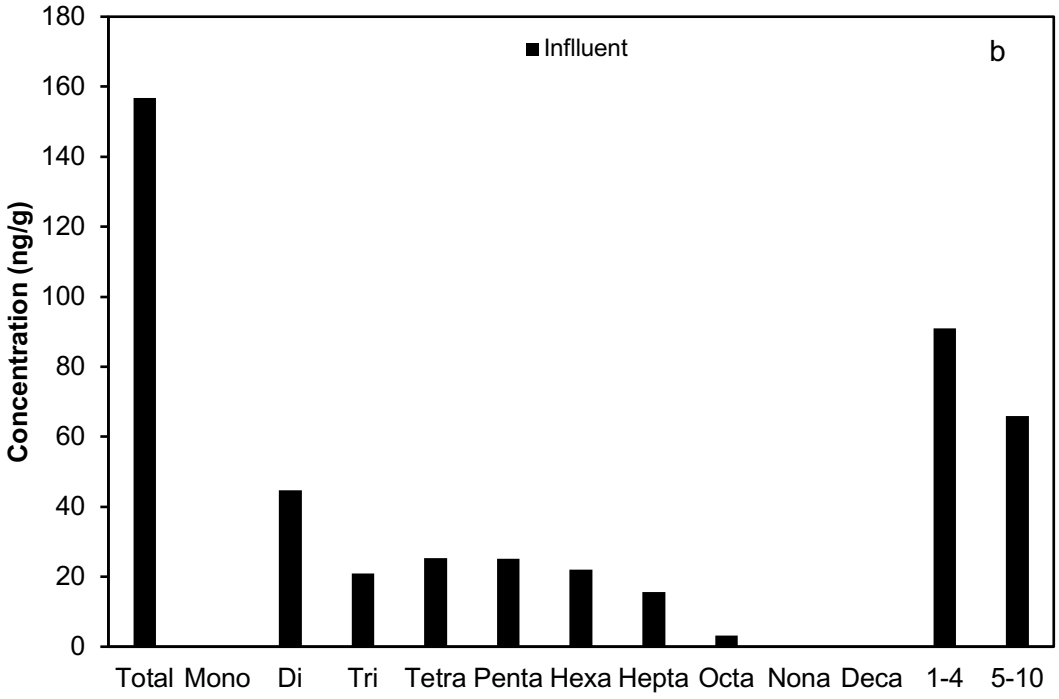
Hexa
Hepta
Octa
Nona
Deca
1-4
5-10


Inflluent
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Concentration (ng/g)
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Octa
Nona
Deca
1-4
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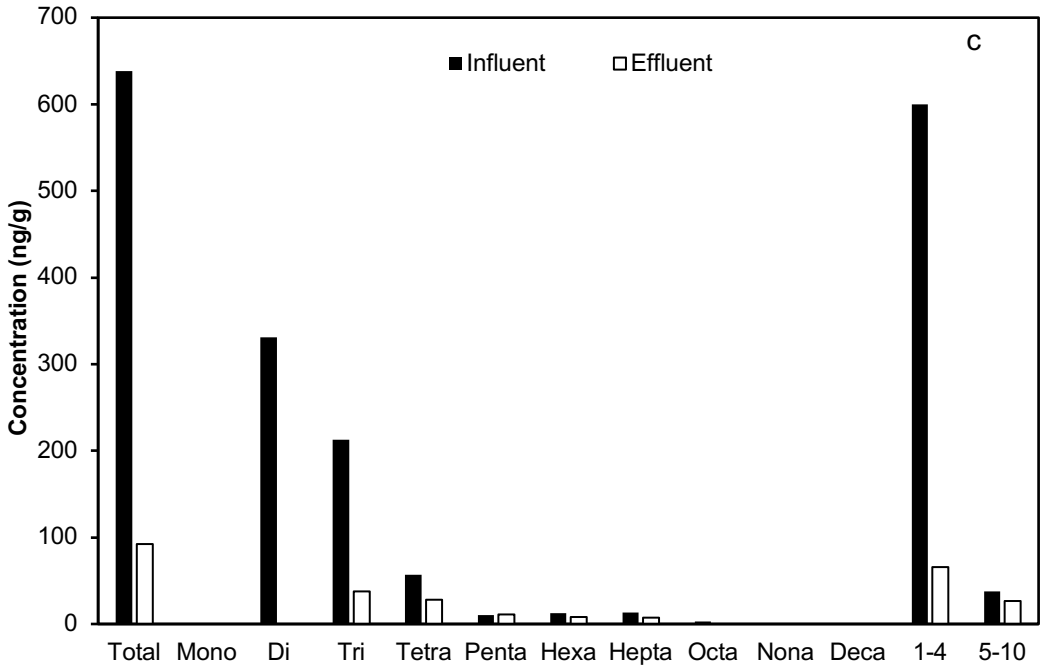

Figure 4-4. Concentrations of particulate total PCBs, homologs, mono- to tetra-CBs, and penta- to deca-CBs in stormwater influent/effluent samples at the on-campus bioretention system from Events III (no effluent) (a), X (no effluent) (b), and XI (c). Error bars represent the standard deviation from two samples (a).

4.3.5 Total PCB concentrations, loads, and reductions via bioretention

Using the PCB concentrations from Event XI (638 ng/g and 92 ng/g) and TSS load data from previous study (Houng and Davis, 2009), PCB load reduction was estimated (Table 4-4). These calculations indicate that, dependent on TSS removal efficiencies, bioretention can reduce particulate-bound PCB export by about 0.36 to 0.76 g/ha-yr (99.0–99.6%). The total load reduction of PCBs in both phases could be 0.86–7.16 g/ha-yr. The load reduction of PCBs via the particulate phase was lower compared to dissolved PCBs due to a higher mass of PCBs in the liquid phase compared to the particulate phase. The mass contribution of lower chlorinated congeners was underestimated in previous studies because these congeners were not included

when reporting total PCBs (Hwang and Foster, 2008, Zgheib et al., 2011b). Instead, in this study, concentrations of all the 209 PCBs were recorded and di- to tetra- CBs were detected at higher concentrations than highly chlorinated PCBs. As a result, although the removal of particles in stormwater can reduce the mass of PCBs, dissolved PCBs must be further investigated.

Table 4-4. TSS data used and estimated PCB load reduction via TSS removal.

Location	TSS load in (kg/ha-yr)	TSS load out (kg/ha-yr)	Estimated PCB load in (mg/ha-yr)	Estimated PCB load out (mg/ha-yr)	Estimated PCB load reduction (mg/ha-yr)
College Park, MD	1190	37	759	3.4	755.6
Silver Spring, MD	759	3.4	364	3.5	360.5

Using 67.5–755 ng/L from this study as the concentration of dissolved PCB in the influent and the equation in Section 4.2.8, the estimated annual PCB loading from dissolved phase ranged from 0.58 to 6.52 g/ha-yr. Assuming the effluent volume is 50–80 % of the influent volume for this bioretention cell (Li et al., 2009), the annual PCB mass load in the dissolved phase after treatment from this bioretention cell was 0.08–0.40 g/ha-yr using the effluent dissolved PCB concentration in ng/L (18.0–57.8 ng/L from this study). This estimation indicated that bioretention has a potential to reduce dissolved PCBs by 0.5 to 6.4 g/ha-yr. The load

reduction of dissolved PCBs in stormwater via the bioretention ranges from 86.2 to 98.8%. When taking both the dissolved phase and particulate phase into consideration, the bioretention could reduce PCBs by 0.86–7.2 g/ha-yr (total PCB load reduction: 91.5–98.4%). In order to meet the TMDLs in MD for PCBs, the required load reduction (%) from stormwater ranged from 51.6–93.0% (MDE, 2011, MDE, 2012, MDE, 2016). The percentage of load reduction calculated in this study met the required load reduction, indicating that bioretention can help to meet PCB TMDLs.

4.4 Conclusions

In order to evaluate the performance of a bioretention cell in treating PCBs in stormwater, the presence of PCBs in stormwater influent and effluent samples was evaluated. The partitioning of PCBs between dissolved phase and particulate phase was also studied. Our results showed that bioretention effectively decreased concentrations of TSS and PCBs (both dissolved phase and particulate phase) in stormwater.

- Both dissolved and particulate PCB concentrations varied between events, ranging from 67 ± 17 to 755 ± 23 ng/L, which was mainly attributed to the dry period prior to the rain event.
- Particle-bound PCBs accounted for 2.6–17.1% of total PCBs detected in stormwater. As a result, the dissolved phase should not be ignored when studying and removing PCBs from stormwater.
- Dissolved PCB concentrations were significantly decreased in the effluent compared to influent by 65–92.3% after bioretention treatment.

- Bioretention is effective in removing PCBs from stormwater via adsorption and TSS removal, with a load reduction at 91.5–98.4%.

Chapter 5. Polychlorinated biphenyls in roadway paint scrapings with focus on 3,3′-dichlorobiphenyl (PCB 11)

Abstract

Yellow and white paint samples collected from roadway lines and curbs were studied to assess the concentrations of polychlorinated biphenyls (PCBs) in road paints. Total PCB concentrations ranged from 162 ± 19.3 to 203 ± 22.1 ng/L in yellow paints, whereas white paints contained lower concentrations of PCBs (7.80 ± 0.01 ng/g to 60.1 ± 6.93 ng/g). 3, 3′-dichlorobiphenyl (PCB 11) is a marker of non-legacy PCB contamination and accounted for 17%–91% of the total concentration of PCBs in yellow paints, but this congener was below the detection limit in white paints. Another congener that has been reported as a major congener in pigments, 2,2′,5,5′-tetrachlorobiphenyl (PCB 52), was also detected in both the yellow and white road paint samples. PCBs may be transported into the urban environment via the release of colloidal paint particles after binder degradation or through volatilization. The results from this study show that paints used on roadways contain PCBs, particularly yellow paint, and thus roadway paints could contribute towards the total level of PCBs in stormwater and the urban aquatic environment. More research is needed on the mass contribution from road paints on stormwater PCBs and their leaching characteristics.

5.1 Introduction

Polychlorinated biphenyls (PCBs) consist of a group of 209 organic chlorine compounds widely used in the U.S. from 1929 to 1979. Aroclor is the trade name for industrially-produced PCB mixtures, (Kimbrough, 1995) but PCB 11 (3,3′-dichlorobiphenyl) is a common non-Aroclor PCB congener that has been detected in various environments including urban air (Hu et al., 2008), stormwater (Cao et al., 2019), wastewater and commercial products (Guo, 2013) after the 1979 (U.S) ban (Vorkamp, 2016). PCB 11 is a by-product from manufacturing of diarylide derived yellow pigments and is therefore emerging as a marker of non-legacy PCB contamination (Rodenburg et al., 2010, Grossman, 2013). PCB 11 has been detected as one of the most abundant congeners in stormwater sediment samples collected in urban areas (Cao et al., 2019). Another congener, PCB 52 (2,2′,5,5′-tetrachlorobiphenyl), was also detected in azo-type pigments (Hu and Hornbuckle, 2010, Liu et al., 2016). Unlike PCB 11, PCB 52 is an important component of Aroclors 1016, 1242, 1248 and 1254 (ATSDR, 2000).

PCB 11 toxicity has often been ignored, since it is not included in the Aroclor mixtures. This congener can alter neuronal morphogenesis at low levels (0.22 ng/mL) in rats (Sethi et al., 2017) and its metabolites (OH-PCB 11 and OH-PCB 11 sulfate) can significantly alter the growth of cells involved in cognitive and higher-order behaviors in neonatal rats (Sethi et al., 2017). Other studies have also shown altered neuronal morphogenesis at 1 fM of PCB 11 (~0.22 ng/mL) (Sethi et al., 2017). Therefore, PCB 11 may represent an underestimated problem in the environment that warrants further study. Additionally, PCB 52 can cause cyto- and genotoxicity and this congener has also induced pathologic changes in Rhesus monkeys at a dietary dose of 60 µg/kg/day for 133 days (McNulty et al., 1980).

Review of the current literature indicated limited information identifying sources and fate of PCB11 in the environment (Guo, 2013, Vorkamp, 2016). Urban stormwater represents a growing concern as a PCB source and many jurisdictions are addressing PCB pollutants loads and reductions as part of Total Maximum Daily Load restrictions (Zgheib et al., 2012, David et al., 2015, Gilbreath et al., 2019, Wu et al., 2019). Therefore, additional research is needed to identify sources of PCB 11 as well as other PCBs in urban areas and to minimize their release and accumulation. Prior study has found that the concentration of PCB 11 decreased significantly after removing yellow flakes (apparently peeling from roadway markings) from stormwater sediments (Cao et al., 2019). Since PCB 11 is a by-product of the manufacturing of pigments, roadway paints may be an important source in urban areas. Thus, due to their wide use, roadway paints merit study as a possible urban PCB source. The objectives of this study were 1) to assess the concentration, mass, and congener pattern of PCBs in roadway paints with different colors, yellow and white; 2) to specifically evaluate the concentration and relative abundance of PCB 11 and PCB 52 in different roadway paints; 3) to explore the potential mechanisms of PCB immobilization from paints as PCB sources to urban stormwater and the aquatic environment.

5.2 Materials and methods

5.2.1 Sampling sites

Scraped paint samples were collected from seven on-campus sites at University of Maryland (UMD), College Park from April to June 2019. A clean knife with sharp blade was used to scratch the paint samples from traffic and curb lines. Flakes peeling off painted curbs were collected by hand. Among the seven samples, three samples contained yellow flakes peeling from curbs: 1) near a bioretention cell stormwater control measure (Yellow1:

38°59'36.8"N 76°56'20.4"W), 2) near a loading dock of research/educational building 1 (Yellow2: 38°59'20.6"N 76°56'16.7"W), and 3) along a road near research/educational building 2 (Yellow3: 38°59'35.5"N 76°56'28.9"W). One yellow sample was scraped from the yellow traffic line near an apartment building near the UMD campus (Yellow4: 38°59'34.4"N 76°56'00.4"W). Additionally, three samples were scraped from white traffic lines on roads (W1: 38°59'37.1"N 76°56'13.8"W) and parking lots (W2: 38°59'25.4"N 76°56'29.7"W, W3: 38°59'35.3"N 76°56'23.1"W). In the laboratory, the collected flakes were ground into smaller pieces (< 2 mm) using a mortar and pestle and stored at room temperature.

5.2.2 PCB extraction and analysis

PCBs in the paints were extracted using microwave assisted extraction (MAE) (MARS 6, CEM, U.S.). Briefly, two grams of ground sample was added into each extraction vessel and clean sea sand (Merck, U.S.) was used as laboratory blank control. Surrogates standards [tetrachloro-m-xylene (TCMX), 2,4,6-trichlorobiphenyl (PCB 30) and 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB 204)] were added into each sample prior to MAE. The filter papers and paint samples were extracted using 20 mL hexane (95% n-hexane for organic residue analysis)-acetone (HPLC grade, Honeywell) (1:1) and cleaned up according to Cao et al.(Cao et al., 2019) Briefly, the extracts were loaded onto a column filled with glass wool (Acros Organics, Germany), prepared alumina (Fisher Scientific, U.S.) and sodium sulfate (Fisher Scientifc, U.S.). 15 mL of hexane were used to elute the column and the effluent was collected for further analysis.

5.2.3 PCB quantification

Samples were analyzed and PCBs were quantified using a gas chromatography/electron capture detector (GC-ECD) (7890B, Agilent Technologies, U.S.) equipped with an Agilent J&W HP-5ms column (60 m × 250 μm × 0.25 μm). The injection volume was 1 μL with helium as the carrier gas. The temperature program was: initial temperature at 70 °C, 7 °C/min to 180 °C, 1°C/min to 225 °C, 5.8 °C/min to 285 °C, held at 285 °C for 20 min, 11.5 °C/min to 300 °C and held at 300 °C for 10 min.

Target compounds (209 PCBs), surrogate standards, and internal standards [4-bromobiphenyl (4-BB) and 2,2′,4 ,5,5′-pentabromobiphenyl (penta-BB)] were purchased from AccuStandard (U.S.) and Restek (U.S.). All laboratory materials were made either from glass or PTFE to avoid sample contamination and adsorption to the materials.

The applied GC-ECD method was able to identify 209 PCB congeners present in 131 separate peaks. The detection limits for PCB congeners ranged from 0.0025 to 1 ng/g for the paint samples. The method detection limits were calculated by dividing the instrument detection limits (0.005 to 2 ng/mL) by sample masses.

5.2.4 Statistical analysis

For the comparison of three or more groups of data, a pairwise t-test was performed. Holm's sequential Bonferroni method was applied to correct the results by reducing the possibility of type I error.

5.3 Results and discussion

5.3.1 PCBs in yellow paints

During the extraction of the yellow roadway paints, at least two different yellow colors of the extracts were observed indicating that different paint products were used. These paints could be manufactured from different batches or originate from different manufacturers. Analysis of concentrations of total PCBs, homologs, mono- to tetra-CBs, and penta- to deca-CBs in yellow paint samples from different sites showed that the total PCB concentrations per g was similar, ranging from 162 ng/g to 203 ng/g (Figure 5-1). Hu and Hornbuckle (2010) tested 33 commercial paint pigments and found that PCBs were primarily detected in organic pigments at 2 to 200 ng/g fresh weight. The level of PCBs found in azo-type pigments ranged from 7 μg/kg to 740 mg/kg (Anezaki and Nakano, 2014). Azo-pigments can be produced in all colors but the most important ones are yellow, orange and red (Vorkamp, 2016). All concentrations in this study were in this range (Figure 5-1). For the yellow paint samples, the concentration of mono- to tetra-CBs (139–199 ng/g) was significantly higher than penta- to deca-CBs (3.95–38.6 ng/g) ($p < 10^{-6}$). Yellow2 had the highest concentration of penta- to deca- CBs, at 38.6 ± 6.00 ng/g. All other samples contained penta- to deca-CBs below 12.1 ng/g, indicating that lower chlorinated PCBs are more likely to be found in paints.

The homolog distributions for Yellow1 and Yellow3 were similar, with di-CBs and tetra-CBs dominant, indicating they could be the same paint. The homolog distributions of the other samples were different due to different types of pigments or different manufacturing processes (Hu and Hornbuckle, 2010). Yellow1 and Yellow3 congener distributions are similar to those of two mono-azo yellow pigments, where di-CBs were the most abundant, with limited presence of higher chlorinated PCBs (Hu and Hornbuckle, 2010). However, in Yellow2, tetra-CBs, penta-CBs and di-CBs were the most dominant. In Yellow4, di-CBs had the highest concentration, with other homologs below 7.5 ng/g (close to the quantification limit of 0.0025 to 1 ng/g).

The average number of chlorines per biphenyl ranged from 2.1 ± 0.02 to 3.6 ± 0.01, where Yellow1, Yellow3, and Yellow4 all had chlorine numbers smaller than 2.5 because of the high abundance of di-CBs. The different homolog distribution for Yellow2 caused an increased average number of chlorines per biphenyl, which was significantly different from other samples ($p < 10^{-6}$).

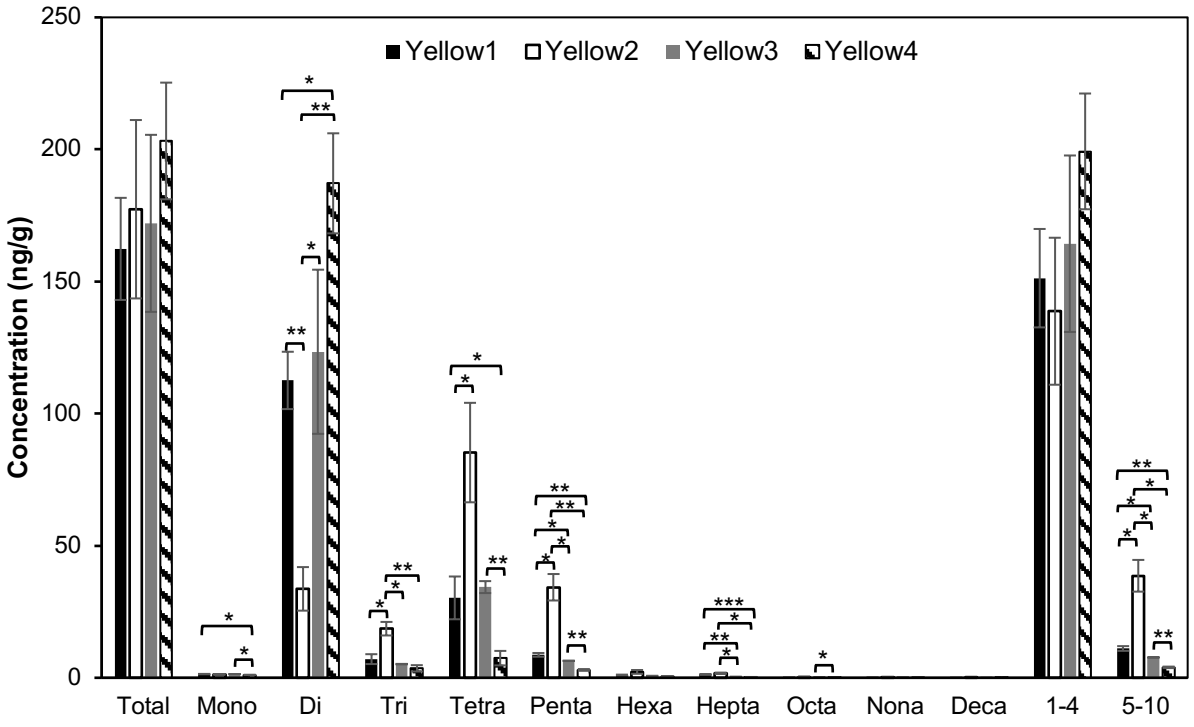

Figure 5-1. Concentrations of total PCBs, homologs, mono- to tetra-CBs, and penta- to deca-CBs in the yellow paint samples (significance levels: * < 0.05; ** < 0.01; *** < 0.001).

5.3.2 PCB 11 and other dominant congeners in yellow road paints

PCB 11 was detected in all four paint samples, with a minimum concentration of 30.8 ± 8.17 ng/g (Yellow2) (Figure 5-2) and highest of 185 ± 18.7 ng/g (Yellow4). The concentration of PCB11 was 182 ± 10 ng/g in yellow flakes separated from stormwater sediment samples (Cao et

al., 2019). The relative abundance of PCB 11 in Yellow2 was 17.4%. In contrast, the relative abundance of PCB 11 ranged from 68.4% to a high of 91.1% in the other three samples, indicating that PCB 11 was the most frequently detected congener in paint samples Yellow 1, 3 and 4.

During the manufacturing of azo pigments, the raw materials and intermediate products include compounds such as chlorinated aniline and chlorinated benzidines (Hu and Hornbuckle, 2010). These chlorinated compounds have the potential to produce PCBs during side-reactions and PCB 11 can be synthesized from 3,3′-dichlorobendizine (Hu and Hornbuckle, 2010, Anezaki and Nakano, 2014).

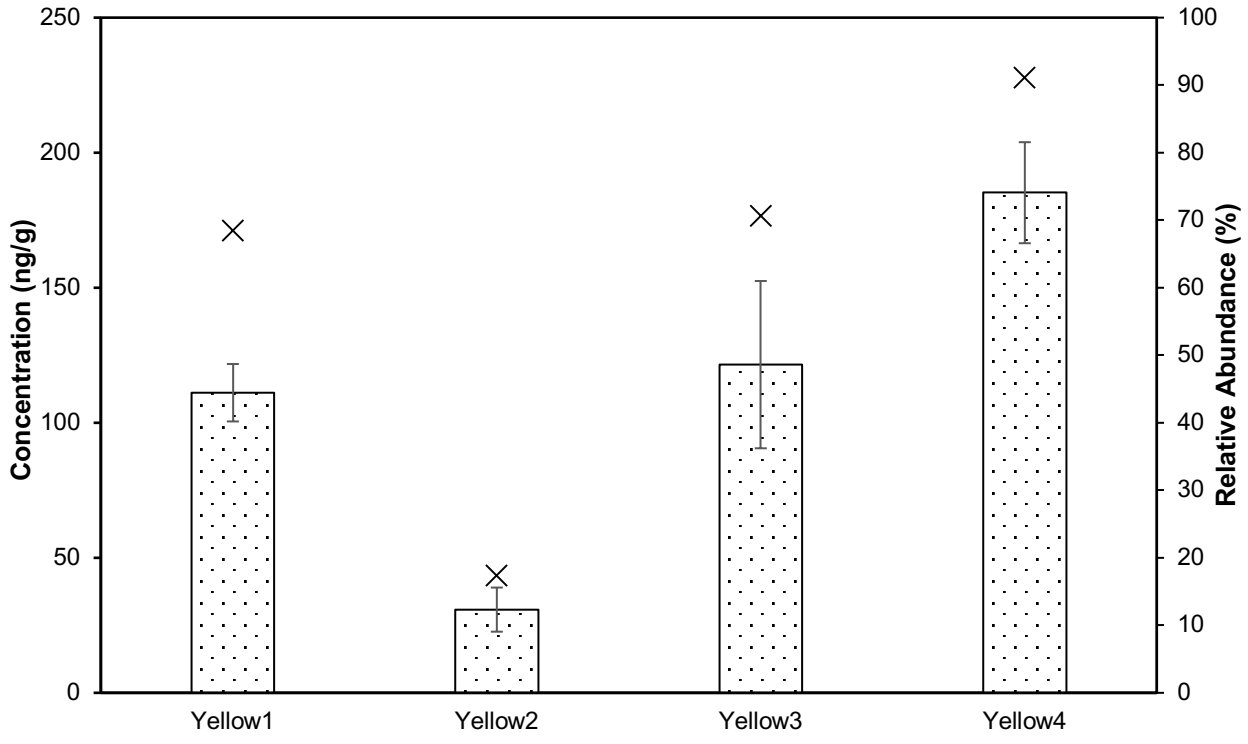

Figure 5-2. Concentration and relative abundance of PCB 11 in yellow paint samples (bar represents concentration (left axis), x represents relative abundance (right axis)).

PCB 52 can be synthesized from 2,2′,5,5′-tetrachlorobendizine (Anezaki and Nakano, 2014). It was detected up to 9.46 ± 0.73 ng/g in Yellow4. Concentrations of PCB 52 were lowest in Yellow1 (1.36 ± 0.52 ng/g) and Yellow3 (0.88 ± 0.34 ng/g). Other detected congeners with concentrations above 10 ng/g were PCB 25 (2,3′,4-trichlorobiphenyl), PCB 40 (2,2′,3,3′-tetrachlorophenyl), PCB 96 (2,2′,3,6,6′-pentachlorobiphenyl) and PCB 103 (2,2′,4,5′,6-pentachlorobiphenyl). PCB 25 was one of the most abundant congeners found in an organic red colorant (Jahnke and Hornbuckle, 2019). PCB 40 was one of the major congeners found in one polycyclic-type pigment (Anezaki and Nakano, 2014). The frequent detection of such congeners indicates that PCBs can be produced during the manufacturing of various types of pigments.

5.3.3 PCBs in white paints

The total PCB concentrations in white paint (Figure 5-3) were lower than in yellow paint samples ($p < 10^{-8}$). The total PCB concentration ranged from 7.8 ± 0.9 to 60.1 ± 6.9 ng/g in the three white samples and tri- to penta-CBs were the most dominant. No PCBs were found in the white pigments tested by Hu and Hornbuckle (2010), where the total PCB concentration was 0.03 ng/g of colorant in the white colorant. In their study, the most dominant congener was PCB 25. In this study, PCB 25 was found in White3, at 3.2 ± 0.17 ng/g. In contrast to the yellow paints, PCB 11 was not detected above the detection limit in any of the white samples, indicating that PCB 11 was not a byproduct during the manufacturing of white pigments. PCB 52 was detected in White3 at 1.32 ± 0.21 ng/g. White2 had the lowest number of chlorines per biphenyl (2.70 ± 0.30) of the three white pigments, while the other two had similar chlorine numbers (4.1 ± 0.05), which is higher than the yellow pigments. The variability in total PCB concentrations,

homolog distributions as well as chlorine numbers per biphenyl clearly shows the difference in composition for different types of pigments.

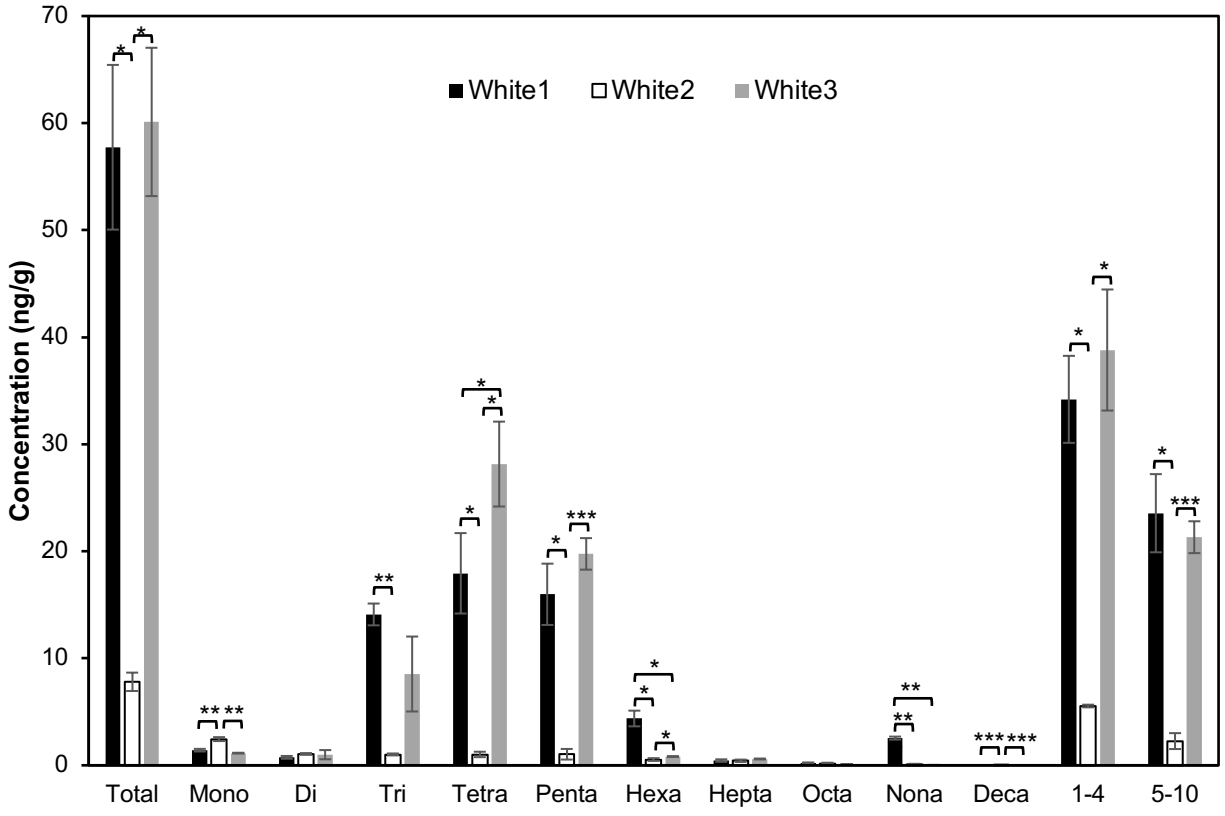

Figure 5-3. Concentrations of total PCBs, homologs, mono- to tetra-CBs, and penta- to deca-CBs in white paint samples scraped from traffic lines (significance levels: * < 0.05; ** < 0.01; *** < 0.001).

5.3.4 Potential mechanisms for PCB mobilization from paints

Rodenburg et al. (2010) presented evidence that pigments in consumer goods caused dispersion of PCB 11 throughout the environment at problematic levels. Roadway paints as a non-legacy PCB source has not been considered and mechanisms for PCBs mobilizing from painted surfaces into the urban environment have not been discussed. Due to the complexity of

the paint matrix and the environmental transport processes, several mechanisms could be involved.

Paints consist of binder, pigments, extenders, solvents, and additives (Talbert, 2007). Binder is a polymer added to hold the pigments in place. When the paint binder is destroyed or photodegraded, paint colloids can be released from the painted surface (Barnes and Davis, 1996). These colloids are mobile and can be transported to other environments by stormwater. During the process, PCBs, especially those less chlorinated, could be dissolved in stormwater, since they have lower hydrophobicity.

Surface/air transport can be a major pathway of PCBs release from paint. Jahnke and Hornbuckle found that all PCB congeners in applied paints can be volatilized (Jahnke and Hornbuckle, 2019) and accumulated on surface films formed on nearby buildings and washed away during rain events (Diamond et al., 2000, Lerner, 2002, Diamond and Hodge, 2007). Atmospheric PCBs released from roadway paints could also contribute to PCBs in stormwater via wet deposition, which could transport PCBs to surface waters. Jahnke and Hornbuckle (2019) also found that the presence of water accelerated the emissions of PCBs from colorants due to the full miscibility of colorants in water and the physical-chemical properties of the congeners.

Another potential pathway is physical erosion of paint flakes peeling off the painted surface, where stormwater can transport the released flakes. Yellow flakes have been observed in stormwater sediments and PCB 11 was abundant in these samples (Cao et al., 2019).

Due to its relative high volatility, PCB 11 can contribute to the amount of airborne PCBs (Hu et al., 2008, Hu and Hornbuckle, 2010). Although PCB 11 was found at high concentrations in roadway paint samples, it can be aerobically degraded after release into the environment via

dihydroxylation with 5,6-dihydrodiol (80%) and 4,5-dihydrodiol (20%) as two degradation products.(Haddock et al., 1995) The process could be catalyzed by biphenyl 2,3-dioxygenase, which is present in strains like *Pseudomonas pseudoalcaligenes* KF707 and *Burkholderia* sp. LB400 (Pieper, 2005).

Only yellow and white pigments were evaluated in this study. Higher concentrations of total PCBs have been found in green pigments (284 ng/g) than all other pigment colors, with PCB 209 as the most abundant congener (Jahnke and Hornbuckle, 2019).

While roadway paint contains PCB 11, it is likely not the only source of PCB 11 and other congeners in the urban environment. PCB 11 has been detected in commercial goods like newspapers, magazines, and cardboard boxes (Rodenburg et al., 2010, Guo et al., 2014) and could be released from discarded materials and be transported to waterways (Guo et al., 2014). Mass contributions of PCBs from roadway paints in urban areas are not clear. The leaching potential of pigments of different colors in road paints, and the transport pathways into the urban environment needs additional study. The high detection rate of PCB 11 in various environmental samples (air, water, and stormwater sediments) could be caused by their presence and release from paint pigments. Based on the concentrations of PCBs found in roadway paints as well as paint/air partition coefficients (Jahnke and Hornbuckle, 2019), their concentrations in water bodies may pose risk to aquatic life due to bioconcentration. In addition with the rising evidence of the toxicity of the low molecular weight congeners (Espandiari et al., 2003), more information is needed on the composition of roadway paints.

Chapter 6. Conclusion and recommendations

6.1 Conclusions

Surface sediment and bioretention media samples were collected to analyze the concentration of 209 PCB congeners as well as homolog distribution to evaluate the occurrence and removal of PCBs in urban stormwater. The presence of microorganisms capable of PCB transformation were tested in these samples. Additionally, the performance of an on-campus bioretention cell was evaluated, with emphasis on PCB removal.

6.1.1 Occurrence of PCBs in surface sediments and bioretention media

The average concentration of total PCBs ranged from 9.7 ± 1.6 ng/g to 51.6 ± 5.6 ng/g in the surface sediment samples collected in urban areas. Land use had an impact on PCB concentrations in urban areas. Higher average PCB concentrations were found near dense urban areas (39.8 ± 10.5 ng/g) and residential areas (35.3 ± 6.2 ng/g). Old buildings were found near these areas and they could be a source of PCBs due to the PCBs added into caulking and sealants before the 1970s. The PCBs found in these dense urban and residential areas were above the interim sediment quality guideline (ISQG) for sediment-dwelling organisms and could cause potential toxicity for aquatic organisms. The concentration of PCBs was positively related with the total organic carbon content (TOC) in the collected stormwater sediments. The PCB concentration varied with the particle size of the sediments. Particles > 75 μm had lower PCB concentrations than particles ≤ 75 μm ($p < 0.01$). However, PCBs sorbed to the larger particles made up the greatest mass by more than 80%. This indicated that removal of larger particles, which is easier to perform, could remove more than 80% of PCBs.

In the surface sediment samples, the most dominant homologs were penta- and hexa-CBs. The average number of chlorines per biphenyl in surface sediments ranged from 3.63 ± 0.21 to 5.39 ± 0.14. PCBs found in the surface sediment samples could be either mixtures of several Aroclors or biodegraded products of Aroclors. Some PCB congeners detected at high concentrations were also abundant in Aroclor 1254, indicating that Aroclor 1254 could have contributed to the PCB contamination in these samples.

Due to steric hindrance, chlorines at *ortho* positions are less likely to be replaced with hydrogen (i.d. dechlorination). Thus, the increase in the level of unflanked *ortho*-chlorinated congeners is an indicator of PCB biodegradation via organohalide respiration. The abundance of unflanked *ortho*-chlorinated biphenyls varied among the sites (0.41 ± 0.03% to 4.85 ± 0.42%). The abundance of unflanked *ortho*-chlorinated biphenyls found in the surface sediment samples were higher than the abundance of PCBs in Aroclor 1254 (0.03%) thus indicating that organohalide respiration occurred or PCB at these sites were from lowly chlorinated Aroclor mixtures.

For bioretention media core samples (collected from an on-campus bioretention system), the total PCB concentration ranged from 11.6 ± 1.2 ng/g to 38.4 ± 2.3 ng/g. The concentration of PCBs decreased as the depth increased. In addition, the concentration of PCBs was lower at the locations toward the end of the bioretention compared to the inlet. Only the surface sample at the inlet entrance reached a value above the ISQG and thus could cause adverse effects on dwelling-organisms. This indicated that bioretention is efficient in retaining PCBs and more filtration and sorption is taking place in the upper layer of the material. Spatial distribution of PCBs also indicated that shallow cell design (20 cm) is adequate for treating PCBs in stormwater.

The average number of chlorines per biphenyl in the bioretention media cores was 4.41 ± 0.24. No relationship was found between depth or distance from the inlet and the average chlorine numbers indicating that the number of chlorines per biphenyl had no spatial trends in the bioretention cell. The abundance of unflanked *ortho*-chlorinated biphenyl in the bioretention media core samples (1.65 ± 0.04% to 3.19 ± 2.71%) were in the same range as detected in the surface sediment. The higher level of *ortho*-chlorinated congeners than the maximum level found in Aroclors in five of the eleven samples indicated the occurrence of anaerobic organohalide respiration. A higher level of organohalide respiration could take place in the lower core sections because the fraction of *ortho*-chlorines increased with depth at each sampling location in the bioretention cell.

6.1.2 Organohalide respiration in bioretention core

Results from molecular analyses of bioretention samples indicated that bacteria capable of PCB biotransformation were present in both surface sediment and bioretention core samples. Higher bacterial abundance was found in surface layers compared to lower layers of the bioretention cell. The anaerobic organohalide respiring bacteria *Dehalogenimonas* were detected in all samples with gene copy ranging from 9 to 3.6×10^3/g sediment. The low abundance (0.0014–9.2% of total bacteria) of putative organohalide respiring bacteria in this environment indicated that low abundance of *Dehalogenimonas* was enough for PCB transformation or they were not the major bacteria performing that. In some samples both bacteria capable of organohalide respiration and bacteria performing aerobic PCB degradation were detected. Therefore, both aerobic and anaerobic PCB transformation could happen in the bioretention cell under different conditions. Identification of these bacteria showed that the most frequently

detected phyla in soil (such as Actinobacteria, Proteobacteria and Chloroflexi) were also the most abundant in stormwater sediments and bioretention media. Bacteria from the genera *Dehalococcoidia*, *Dehalobium*, *Dehalogenimonas* and *Dehalobacter* were found in six of the seventeen samples. *Dehalococcoides mccartyi*, a species confirmed capable of organohalide respiration of Aroclor 1260 (Wang and He, 2013), was found in the surface sediment from a stormwater control measure (SCM) in the commercial area. Bacteria within the genus *Rhodococcus* (capable of aerobic PCBs degradation) (Kim and Picardal, 2001), were detected in surface sediment and media core samples.

To test if the bacteria capable of PCB transformation were active, RNA from surface sediment and the top layer of the bioretention media at UMD was collected. The RNA results showed that putative organohalide respiring bacteria were active in both the surface sediment sample and surface bioretention media samples collected at UMD. Bacteria capable of PCB ring deoxygenation and/or cleavage also showed activity at the top layer in the bioretention cell. The presence of active anaerobic organohalide respiring bacteria and active PCB transformation under both aerobic and anaerobic conditions in the bioretention cell. The expression of such genes showed the potential that these bacteria are actively biotransforming PCBs in the bioretention cell.

6.1.3 Fate of PCBs in bioretention

PCBs in stormwater before and after bioretention treatment were monitored. The concentration of dissolved PCBs in stormwater varied between events, ranging from 67 ± 17 to 755 ± 23 ng/L. The concentration of dissolved PCBs decreased by 64–92% after the bioretention treatment, thus showing that bioretention is effective in removing dissolved PCBs from stormwater. PCB concentration in the particulate phase (157–638 ng/g) was higher than the

concentration found in the surface sediment samples collected in urban areas. This could be due to the smaller size of the particles in stormwater. In addition, bioretention treatment removed 52.1–98.2% of TSS in stormwater. Therefore, more than 50% of particle-bound PCBs was in this case removed from stormwater via TSS removal. Particulate-bound PCBs accounted for 2.6–17.1% of total PCB mass in stormwater, indicating that dissolved PCBs cannot be ignored when treating PCBs in stormwater.

6.1.4 Concentration of PCBs in road paints

PCB 11 is one of most frequently detected congeners from different environmental matrices and an unintentional byproduct from the manufacturing of yellow pigments. It also had a high detection rate in this study. PCB 11 was found in all of the stormwater samples, both the liquid phase and sediment samples. The relative abundance of PCB 11 ranged between 1.3% and 30.9% of total PCB concentration in the surface sediment samples and bioretention media samples. PCB 11 accounted for 20.5% of the total dissolved PCBs in stormwater. Yellow paint flakes were noted in the surface sediment samples collected near UMD and the concentration of PCB 11 was significantly lower after picking out the yellow flakes ($p < 0.05$).

Yellow traffic line paint could be a major source of PCB 11 in stormwater. Different commercial paints could be used at different locations and thus contain different components. Yellow road paints were scratched from different sites on the UMD campus and the concentration of total PCBs ranged from 162 ± 19 ng/g to 203 ± 22 ng/g in these flakes. The concentration of PCB 11 found in the yellow road paints ranged from 30.8 ng/g to 185 ng/g (17.4% to 91.1% of total PCBs). PCB 11 accounted for more than 68% of the total PCBs in three of the four samples. PCB 25, PCB 40, and PCB 52 were also found in the yellow road paints in

high concentration. White paints had reduced total PCB concentrations (7.80 ± 0.01 ng/g to 60.1 ± 6.93 ng/g) than yellow paints and the homolog distribution was different. Abundant congeners including PCB 25 and PCB 52 were found in white paints at a high concentration (PCB 25: 3.02 ± 0.17 ng/g, PCB 52: 1.32 ± 0.21 ng/g). PCB 11 was below the detection limit in the white paint samples, indicating that PCB 11 was not related to white pigment. The results indicated that road paints could be important source of PCBs, especially PCB 11, in urban areas.

6.1.5 Estimation of PCB loads and load reductions in urban areas

When solely considering the dissolved phase, the estimated annual PCB loading from stormwater ranged from 0.58 to 6.52 g/ha-yr. The annual PCB mass load in the effluent from a bioretention cell was 0.08–0.40 g/ha-yr. This estimation indicated that BMPs have a potential to reduce dissolved PCBs by 0.50 to 6.44 g/ha-yr. For the particulate phase, bioretention have a potential to reduce PCB export by 0.36 to 0.76 g/ha-yr via TSS removal. The total load reduction of PCBs in both phases could be 0.94–7.28 g/ha-yr (91.5–98.4%). The estimated load reduction indicated that bioretention treatment was a promising way to remove PCBs from stormwater in order to meet PCB TMDLs.

The sources and receptors of PCBs in urban areas are shown in Figure 6-1. The highlighted concentrations and masses result from this study. Due to release from old buildings, dense urban areas and residential areas are more important PCB sources than greenspace. The estimated annual loading of PCBs from stormwater ranged from 0.94 to 7.28 g/ha-yr. This indicated that stormwater was an important source of PCBs in this urban area. Road paints also contained a high concentration of PCBs, but the mass contribution from road paints was not quantified. Via adsorption and TSS removal, bioretention cells have the potential to remove

0.86–7.16 g/ha-yr PCBs (91.5–98.4%). In addition, PCBs retained in the bioretention media could be transformed biologically. The presence of putative organohalide respiring bacteria and PCB degrading bacteria indicated the potential of PCB transformation in the evaluated bioretention cell. However, the mass reduction of PCBs via biotransformation could not be quantified because the degradation products of PCBs were difficult to detect. Anaerobic organohalide respiration does not reduce the concentration of PCBs, instead it reduces the level of chlorination, which is a required step for aerobic PCB degradation to take place. Aerobic PCB degradation will reduce the concentration due to the cleavage of the ring structure and the subsequent mineralization to CO_2.

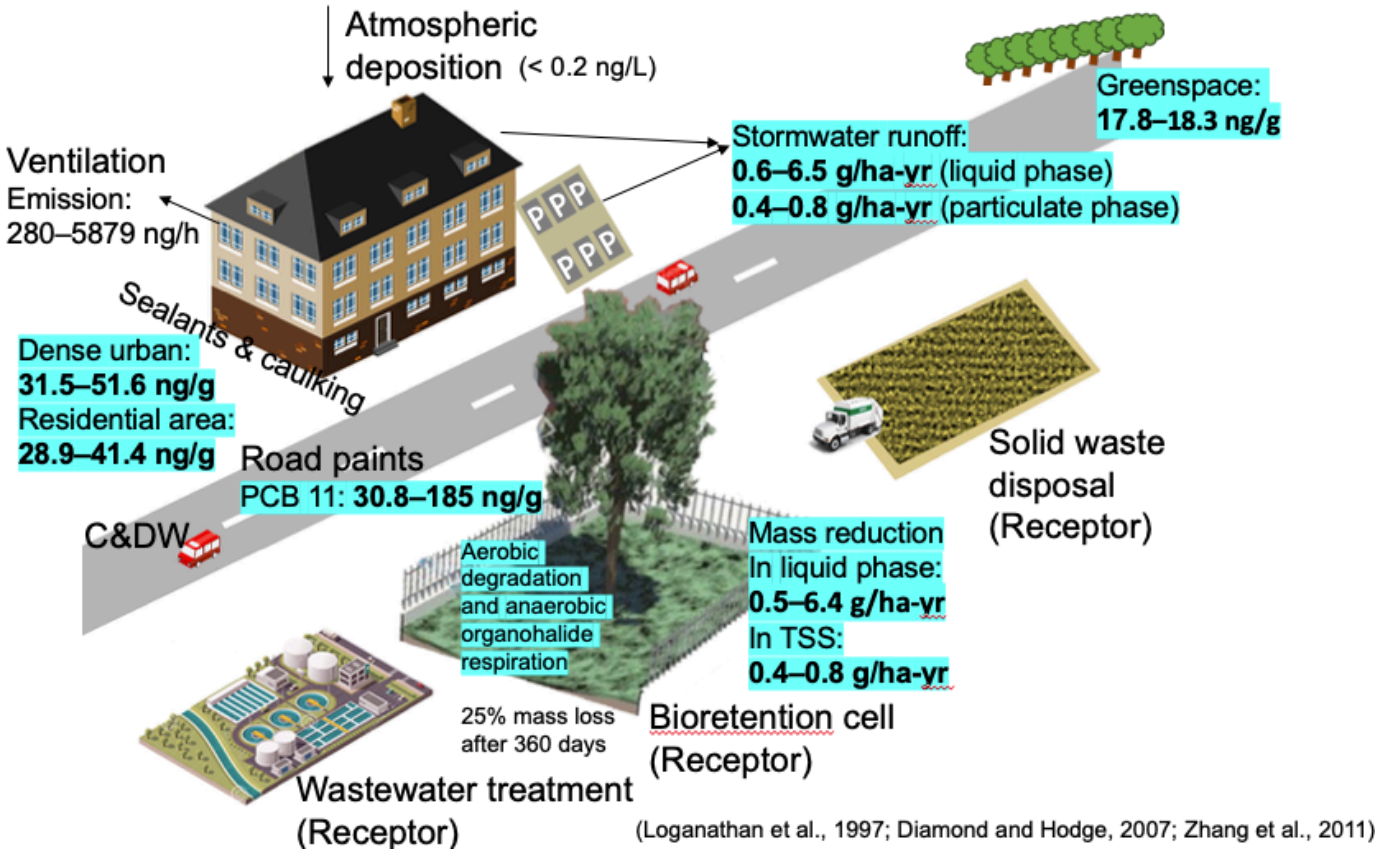

Figure 6-1. Sources and receptors of PCBs in urban area.

6.2 Recommendations for PCB removal

Bioretention is effective in reducing the concentration of dissolved PCBs as well as PCBs sorbed to particles. PCBs (based on mass) were to a larger extent accumulated at the top layer of the bioretention media compared to deeper parts of the geomedia, thus shallow bioretention cell design would enhance PCB removal in stormwater. This also means that when maintaining the bioretention cell, replacement of the top layers (5−10 cm) should be performed more frequently than lower layers. This will also help maintain bioretention infiltration rates (Davis et al., 2009).

With information on the two biotransformation pathways of PCBs, new SCM designs can be developed that can target PCB reduction without compromising removal of other contaminants in stormwater. Dynamic aerobic and anaerobic conditions would improve PCB biotransformation efficiency by first transforming the highly chlorinated PCBs into less chlorinated PCBs under anaerobic conditions and then further degrading these procedures under aerobic conditions, thus obtaining complete mineralization of the organic compounds. Anaerobic conditions could be achieved by implementing internal water storage in bioretention facilities. In other studies, internal water storage improved the rate of denitrification significantly thus a combined effect could be obtained (Igielski et al., 2019). A shallow wetland could follow a bioretention cell to retain and treat PCB contaminated effluent (Figure 6-2). At the same time, the less-chlorinated PCBs remaining in the bioretention media are ready to be degraded when the media becomes aerobic.

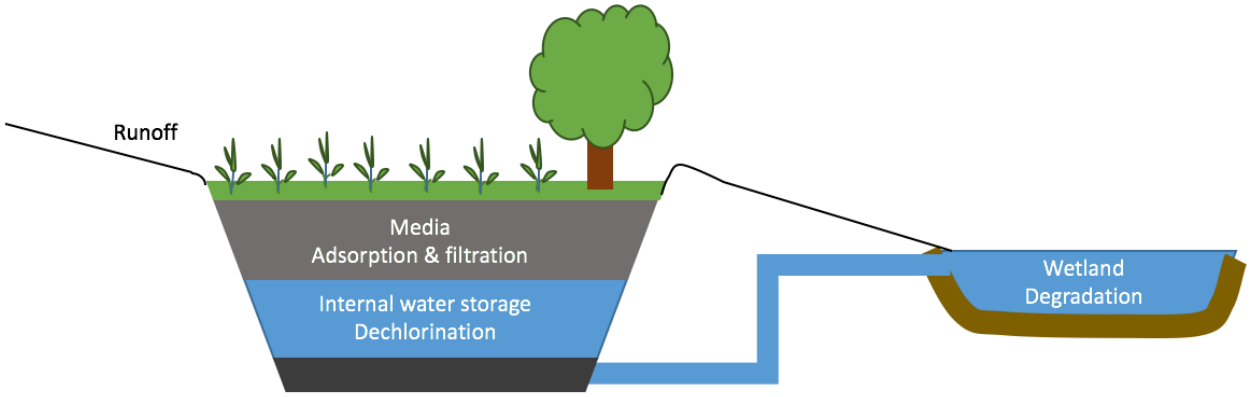

Figure 6-2. Design of a bioretention with internal water storage followed by a wetland to treat stormwater PCBs.

6.3 Recommendations for further research

Microbial communities of active microorganisms capable of organohalide respiration can be studied and compared to DNA results. RNA results can provide more specific information about the microbial activity taking place in bioretention media. In addition, mesocosms can be set up using the collected bioretention media to examine if the microorganisms in bioretention are capable of PCB biotransformation. More research is needed to design a SCM with anaerobic condition preceding aerobic conditions that can promote effective PCB biodegradation.

In this study, only two storm events were analyzed for PCBs. More stormwater samples as well as rainfall data should be collected. Concentrations of PCBs in both dissolved phase and particulate phase need to be compared to find out the contribution of each phase. With rainfall information as well as influent and effluent volumes, total PCB mass as well as annual PCB mass load can be refined. Reduction in PCB mass load can also be refined with the above information. Such information can help evaluate the effectiveness of bioretention in PCB

removal from urban stormwater runoff. Information from this future study will help complete figure 5-1 by estimating the mass load of PCBs from stormwater runoff in urban area more accurately.

Relative abundance of PCB 11 was up to 90% in one of the yellow paints scratched from a yellow traffic line. Information on the concentration of PCBs in yellow paints from different manufacturers is needed. Leaching characteristics of paints also needs to be studied using synthetic stormwater to avoid interference from real stormwater. The paint with the least PCBs and lowest leaching possibility should be recommended for public use. Loading of PCBs from road paints as well as construction and demolition waste (C&DW) should also be estimated to better evaluate the contribution of different sources to PCBs in urban areas (Figure 5-1). Only by understanding the importance of various sources, more specific measures can be taken to control PCBs in urban areas.

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