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ASSESSING ONGOING SOURCES AND FATE OF DISSOLVED POLYCHLORINATED BIPHENYLS (PCBs) IN A STREAM

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POLYCHLORINATED BIPHENYLS (PCBs) IN A STREAM

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In Partial Fulfillment
of the Requirements for the Degree
Doctors of Philosophy
Environmental Engineering and Earth Sciences

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by
Viet D. Dang
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Accepted by:
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ABSTRACT

Polychlorinated biphenyls (PCBs) contamination remains a concern due to their persistence and the risk to human health associated with them. Once released into the environment, these substances are mainly associated with sediment particles and sediment organic carbon matter. They bioaccumulate in organisms via contaminated food and only a small faction is desorbed from sediment into the water column. Given the discontinuity of active discharge, PCBs are currently entering the water via groundwater inputs, soil erosion, and/or ongoing leakage from the point source. It is the dissolved form that 1) can be either absorbed by biofilm attached to stream leaves, which is a basal resource of stream food webs, and 2) can cross the air-water interface and sorb to leaves while they are still on the plants. Many studies have investigated the fate and transport of PCBs in fish and consumers at higher trophic levels. However, limited knowledge exists on the source and fate of bioavailable PCBs as a result of the complexity in accurately measuring the dissolved fraction. This research thus aimed to provide insight into the potential of ongoing sources and the entry of dissolved PCBs to a stream food web. Studies were conducted in Town Creek, a recipient of the Twelvemile Creek/Lake Hartwell Superfund site, SC. The system is a second order stream contaminated with PCBs from a former Sangamo Weston capacitor manufacturer (S-W) in Pickens County, SC.

Assessing potential ongoing sources of dissolved PCBs were based on total PCB concentrations, congener composition profile, and chiral signatures with collection by polyethylene passive samplers (PEs). The concentrations of PCB in the PEs at 0 km

downstream of the plant were 2-5 fold lower than downstream PE concentrations. The congener profile in the PEs 0 km downstream of the S-W site was similar to that of a mixture of Aroclors 1016 and 1254 (4:1 v/v), historically released from the S-W plant. Chiral PCBs 91 and 95 were the only two congeners detected in the passive samplers. While the enantiomeric fraction (EF) values for PCB 91 were non-racemic (>0.5), the EFs for PCB 95 were either racemic (=0.5) or nearly racemic. Increasing PCB concentrations downstream and a composition of predominantly low-weight congeners in the PEs at 0 km downstream of the plant site suggested ongoing sources of dissolved PCBs from the original S-W facility to the system. Consistent EF values were likely indicative of aerobic biotransformation of dissolved PCBs, but did not provide strong evidence for potential ongoing PCB inputs into the system.

Uptake rates of dissolved PCBs by microbial decomposing leaves were monitored by deploying fresh leaves in the streambed. Four individual leaf species including three deciduous species (*Acer rubrum*, *Quercus rubra* L., *Liriodendron tulipifera*) and and one evergreen (*Rhododendron maximum*) were used due to their different leaf quality. Uptake rates and PCB concentrations were significantly changed with time for the deciduous species, but not for the evergreen rhododendron. The deciduous species was subject to microbial colonization which drives microbial decomposition at a greater extent, and thus faster uptake of PCBs, than the evergreen rhododendron. A positive correlation was observed between PCB concentrations and lipid content in all four species, but the correlations were not strong. PCB homologue distribution was time consistent among

species and in a good agreement with congener patterns of dissolved PCBs in the passive samplers.

The sources and magnitude of PCBs in the evergreen rhododendron next to a contaminated stream were investigated due to its long-lived foliage and an elevated PCB concentration measured in fresh leaves. Leaves were collected from the same shrubs in Town Creek throughout three seasons (fall 2010, winter and spring 2011). Concentrations were similar between fall and winter, and then decreased in spring. High concentrations in fall were likely related to high flow rate and seasonal precipitation, while increased revolatilization of low chlorinated congeners with increasing temperature could result in decreased PCB concentrations in spring. Two other evergreen species (American holly and blue spruce) collected 1 km upstream of the S-W plant site had PCB concentrations below the detection limit. In addition, congener pattern in rhododendron leaves was similar to the PEs, while it differed from the congener distribution in surficial soils. These observations suggest that the source of PCBs in the rhododendron is through volatilization of dissolved PCBs in the water rather than uptake from soil or atmospheric long-range transport.

Dissolved phase organochlorine compounds are of great concern due to their ability to transport to biological membrane. This dissertation proposed and examined a conceptual model to better understanding the potential for current sources and fate of dissolved PCBs in a small stream. Results from this research demonstrate the knowledge gaps about the entry of contaminants to food webs. The study will also serve as fundamental to assess source control and human health risks.

DEDICATION

This manuscript is dedicated to my family for supporting me throughout the graduate school.

ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

Background

Persistent organic pollutants (POPs) are chemical substances that persist in the environment, bioaccumulate through the food web, and pose a toxic effect on ecosystems and human beings. While there are many POPs of interest, this dissertation focuses solely on polychlorinated biphenyls (PCBs). They are a mixture of up to 209 congeners that differ in the number and location of chlorine atoms around the biphenyl molecule. The basic structure and numbering of PCBs are shown in Figure 1.1. They were produced by chlorination of biphenyls, and their commercial production started about 60 years ago (Ivanov and Sandell, 1992; Rantanen, 1992). Total estimated global production of PCBs was 1.1×10^9 kg through 1980 (Erickson, 1997). Their commercial productions were marketed with respect to percentage of their chlorine content (by weight) and were available under several trade names, for example, Aroclor (Monsanto, USA); Clophen (Bayer, Germany); Kanechlor (Kanegafuchi, Japan); Phenoclor and Pyralene (Prodolec, France) (Erickson, 1997). PCBs are either oily liquids or solids and have no smell or taste (ATSDR, 2000). Some PCBs (e.g., mono-, di-, and tri-chlorobiphenyls) are more volatile than higher chlorinated congeners and may exist as a vapor in air. Physicochemical properties of PCBs, like inflammability or electric conductivity, made them available in a wide field of application. Thus, PCBs have been used as dielectric fluids in coolants and lubricants in transformers, capacitors, and other electrical equipment (USEPA, 1976).

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Like dioxin, PCBs are persistent in the environment, resistant to biodegradation, and

prone to accumulate in food webs.
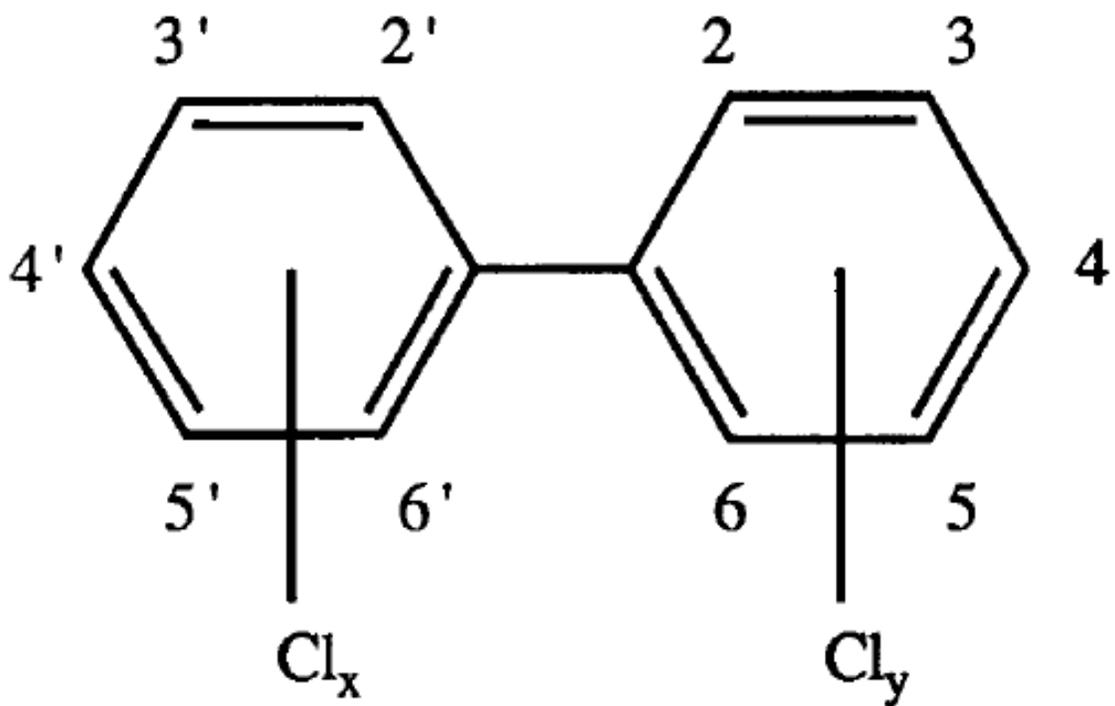
Figure 1.1. The basic structure and numbering scheme for PCBs. X = 1 to 5, Y = 1 to 5,
$X + Y \geq 1$.

Conceptual model

A conceptual model of the sources and fate of PCBs in aquatic environments can be presented in Figure 1.2. Due to their degree of hydrophobicity, PCBs enter stream systems mainly through particle transport. Currently, they are likely entering through groundwater, erosion from lands, or ongoing leakage from the original source. Although a large amount of PCBs resides in the sediment, which will be ultimately exported downstream, a small but significant fraction is desorbed into the water column. This dissolved fraction is readily bioavailable and can be transported into biological membranes of aquatic organisms via passive diffusion and may exert toxic effects (Suffet et al., 1994; Escher and Hermens, 2004). Furthermore, the dissolved fraction can be absorbed by decomposing leaves in the stream. After these leaves enter streams, they are colonized by biofilms consisting mainly of bacteria and fungi. These so-called "conditioned leaves" are an important source of carbon and nutrient to stream food webs; and therefore, they may also be an important pathway to transfer contaminants into stream food webs. Given the high Henry's law constants for some of the congeners, the dissolved PCBs can also volatilize to the atmosphere and sorb to leaves, while they are still on the plant. This in turn is the first step of a pathway to transport contaminants to land-based food webs. It is thus critical to understand the dissolved phase because it causes risk to human through the entry of contaminants into the food webs and through inhalation due to volatilization. In this dissertation, a core focus was to better understand the sources and fate of dissolved PCBs in a stream because their strong hydrophobic

character has complicated sample collection, extraction, and analysis, making understanding the behavior of dissolved PCBs difficult to discern.
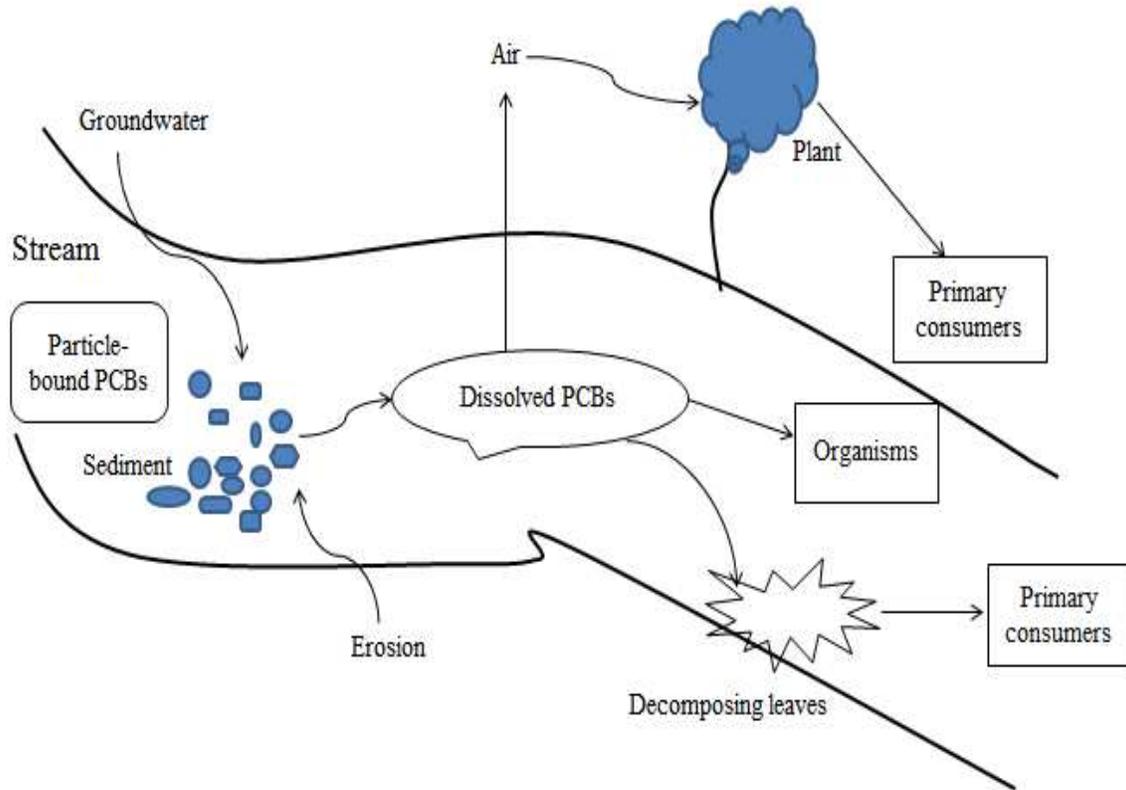
Figure 1.2. Generic pathways of dissolved PCBs into a stream system.

Study area

The Sangamo Weston Superfund site is located in the Piedmont physiographic province of South Carolina and Georgia, a broad plateau area ranging in elevation from 400 to 1,400 ft above sea level (USEPA, 1994). Sangamo Weston Inc. (S-W) opened a capacitor manufacturing plant in Pickens County, SC, in 1955 (USEPA, 1994). The plant

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used several varieties of PCB-containing dielectric fluids in its manufacturing processes. Manufacturing operations continued until 1987 when Sangamo Weston was sold to Schlumberger Industries (USEPA, 1994). The greatest quantities of PCBs used during its operation consisted of Aroclors 1242, 1254, and 1016. Waste disposal practice included on-site land burial as well an unspecified amount of PCBs buried in six satellite disposal areas (Brenner et al., 2004). Between 1955 and 1977, more than 400,000 lbs of PCBs was discharged with effluent from the S-W plant directly into Town Creek, a tributary of Twelvemile Creek, which is in turn a tributary of Lake Hartwell reservoir (USEPA, 1994). A map of the Sangamo-Weston/Twelvemile Creek/Lake Hartwell Superfund site is presented in Figure 1.3. Twelvemile Creek watershed is also the source of drinking water for the public water supply systems owned and operated by the Town of Pickens, which serves around 43,000 people (USEPA, 1994).
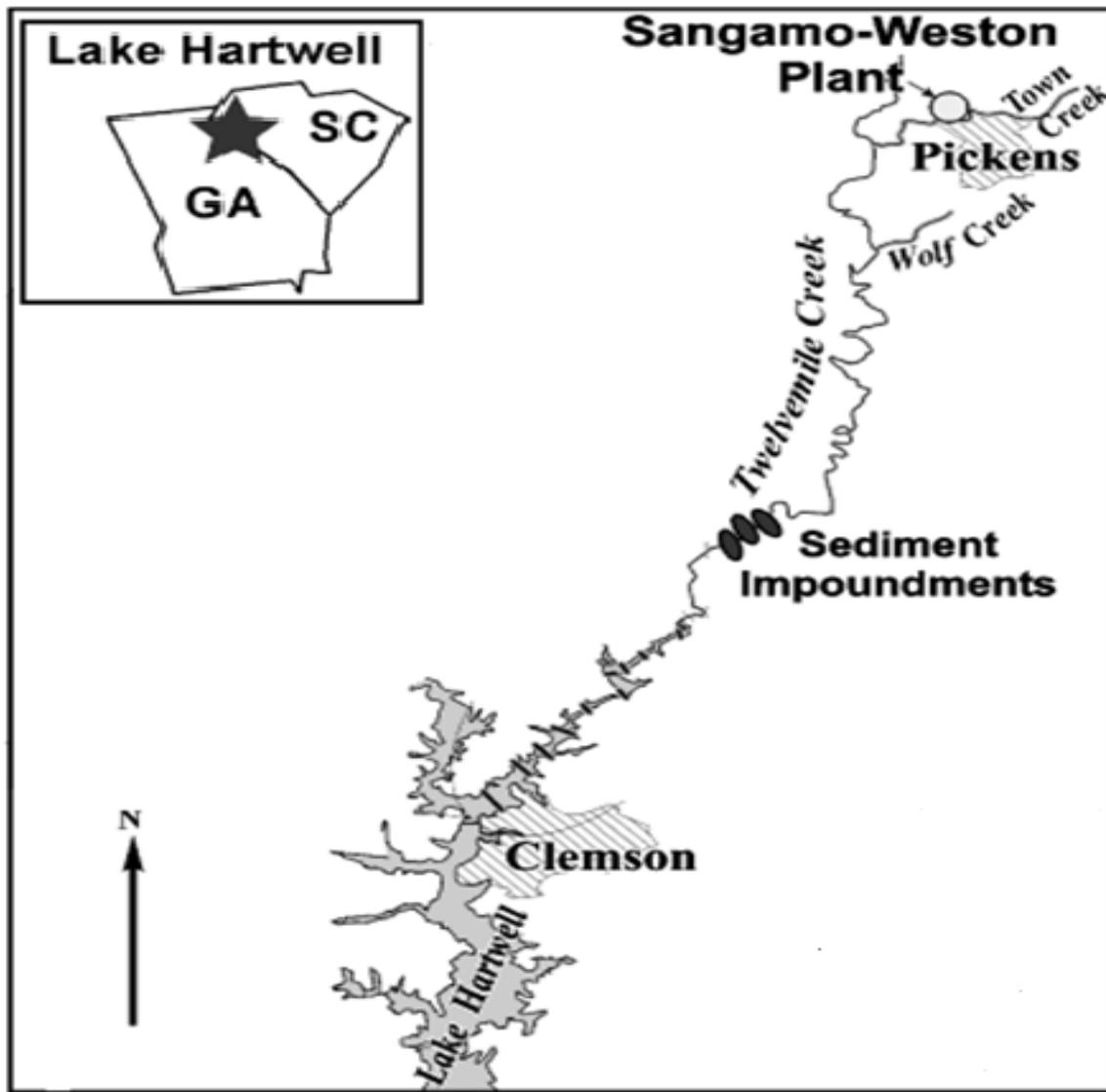
Figure 1.3. Map of the Sangamo-Weston/Twelvemile Creek/Lake Hartwell Superfund site (Brenner et al. 2004).

Initial studies of surficial and core sediment of the Twelvemile Creek watershed and Lake Hartwell were conducted by South Carolina Department of Health and Environmental Control (SCDHEC) and several Clemson graduate students from 1976 through the late 1980s (USEPA, 1987). PCB concentrations were highest in surficial sediments collected near the discharge point of the plant on Town Creek. The concentrations generally decreased with increasing distance downstream from the S-W plant site. PCB sediment concentrations in core samples were highest in samples collected from the Twelvemile Creek Arm of Lake Hartwell. In addition, PCBs were detected in Lake Hartwell fishes at levels above the safe tolerance level (5 ppm) at that time (EPA, 1987). The level of concern was lowered to 2 ppm in 1984 by the U.S Food and Drug Administration (FDA) (SCDHEC, 1987). An additional recommendation was issued that all fishes over three pounds taken from any portion of Lake Hartwell not be consumed (SCDHEC, 1987).

Recent investigations report high levels of PCBs throughout Twelvemile Creek system (Dang, 2007; Walters et al., 2008). Samples collected in Town Creek had consistently the highest total PCBs among food web compartments (Walters et al., 2008). Fish PCB concentrations still exceeded 2000 ng/g wet wt, which is the current federal advisory for fish consumption. These findings suggest an ongoing PCB sources from the plant site to Town Creek because if this source had been terminated, low levels throughout the system would be expected. Results from these studies will be in part supplemental to my following research hypotheses.

<u>Research hypotheses</u>

The main objectives of this dissertation were to assess potential ongoing inputs of dissolved phase PCBs into a stream system and to provide insight into the fate of PCBs at a base of food webs. I proposed three hypotheses in this research:

1)   There were additional sources of PCB from the vicinity of the S-W facility entering to Town Creek water system as a result of a) increased PCB concentrations with increasing downstream distance, b) similarity of congener profile in the water at the contamination source and historical discharge from the plant, and c) racemic mixture near the source and deviation from racemic mixture of chiral PCBs downstream of the source.

2)   Leaves with higher quality of nutrient (e.g., lower initial C:N and lignin content) would be microbially colonized more rapidly, which in turn absorbed dissolved phase PCB at a greater extent, than the ones with lower nutritional quality.

3)   Evergreen leaves next to a contaminated stream were contaminated with dissolved PCBs in the water column due to volatilization rather than soil uptake or long-range atmospheric transport and the temporal PCB variation in the leaves was related to stream discharge, precipitation, and air temperature.

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CHAPTER 2

ASSESSING ONGOING SOURCES OF DISSOLVED PHASE PCBs

IN A CONTAMINATED STREAM.

Abstract

Few studies assess the potential of ongoing sources of "fresh" PCBs to aquatic systems when their direct discharge to the environment has been eliminated. Here, I used passive sampling methods to investigate the fate and source of dissolved phase PCBs in a stream with a legacy of severe contamination. I used single-layered low density polyethylenes (PEs) to measure total PCB concentrations, congener profiles, and enantiomeric fractions (EFs) in the stream and to provide multiple lines of evidence for assessing ongoing inputs of PCB to the system. PCB concentrations were well above upstream background levels and significantly increased with distance as downstream PE concentrations were almost five times greater than PE concentrations at the contamination source. PCBs in the PEs at 0 km downstream of the site of a former capacitor manufacturer were dominated by low K_{ow} congeners, similar to those indicative of the mixture of Aroclors 1016 and 1254 (4:1 v/v) historically released from the plant. PCBs 91 and 95 were the only two chiral congeners detected in the PEs downstream. EF values were non-racemic for PCB 91, while the values were either racemic or close to racemic for PCB 95. Increased PCB concentrations with distance and a congener composition of predominantly low-weight congeners in the PEs at 0 km downstream of the plant site suggested an ongoing PCB source from the plant site. Chiral signatures

suggested aerobic biotransformation of dissolved PCBs, but did not shed any light on possible ongoing PCB inputs to the water column.

Introduction

Aquatic environments receive inputs of organic pollutants from current and historical industrial sources. Although PCBs have been banned for several decades, their degree of hydrophobicity makes them persistent and soluble in fats, and therefore, capable of accumulating in the biological tissues (USEPA, 2007; NRC, 2001). PCB remedial action at contaminated sites is thus necessary to mitigate risks to the entire ecosystems and to human health. Recommended clean-up activities typically involve the removal of contaminated soils and sediments to reduce or eliminate exposure pathways. Broadly referred to as "source control", these efforts that are aimed at limiting PCB entry into environmental systems must be completed before additional clean-up steps can be effective (USEPA, 2004; NRC, 2003). Although PCBs generally accumulate in sediments, it is the dissolved form that enters bacteria and plants at the lowest levels of aquatic food webs (Colman, 2000). Therefore, understanding the dissolved fraction of environmental pollutants is critical for assessing source control and potential biological impacts.

Application of passive sampling devices (e.g., low-density polyethylene, PE; semi-permeable membrane device, SPMD; and solid phase microextraction, SPME) to study the bioavailability of hydrophobic contaminants has been widely demonstrated (Huckins et al., 1993; Booij et al., 2003; Carls et al., 2004). Their operation is based on

the free flow of analyte molecules from the sampled medium to a receiving phase in a sampling device (Adams et al., 2007). The accumulation of analytes in passive samplers continues until equilibrium is established in the system, or until the sampling period is completed. Passive samplers are extensively used in environmental assessments because these methods are more accurate for measuring dissolved PCB concentrations than biota and sediments where biotransformation can occur (Vrana et al., 2005; Huckins et al., 1990; Verweij et al., 2004), and because they accumulate a range of hydrophobic organic contaminants including PAHs and PCBs (Huckins et al., 1993; Booij et al., 2003).

Many organic contaminants (e.g., agrochemicals, pharmaceuticals, and organochlorines) are present in the environment as enantiomers that are similar in structure but not super-imposable due to restricted rotation (Eljarrat et al., 2008). Enantioselective analysis of chiral compounds is a proven tool for source apportionment and chemical characterization (Bidleman and Falconer, 1999; Bidleman et al., 2004). Chiral signatures can provide insight into the biotransformation of contaminants in aquatic food webs because only biological processes, but not physical and chemical processes in an achiral environment, will change enantiomer compositions (Kallenborn and Huhnerfuss, 2001). Chiral signatures of the enantiomers are defined by the term, enantiomeric fraction (EF). If its optical rotation is known, the EF is calculated by the peak area determined by gas or liquid chromatography of the (+)-enantiomer divided by the sum of the peak area of (+) and (-)-enantiomers. Otherwise, EF is defined by the peak area of the first eluting enantiomer divided by the total peak areas of the first and second eluting enantiomers. Therefore, non-racemic EFs ($\neq 0.5$) indicate biotransformation of

contaminants or that a weathered contribution serves as a predominant source, while racemic EFs (=0.5) indicate either the lack of enantioselective degradation in the aquatic system or the chemicals are emitted as "fresh" components, such as an unweathered pollutant (Padma et al., 2003).

PCB congener-specific and chiral analyses in passive samplers have been increasingly used to assess the sources of PCB in water. For instance, the congener profile in passive samplers was monitored to distinguish between atmospheric deposition and in situ sediment remobilization as sources of dissolved PCBs (Litten et al., 1993; USGS, 2003; Allan and Rannekley, 2011). Recent research has also employed chiral analysis to provide insight into the sources of PCB in the water column (Asher et al., 2007; Dang et al., 2010). In this study, I applied passive sampling methods and analytical tools (e.g., total concentrations, congener profile, and chiral signatures) to investigate the potential for ongoing PCB sources at a small stream that is part of the Sangamo-Weston Superfund site. The site was heavily contaminated with PCBs released from the former capacitor manufacturing plant. Although previous studies report decreased concentrations with increasing distance from the plant site, high concentrations of PCBs were consistently observed in surficial sediment and biota collected below the discharge point of the plant (Walters et al., 2008, URS, 2004, 2011). These results led me to hypothesize that there were ongoing sources of dissolved PCBs from the plant site entering the stream, while sediment remobilization was a minor contribution to dissolved PCBs in the water. If PCBs are still leaking from the original S-W plant site, I expect that: 1) PCB concentrations in the dissolved phase near these sources are higher than background

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levels, 2) congener pattern of dissolved PCBs near the discharge point will resemble congener distributions in fresh or historical discharge source, and 3) chiral signatures of dissolved PCBs should indicate a racemic mixture near the sources and deviate from racemic with distance from the source due to biotransformation processes associated with cycling of PCBs within the stream. The combination of total PCBs, congener distribution, and EF values with collection by passive samplers will be thus a promising application to study additional inputs of PCB to aquatic systems.

Materials and methods

Passive sampler preparation

I used low density polyethylene (LDPE) membrane as the in situ sampler because it is inexpensive, quickly reaches equilibrium which reduces the effect of biofouling, and is durable under extreme environmental conditions (Verweij et al., 2004; Booij et al., 2002; Wennrich et al., 2003). In general, LDPE tubing (film thickness of 50μm) was purchased from Brentwood Plastics, Inc. (St Louis, MO). I prepared single-layer sheets (5 cm long, 2.5 cm wide) by cutting along the edges of the tubing (Figure 2.1), and pre-cleaned the PEs by sequentially soaking in dichloromethane for 24 hours, in hexane for 24 hours, and in methanol for 24 hours (Fernandez et al., 2009).

PE deployment devices were constructed using a stainless steel dipping basket (10 × 6 × 6 in). PE sheets were woven on copper wires and then attached to mesh openings of the basket (Figure 2.2). Two metal posts were driven into the streambed and positioned perpendicular to the current. Each basket containing three PE sheets was suspended

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approximately 20 cm below the water surface and secured to the metal posts via copper wires. Undeployed PEs served as field blanks during initial deployment and as laboratory blank for analysis of contamination.

Sediment collection

Sediment from multiple depositional areas from sites D6 to D8 (Figure 2.3) was collected. Approximately 50 g of surficial sediments (5-cm) were sampled using a large metal spoon. Samples were composites and three replicate samples were prepared. The samples were stored in amber jars, and transported to the laboratory for further PCBs extraction and analysis.

Figure 2.1. Single-layer polyethylene (PE) (5 cm long, 2.5 cm wide) used to collect dissolved PCBs.

Figure 2.2. Stainless stain deploying basket. Three PEs were woven on copper wires and then attached to mesh openings of the basket

Study area

My study area was in Town Creek, a tributary of the Twelvemile Creek/Lake Hartwell Superfund site located in Pickens County, SC. Samplers were deployed from 15th December, 2010 to 15th February, 2011 at eight sites located 0 to 3 km downstream from the Sangamo-Weston (S-W) site (Figure 2.3). The plant operated from 1950 through the late 1970s. The former discharge point of the S-W site is located approximately 130 feet downstream of the site D1. There is also an abandoned wastewater treatment plant located about 1 km upstream of sites D6 through D8. The plant was built to treat waste from the S-W plant (Craig Zeller, USEPA region 4, personal communication). Site distances and coordinates are provided in Table 2.1. Town Creek is a third-order stream with widths of 3 to 10 m and depths that range from 0.3 to 1 m (USEPA, 1994). Sediment in the creek is composed primarily of sand, which has low total organic carbon content, ranging from 0.1 to 3.6 % (USEPA, 1994). The flow and depth was below normal due to several years of below normal rainfall.
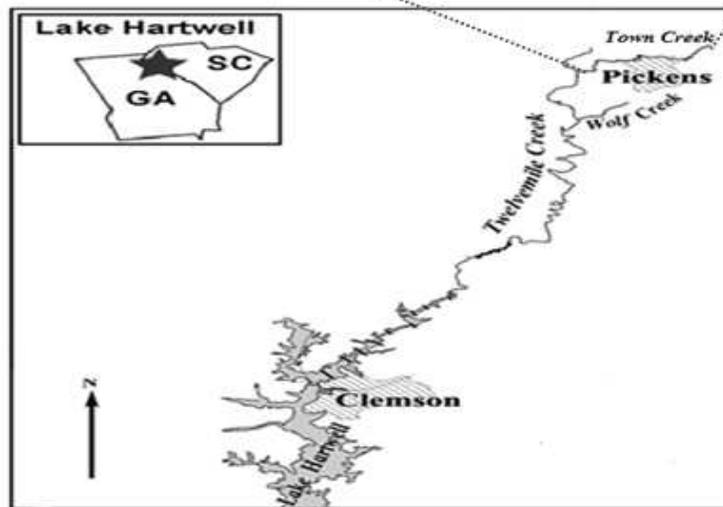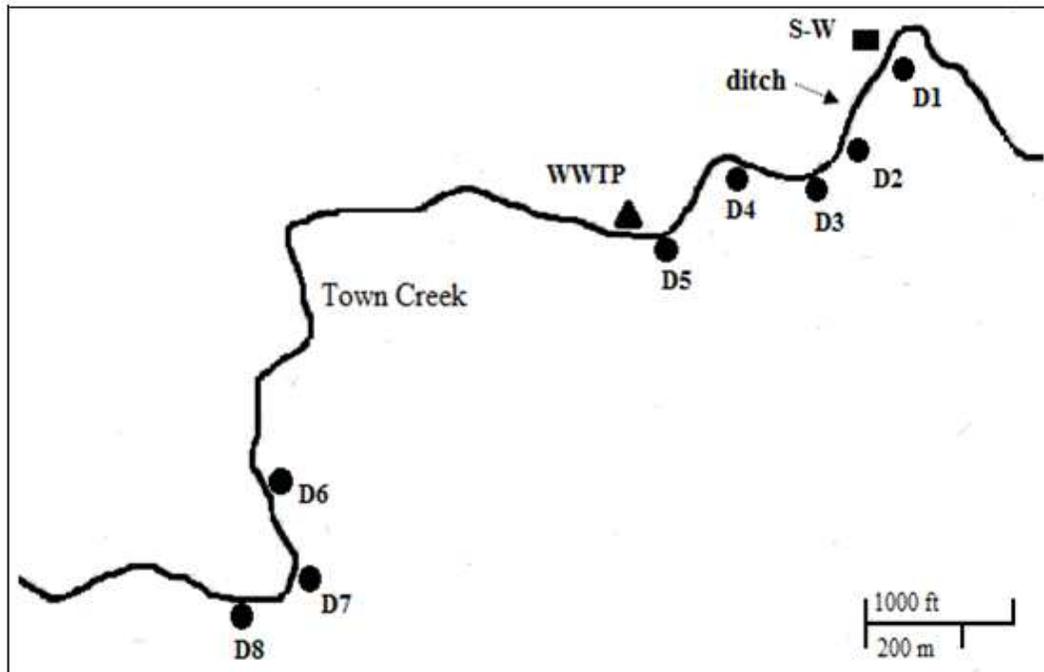
Figure 2.3. Map of Town Creek with PE deployment sites. Solid square and circles indicate the former Sangamo plant (S-W) and sampling locations, respectively. Solid triangle is an abandoned wastewater treatment plants (WWTP). Arrow indicates the discharge ditch from S-W into Town Creek.

Table 2.1. Site distances and coordinates for PEs deployed in Town Creek, SC

Site	Description	Coordinates (DMS)[a]
D1	Town Creek at Sangamo Rd. approximately 150 m below the Sangamo site (130 feet upstream of the discharge ditch)	N34° 53.471' W82° 43.332'
D2	Town Creek at Sangamo Rd. approximately 120 m downstream from site D1 (260 feet downstream of the ditch)	N34° 53.418' W82° 43.381'
D3	Town Creek at Sangamo Rd. and Reece Mill Rd approximately 70 m downstream from site D2 (500 feet downstream of the ditch)	N34° 53.394' W82° 43.418'
D4	Town Creek at Sangamo Rd. and Reece Mill Rd approximately 100 m downstream of bridge from site D3 (830 feet downstream of the discharge ditch)	N34° 53.373' W82° 43.478'
D5	Town Creek at Sangamo Rd. and Reece Mill Rd approximately 170 m downstream from site D4 (1380 feet downstream of the discharge ditch)	N34° 53.416' W82° 43.574'
D6	Town Creek at Shady Grove Rd. approximately 1130 m downstream from site D5	N34° 53.073' W82° 44.191'
D7	Town Creek at Shady Grove Rd. approximately 180 m upstream from site D6	N34° 52.986' W82° 44.141'
D8	Town Creek at Shady Grove Rd. approximately 110 m downstream of bridge from site D7	N34° 52.963' W82° 44.208'

a: Degree, min, and second

Experimental methodology

PE samplers were retrieved after 60 days since my preliminary study indicated that 60 days was enough time for uptake of low-weight congeners to approach equilibrium, but not for heavy-weight congeners. The samplers were returned in a cooler with ice to the laboratory for PCB extraction and gas chromatography (GC) analysis. Any particulate matter and biofilm present on the PEs was gently removed (using tap water) as these may act as accumulation and/or biodegradation sites for PCBs. PCB extraction from PEs was accomplished via dialysis (2×24 hours) in 20 ml dichloromethane. One hundred microliters of surrogate standards containing two non-Aroclor congeners (PCBs 14 and 169) at 2 mg/l were spiked into the PEs prior to extraction. The combined extracts were then eluted through a drying column made of 10 g of baked sodium sulfate. The final extracts were solvent exchanged in isooctane and concentrated to 2 mL for GC analysis. PCBs extraction from sediment was conducted with an Accelerated Solvent Extractor (Dionex, ASE-200) using 1:1 hexane:acetone solvent (USEPA, 2005). Approximately 15 g of air-dried sediment was homogenized with 5 g of baked Na_2SO_4. The mixture was spiked with surrogate standards and loaded into samples cells for extraction. The extracts were cleaned up on a drying column, followed by an aluminum column, and solvent exchanged in isooctane.

Methods of achiral analysis were adapted and modified from previous work (Dang et al., 2010; Dang, 2007). Briefly, total PCBs and congener-specific analysis were conducted with a HP 6890-GC equiped with a RTX-5 column (Restek, Bellefonte, PA; 60m length, 0.25 mm diameter, and 0.25 µm film thickness) and a ^{63}Ni electron capture

detector (ECD). Helium (99.99% high purity) and nitrogen (99.95% high purity) were employed as carrier and make-up gas, respectively. The GC conditions began with the initial oven temperature at 115°C for 2 min. The temperature was increased to 185°C at a rate of 5°C/min and in turn to 260°C at a rate of 2°C/min. It was held at 260°C for 15 min. The injectior and detector temperatures were set at 215°C and 325°C, respectively. The flow rate, anode, and make-up gas were conditioned sequentially by 2.0, 6.0, and 60.0 ml/min, respectively. This quantification method reported 92 peaks (domains) that included 140 target PCB congeners because some congeners co-eluted on the 60-m capillary column (Dang, 2007). To assign unknown peaks and absolute retention times (RTs), a mixture of Aroclors 1016, 1254, and 1260 (1:1:1 w/w/w) was used. The sum of the concentrations of 140-congeners was computed and reported as ΣPCB values. A calibration standard curve was constructed using five levels of 1:1:1 mixture of Aroclors 1016, 1254, and 1260 with two injections at each level. The mean slope (response factors) and intercept were calculated for all targeted PCB congeners. Recalibration was required if a shift in the peak area count of > 5% was observed. A procedural blank (blank solvent), laboratory control blank, field blank, a spiked matrix blank (1:1 mixture of A1016:1254), and a check standard (mid-level standard solution) were included with every GC sequence of 10 to 15 samples per batch. The method of internal standard calibration was applied with two internal standards (aldrin and PCB 204). The chromatogram was divided into two equivalent time segments. The first and second time segments were calibrated relative to aldrin and PCB 204, respectively. To calculate the detection limits, the area of baseline noise over retention time of each congener was first

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determined from three injections of the matrix blank spiked with the lowest concentration of the calibration standard. The instrument detection limits were three times the standard deviations of the baseline noise divided by the slope of the calibration curve.

Chiral PCB congeners were analyzed on an Agilent 6850 GC-ECD and a 30-m Chirasil-Dex column according to the methods described by Wong et al. (2001) and Hall (2004), and modified by Dang et al. (2010). For EF determination, two separate standard solutions were collectively prepared by the use of neat congeners and 1:1:1 mixture of Aroclors 1016, 1254, and 1260 dissolved into isooctane, respectively. Both solutions were analyzed on the GC-ECD using a 30-m Chirasil-Dex column and no interferences with three chiral PCBs (91, 95, and 149) were present. Interferences were defined as a coelution of the enantiomers with another homologous congener. Elution order of enantiomers was obtained from Wong et al. (2000). A check standard was included in every batch of six samples. The EF values for the standards ranged between 0.5 and 5% relative standard deviation (RSD) for all target chiral congeners.

Among-site differences in total PCB concentrations and EF values were compared by one-way analysis of variance (ANOVA), with Tukey honestly-significant-difference post hoc test ($P < 0.05$). Congener composition patterns in the PEs, mixture of Aroclors 1254:1016 (4:1 v/v), and sediment were evaluated using nonmetric multidimensional scaling ordination (NMS, PC-ORD 4.0, MjM Software) (McCune et al., 2002). NMS is an ordination method that is applied when data are not normally distributed or have discontinuous and unequal scales. NMS analysis was run in autopilot mode, which allowed the program to choose the best solution at each dimensionality (McCune and

Mefford, 1999). I used the Sørenson (Bray-Curtis) coefficient as the distance measure in the analysis. I ran the NMS analysis using the mean relative abundances of PCB homologues (proportion of total PCBs) for the PEs and sediment at each site. Relative abundance data were arcsine transformed prior to analysis.

Results and discussion

Deployments and retrieval of PEs was successful except for sites D5 and D7. The samplers at site D5 were lost and the basket containing the samplers at site D7 settled to the streambed and mixed with sediment. Results from these grounded samplers were excluded as they may not represent water column conditions. Stream flow was stable in Twelvemile Creek, the recipient stream of Town Creek, during the study showing only an increase in early February (Figure 2.4). This indicates that PE samplers remained submerged and suspended in the water column during deployment. The dialysis extraction method achieved a recovery of 80.57% ($\pm$ 8.60% SD) and 87.02% ($\pm$ 18.31% SD) for PCB 14 and 169, respectively. Recovery of surrogate standards averaged 75% ($\pm$ 5.20% SD) in sediment samples. The concentrations of PCBs measured in the samplers were reported in ng/g PE since they were integrated over a period of 60 days and were not corrected by the recovery results. The detection limit for total PCBs was 72.2 $\pm$ 31.1 ng/g PE and the concentrations in the deployed samplers were always greater than detection limits.
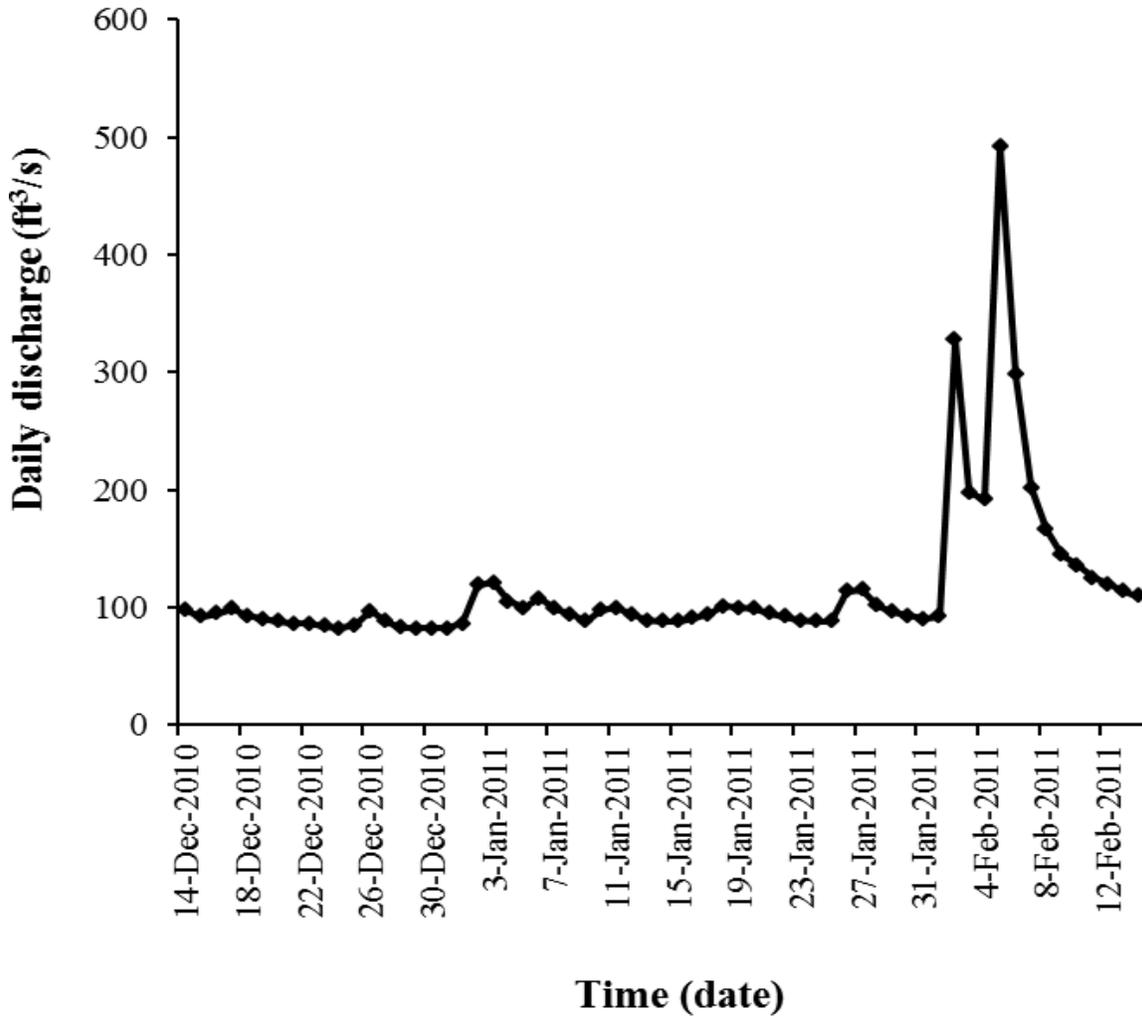
Figure 2.4. Daily discharge data for USGS stream gaging station at Twelvemile Creek near Liberty, SC (station number 0218600).

Total PCB concentrations

Total PCBs in the PEs at different sites are presented in Table A1 (Appendix). In general, average $\sum$ PCB concentrations ranged from 748.8 ng/g PE at site D1 (40 m upstream of the discharge ditch) to 3547.6 ng/g PE at site D8 (1.7 km downstream of the ditch) (Figure 2.5).
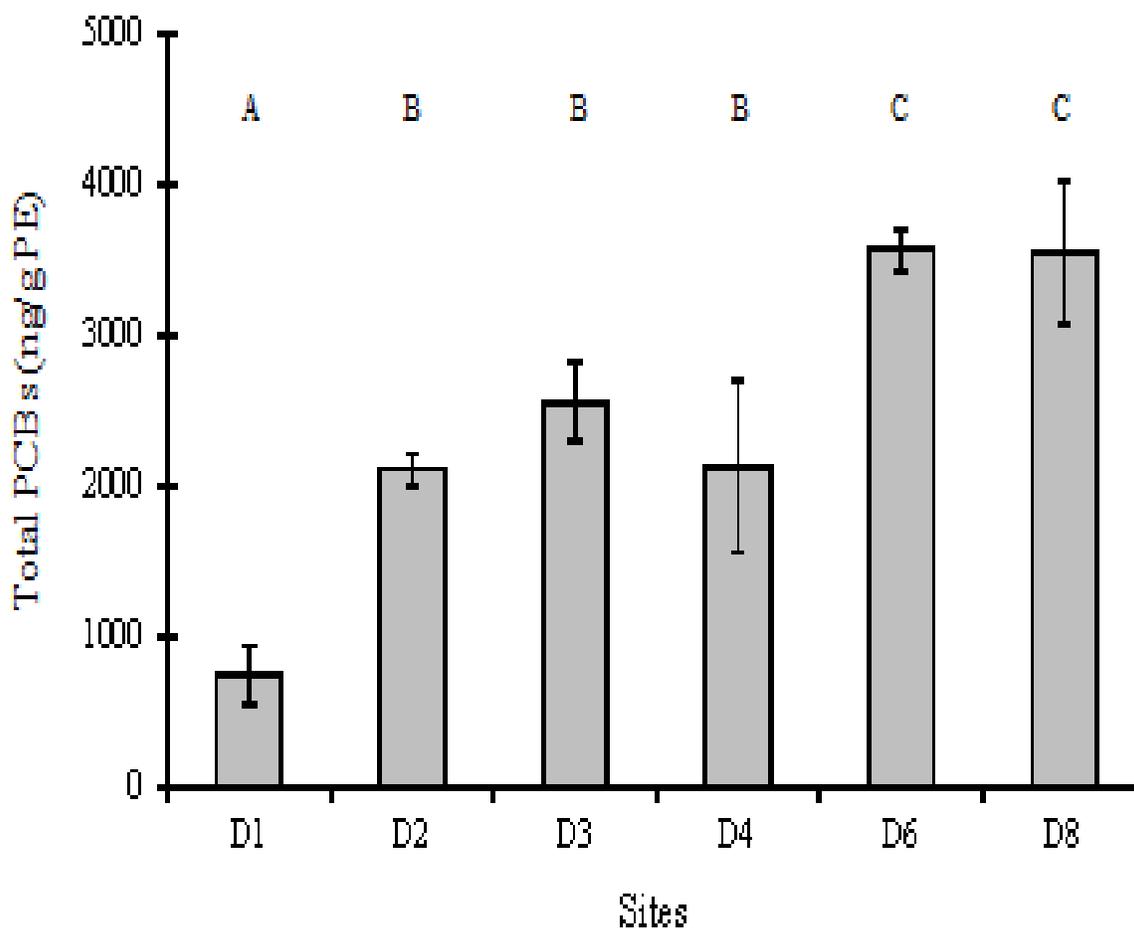
Figure 2.5. Total PCB concentrations (ng/g PE) in PEs at six sites in Town Creek, SC. Concentrations were average of three replicates. Error bars indicate standard deviations. Sites with different letters are significantly different ($\alpha = 0.05$, Tukey posthoc test).

Total PCB concentrations increased fivefold in the downstream direction, with a maximum concentration occurring at sites D6 and D8. Sites D2, D3, and D4 had similar concentrations in the samplers, while the concentrations were not statistically different between sites D6 and D8 ($p<0.05$). The longitudinal variations in $\sum$PCBs suggest two step increases. Concentrations doubled between sites D1 and D2, which fall on either side of the S-W discharge ditch. Concentrations approximately doubled again at sites D6 and D8 which are downstream of the former municipal WWTP. High concentrations downstream of the discharge ditch suggest an ongoing input associated with the original S-W site into the system. If the active discharge (the S-W site) had been slowed or eliminated, decreasing concentrations of dissolved PCBs should be expected throughout the system as a result of dilution by relatively uncontaminated water upstream. Perhaps, the S-W site is still a source, and that multiple pathways are involved. PCB concentrations at site D1 (40 m upstream of the ditch) were well above background levels documented at a reference site approximately 1 km upstream supporting evidence for the entry of PCBs above the ditch (URS, 2004). The doubling in PE concentrations downstream of the discharge ditch suggests that this remains a major pathway for contaminated groundwater migrating from the S-W site. In addition, Walters et al. (2008) conducted a food web study in Town and Twelvemile Creek and measured the highest total PCBs near sites D6 and D8. These findings led them to hypothesize that the abandoned municipal wastewater treatment facility was a potential undocumented source of PCBs to the stream. The facility was built to treat waste from the S-W site (Craig Zeller, U.S. Environmental Protection Agency, personal communication), and it is

possible that the waste treated at the facility likely contained PCBs that could reach the stream via runoff or groundwater inputs.

PCB congener patterns

Among-site differences in congener or homologue distributions also supported the hypothesis of ongoing sources to the stream system. For example, site D1 had an overwhelming fraction of di- and tri-CBs, which is indicative of a recent discharge because these more volatile lower chlorinated PCBs have not yet achieved equilibrium with the atmosphere (Figure 2.6a). Although site D2 is at 0 km downstream of the discharge ditch, I did not observe the presence of di- or tri-CBs. Some di-CBs were observed in the PEs at sites D6 and D8 (Figures 2.6e and 2.6f). Because the study area is shallow and well oxygenated, anaerobic reductive dechlorination of higher chlorinated congeners is negligible. It is more likely that the presence of di-CBs in the PEs at sites D6 and D8 is indicative of a potential fresh source (e.g., groundwater input via seepage in fractured rock or soil erosion from land). Conversely, when moving downstream from site D3 to D4, the data appeared to reflect volatilization of group 1 (containing fewer chlorine atoms) and sorption of group 2 (containing more chlorine atoms) (Figures 2.6b, 2.6c, and 2.6d). A rapid loss of di- and tri-CBs from the water column as reflected in the PEs appeared to occur within a 100 m distance, while steady state modeling conducted by Farley et al. (1994) showed a gradual loss of these low-weight congeners in sediment over a 10 km distance. On the other hand, lack of di-CB downstream of and near the discharge ditch in this study is difficult to explain via the mechanism of volatilization.
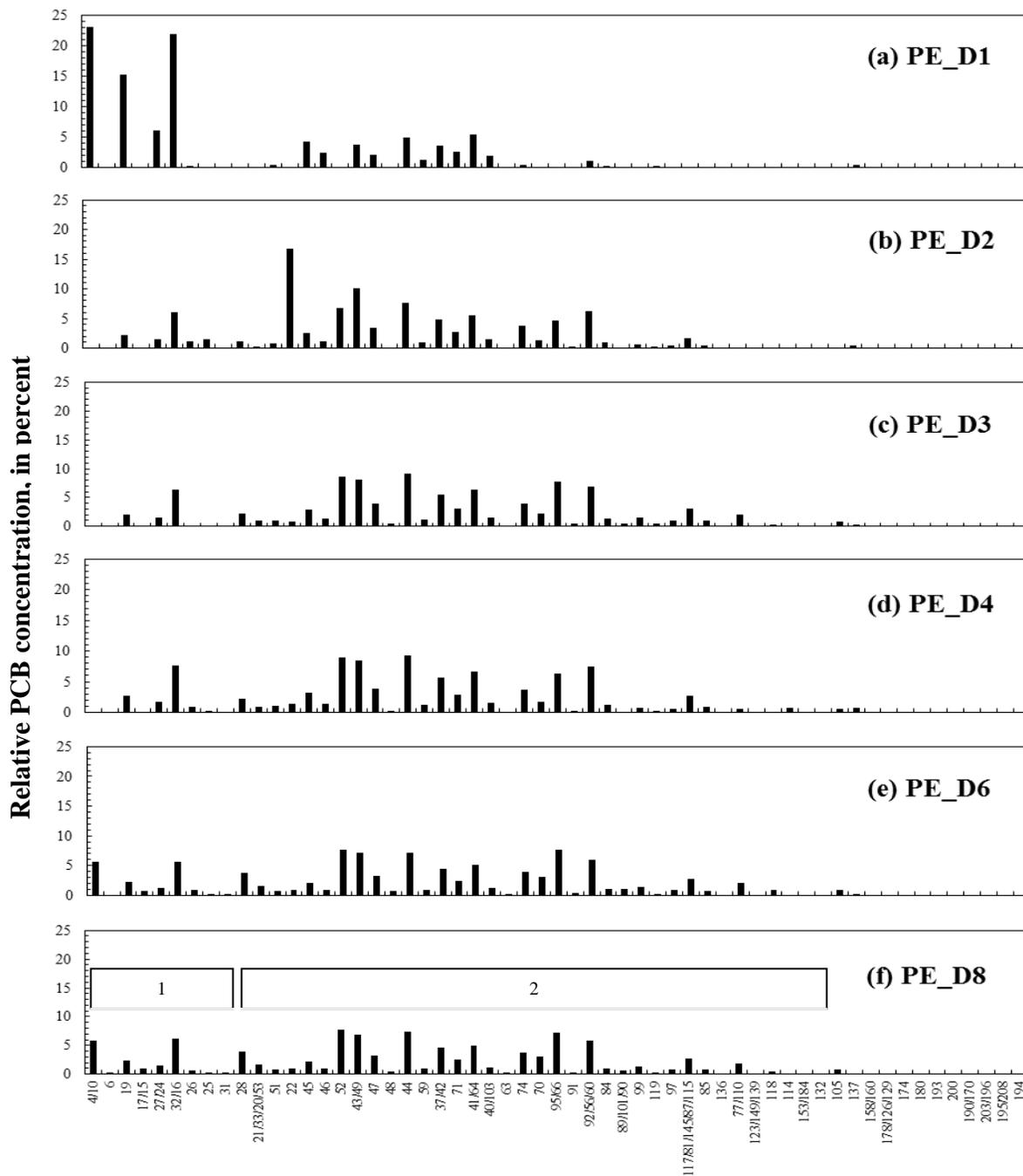
Figure 2.6. PCB congener patterns (87 congeners) in PEs at six locations deployed in Town Creek, SC. Numbers marked by brackets below the axis indicate group 1 (containing fewer chlorine atoms) and group 2 (containing more chlorine atoms), respectively. When more than one congener is displayed for one bar, multiple IUPAC numbers are separated by a slash.

Homologs distribution at site D1 resembles the 4:1 mixture of Aroclors 1016 and 1254 released from the S-W site (Figure 2.7). Walters et al. (2008) also observed greater accumulation of lower chlorinated congeners in different types of organic matter (e.g., periphyton) downstream of the S-W site compared with other sites further downstream. Tetra- and penta-CBs both increased at site D2, and then stayed relatively constant at the remaining sites downstream of the discharge ditch. The increased percent concentrations of tetra and penta-CBs downstream were attributed to the loss of diCBs and decreased percent concentration of tri-CBs. NMS analysis indicated three separate groups (PEs from sites D2 to D8, Aroclors and PEs at site D1, and sediment) in the ordination matrix (Figure 2.8). The congener pattern in the samplers at site D1 and in mixture of Aroclors 1016 and 1254 (4:1 v/v) discharged from the plant was closer to di-+ tri-CB congeners on the plot, while PCB composition patterns in the PEs from sites D2 to D8 represented a high correlation with tetra and penta-CBs. Ordination of higher chlorinated congeners (hexa- and hepta-CBs) showed correspondence with the homolog distribution in the sediment. Similarity of PCB homologues between the PEs at site D1 and Aroclors suggest that a source of relatively non-weathered PCBs from the S-W site is still being released into Town Creek.
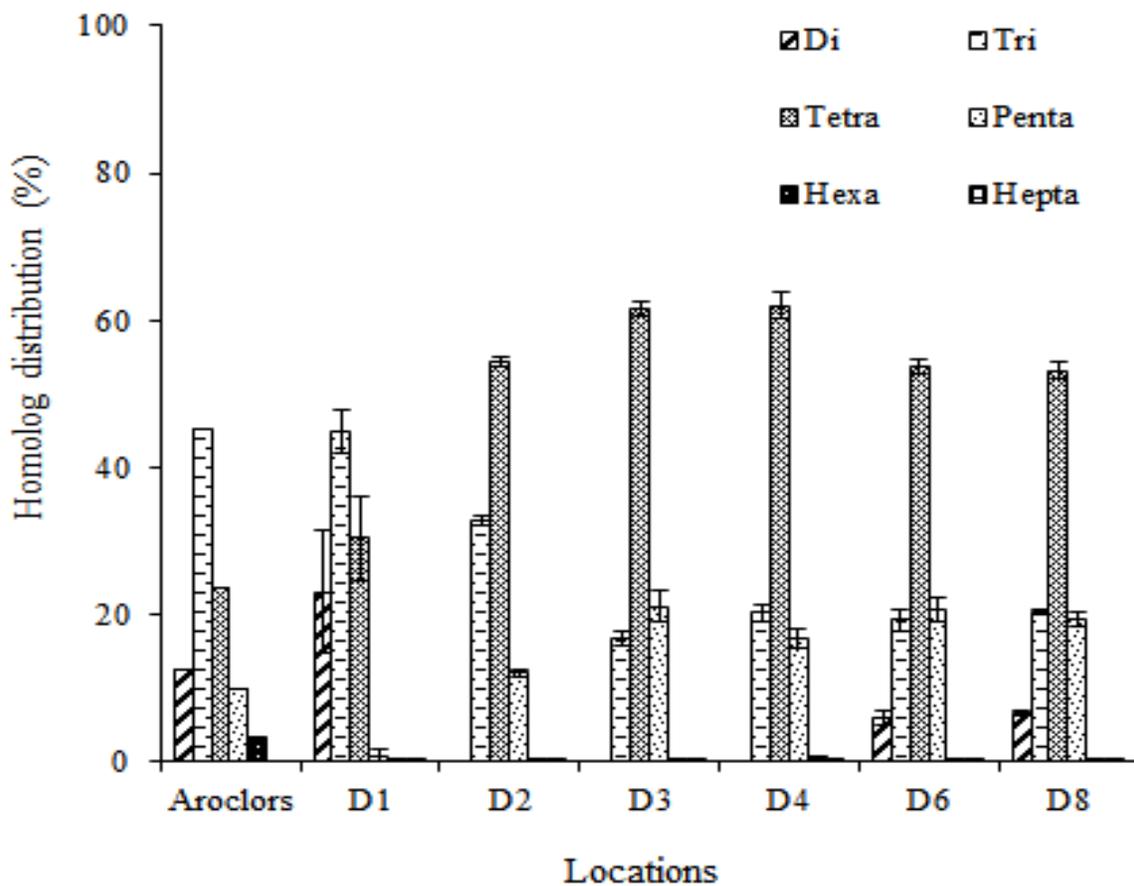
Figure 2.7. PCB homologs distribution (wt %) in Aroclors mixture and passive samplers at six locations on Town Creek, SC. Data for 4:1 mixture of Aroclors 1016:1254 were adapted from Farley et al. (1994). The relative amounts of each homolog were computed as the sum of recovered amounts of congeners of a homolog divided by the total amount of PCB from the sample. Error bars indicate standard deviations.
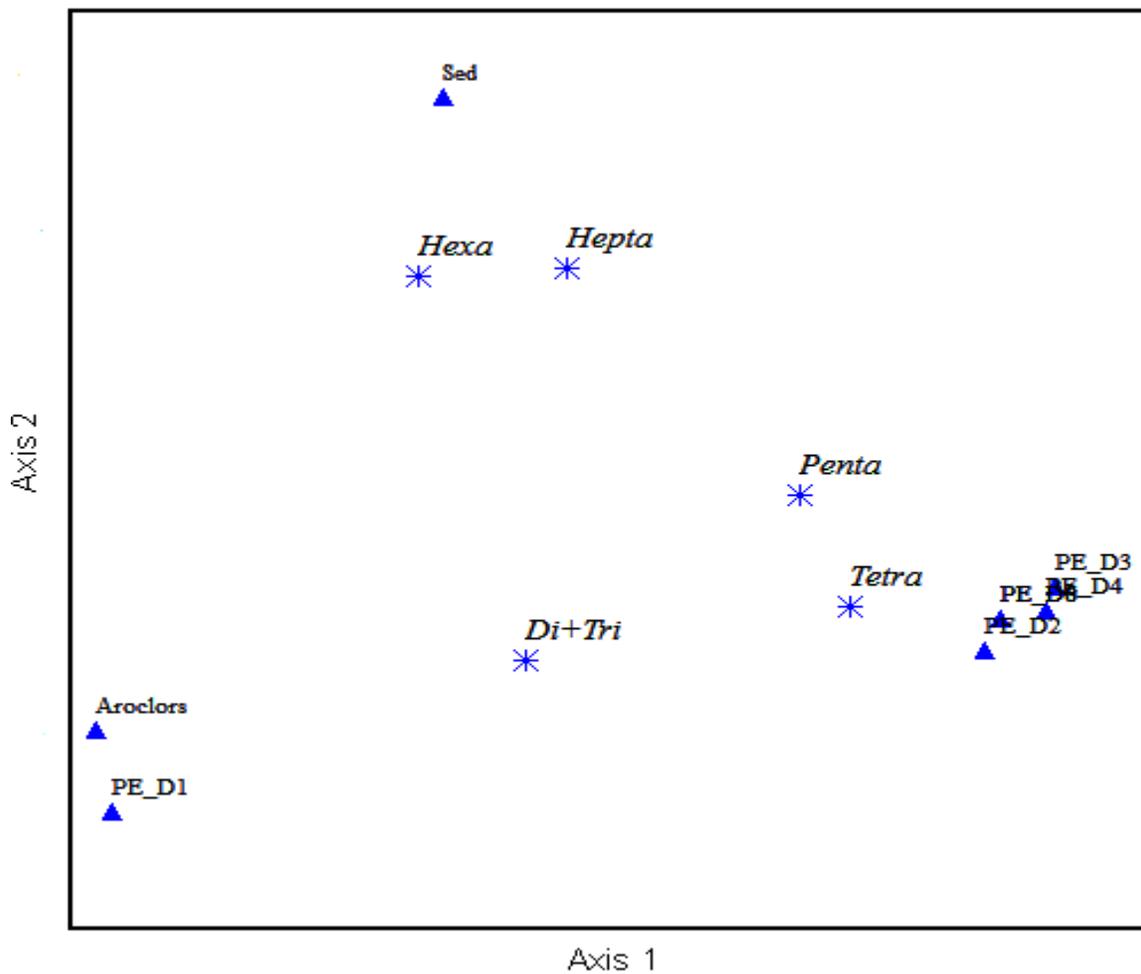
Figure 2.8. Nonmetric multidimensional scaling (NMS) plot of PCB homolog patterns in the PEs and sediment. Aroclors represent a mixture of 4:1 Aroclors 1016: 1254. PE_D1, for example, represents the samplers at site D1. Sites D6 and D8 might be out of order (that is plotting closer to site D1 than other sites), which could result from a partial "resetting" of the longitudinal order of sites due to fresh inputs of di-CBs from the WWTP.

Chiral signatures

EF values, which are near racemic in the vicinity of fresh sources and then depart from racemic with distance from the source will be evidence for ongoing fresh sources to the systems. PCBs 91 (2,2',3,4',6-pentaCB) and 95 (2,2',3,5',6-pentaCB) were the only two chiral congeners detected in the passive samplers. Both PCBs 91 and 95 were below the detection limit in the samplers at site D1, which is the site closest to the former S-W plant. For all other locations, EF values for PCB 91 were significantly non-racemic (>0.5) (Figure 2.9a), while the values for PCB 95 were racemic or close to racemic (Figure 2.9b). Changes in EF with distance were not correlated with total PCB concentrations.

The lack of detectable chiral PCBs 91 and 95 in the samplers at site D1 suggests that the ongoing inputs enter the stream attached to particles. Perhaps, equilibrium has not been established between sorbed PCBs 91 and 95 and the water column at D1. Further downstream (from D2 to D8) these congeners have desorbed enough to be detected in the samplers. The spatial consistency of EF values suggests that biotransformation of PCBs occurs rapidly in the stream or before they enter the system. It is likely that any biotransformation in Town Creek occurs under aerobic conditions because it is a shallow stream. It should be noted that similarity in EF values in the PEs among the sites does not provide any strong evidence to indicate additional inputs of PCB to the system. Different EF patterns between PCBs 95 and 91 could be explained if PCB 95 is not being aerobic degraded in Town Creek sediment, and PCB 91 is degraded. Aerobic degradation of PCBs involves ring cleavage. For instance, in *meta* cleavage, two oxygen atoms are inserted at the *meta* and *ortho* positions of one of phenyl rings by

biphenyl dioxygenase, followed by dehydrogenation to produce 2,3-dihydroxylbiphenyl (Rhee et al., 1999). It is also recognized that PCB 91 has adjacent *meta* and *ortho* potions and therefore is more likely to undergo aerobic biotransformation than PCB 95, which does not have any adjacent *meta* and *ortho* positions. The lack of aerobic biotransformation of PCB 95 is supported by Dang et al. (2010) who observed racemic EF values in samples collected by semi-permeable membrane devices (SPMD) and non-racemic EF values in fine benthic organic matter and coarse particulate organic matter in Twelvemile Creek. Wong et al. (2007) also observed non-racemic EF values for PCB 95 at depth in sediment cores, while the EF values were racemic for the surface sediments collected from Lake Hartwell, which is the receiving water of Town Creek and Twelvemile Creek. Dang et al. (2010) and Wong et al. (2007) both measured non-racemic EF for PCB 91 in the SPMD and the cores, respectively.
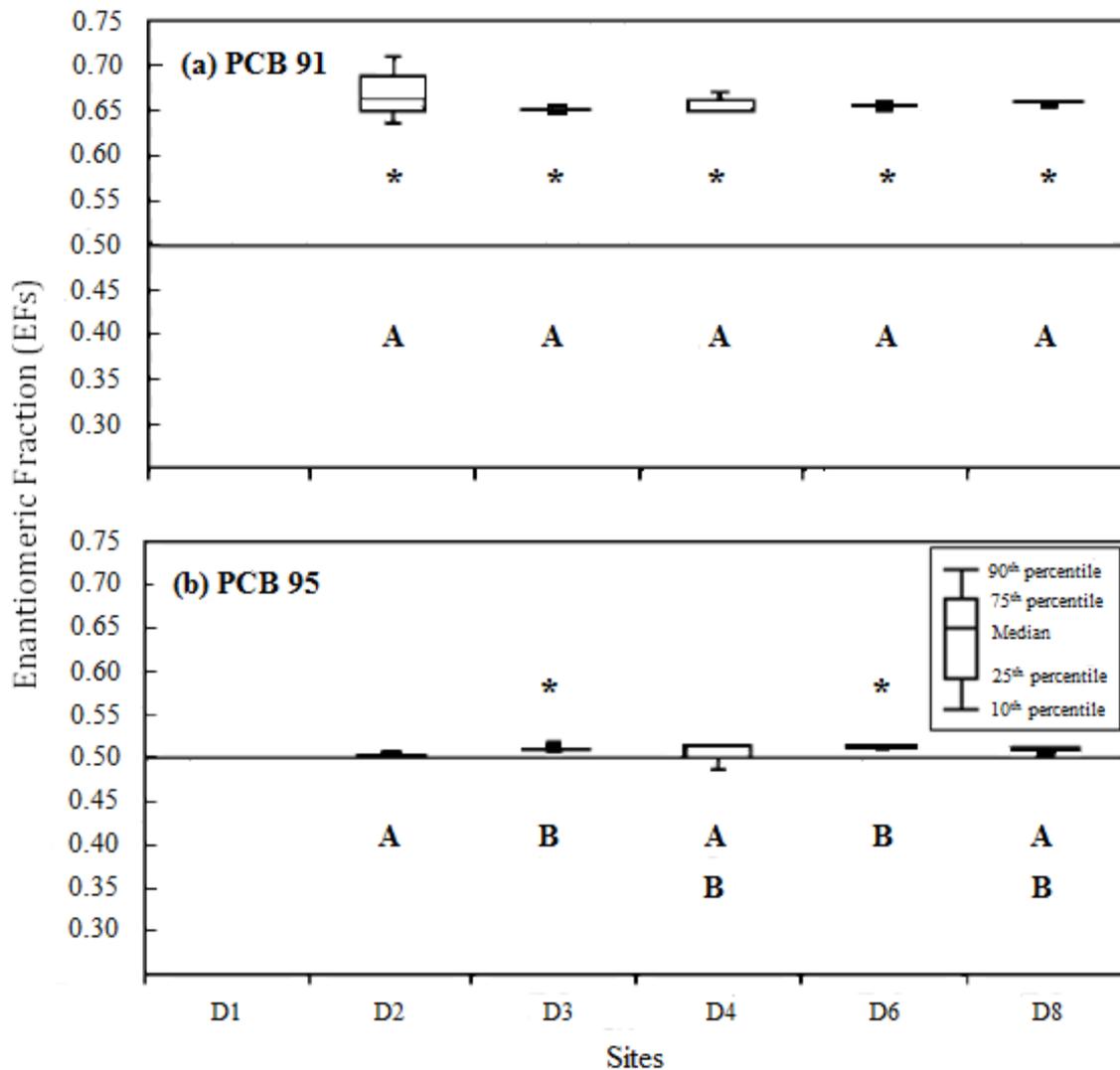
Figure 2.9. Enantiomeric fractions (EFs) for PCBs 91 (a) and 95 (b) in PEs deployed at six locations in Town Creek, SC. Concentrations of both 91 and 95 were not detected at site D1. Values shown mean EFs calculated among three replicate samples. Horizontal line indicates racemic EFs (0.5). Asterisks indicate significantly non-racemic EFs. Sites with different letters are significantly different ($\alpha = 0.05$, Tukey posthoc test).

Conclusion

Three lines of evidence including total PCBs, congener pattern, and chiral signatures were employed to determine ongoing inputs of PCB to the water column of Town Creek. Some lines of evidence were stronger than others, but an overall summary of the evidence includes: 1) the total PCB concentrations increased within a 2-3 km distance from the S-W plant site to the mouth of Twelvemile Creek, 2) the pattern in congener composition in the PEs upstream of the discharge ditch was similar to the congener composition of the mixture of Aroclors 1016 and 1254 (4:1 v/v) released from the S-W plant, and 3) the EF values for chiral PCBs 91 and 95 detected downstream of the ditch were non-racemic and racemic, respectively. Thus, results from total PCBs and congener-specific analysis provide evidence to conclude there is an ongoing source of PCBs to the system. The evidence from the chiral PCB congeners suggests that dissolved PCBs are subject to aerobic biotransformation. However, chiral evidence does not shed any light on possible on-going inputs. This study is crucial because it draws attention to the question of source control, which is often neglected in ongoing monitoring efforts.

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CHAPTER 3

LEAF QUALITY DETERMINES PCB UPTAKE RATES IN STREAMS

Abstract

Microbial decomposition of leaves is an important energy input in lotic systems. Decaying leaf material can be a potential source of organic matter to accumulate hydrophobic pollutants from the surrounding water, and further serves as an important vector of contaminants to higher trophic levels. Leaves with different nutritional quality are subject to different microbial colonization, which will ultimately affect uptake rates of contaminant. In this study, I used three deciduous leaf species including red maple (*Acer rubrum*), northern red oak (*Quercus rubra* L.), and tulip poplar (*Liriodendron tulipifera*), and an evergreen leaf species (*Rhododendron maximum*) for a PCB uptake study based on differences in their initial chemistry. Air-dried individual leaves were loaded in coarse-mesh aluminum bags and deployed for 0 (blank), 20, 50, and 90 days in Town Creek. At day 0, PCB concentrations in maple, oak, and tulip poplar were less than 3.0 ng/g dry wt, while concentration was measured 60.6 ng/g dry wt in rhododendron. Total PCBs changed with time for deciduous species, but were not statistically different for the evergreen rhododendron. The PCB congener pattern was consistent throughout the collection time and resembled the pattern of dissolved PCBs found in polyethylene (PE) passive samplers. Among-species variations in PCB concentrations suggest that the nature of terrestrial leaf material plays a major role in accumulating contaminants.

Introduction

The energy sources to small streams are generally derived from either in-stream (autochthonous) or out-of-stream (allochthonous) inputs. While autochthonous energy is produced via photosynthesis, most allochthonous materials entering rivers are leaves. Leaves are an important source of carbon to stream food webs because they can comprise up to 60% of particulate organic matter during some seasons (Allan, 1995; Fisher and Likens, 1973). They also provide a preferential habitat for microbial biofilms that can further sorb organic contaminants from the surrounding water. These microbial colonized leaves are a food source of detritivores, and thus potential entry points of POPs into the stream food webs. However, a thorough understanding on contaminant transport and the link to food webs in lotic systems is still lacking due to a paucity of evidence for the accumulation of contaminants at the base of the food chain. To date, the few lotic studies have only focused on the pathway of contaminants in periphyton (a complex aggregation of microorganisms growing on solid surfaces), but not leaves, which is crucial oversight given their significance to food webs in forested streams (Berglund et al., 2005; Walters et al., 2008).

It is well-documented that leaves in streams are degraded by physical processes (e.g., burial, abrasion) and biological processes (e.g., microbial conditioning, shredding by invertebrate consumers) to become fine particulate organic matter, which are further transported downstream and available for filter-feeding or collector-gathering insects (Cummins, 1974). Microbial colonization and leaf shredding invertebrates are major mechanisms in processing and accelerating the release of energy stored in the leaf

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materials (Cummins and Klug, 1979). An exception to the primacy of biological mechanisms will occur if the presence of microorganisms and aquatic insects is limited due to environmental inhibitors (e.g., pH, toxicity of contaminants, and high temperature). In addition, the substrate quality of the leaves (e.g., the nitrogen concentration, lignin content, and C:N ratio) is also an important factor affecting breakdown rates. For example, leaves with a lower C:N ratio decompose faster than those having higher mass of overall recalcitrant structural substances (Melillo et al., 1982; Taylor et al., 1989). The result of microbial decomposition of leaves is important to stream food webs because leaf detritus conditioned by microbes forms the base of most stream food chains and contributes in part to food sources for macroinvertebrates (Allan, 1995; Anderson and Sedell, 1979).

Microbially conditioned leaves can serve as a source of organic matter that accumulates pollutants from the surrounding water and sediment, and acts as an important vector for transfer of these contaminants to higher trophic levels. Accumulation of PCBs by conditioned leaves can result from microbial uptake (absorption and surface adsorption) and/or absorption associated with lipid content in both the attached microorganisms and particle substrates (lignin, cellulose, and hemicelluloses). The mechanism of accumulation by microbial decomposition of leaves strongly depends on physicochemical properties of the particular contaminants, properties of the environment containing the leaves, and of the leaves itself (e.g., stages of decomposition, composition of remaining materials in leaf detritus, and the extent of microbial colonization) (Odum and Drifmeyer, 1978). Knowledge is still limited about the nature of leaf litter as an

accumulator of hydrophobic contaminants because there are no known studies that directly address this relationship. While it is obvious that high surface area of leaves covered with a lipid-rich film is perfect for absorbing hydrophobic contaminants like PCBs from the water column, there appears to be a gap in understanding the uptake of PCBs by leaf materials and how it varies among species. Therefore, the main objective of this study was to investigate the importance of individual decomposing leaves on the uptake of PCBs in a contaminated stream draining a forested catchment. I hypothesized that 1) leaves with higher nutritional quality (e.g., lower C:N and initial lignin contents) would accumulate PCBs to a greater extent than lower quality leaves due to greater microbial conditioning, and 2) the source of PCBs in conditioned leaves was primarily from absorption of dissolved PCBs as congener pattern would be similar between PEs and leaves.

Materials and methods

Samples collection

To test my hypothesis, three autumn-shed leaf species including red maple (*Acer rubrum*), northern red oak (*Quercus rubra* L.), and tulip poplar (*Liriodendron tulipifera*) were collected on the ground from multiple locations, while leaves of an evergreen rhododendron (*Rhododendron maximum*) were collected from several plants at one location only. Sampling was performed in late October, 2010, along Town Creek, Pickens County, SC. Upon collection, leaves were then transported to the laboratory for

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sorting and stored in a freezer at -4^0C before use. In addition, surficial sediments were collected and sampling was performed as described in Chapter 2.

Leaf bags preparation

A litterbag method was employed to study PCBs accumulation in deployed leaves. A roll of 8 mesh (2.38 mm) aluminum screening was purchased. The use of a coarse-mesh size would be representative of leaf processing in the environment as it allows access to both the microbial community and macro-consumers. Leaf bags (20×20 cm) were constructed by stapling three edges of the screenings (Figure 3.1). Leaves collected previously were removed from the freezer, and air dried for 48 hours. Approximately 5 grams of each air-dried species were moistened with tap water prior to loading in the prepared litterbags. Four replicate bags of each species at each sampling time were prepared and deployed in late November, 2010 (Figure 3.2). The bags were tied to 10 inch steel chains via copper wires. The leaf bags were put in the streambed for 0 (field blank), 20, 50, and 90 days. Stream leaves from a reference site were also collected for method quantification. After retrieval, the bags were returned to the lab in a cooler with ice. Sediment, debris, and macroinvertebrates were gently rinsed from leaf surfaces with tap water. The remaining materials were stored at -20^0C prior to PCBs extraction and analysis.
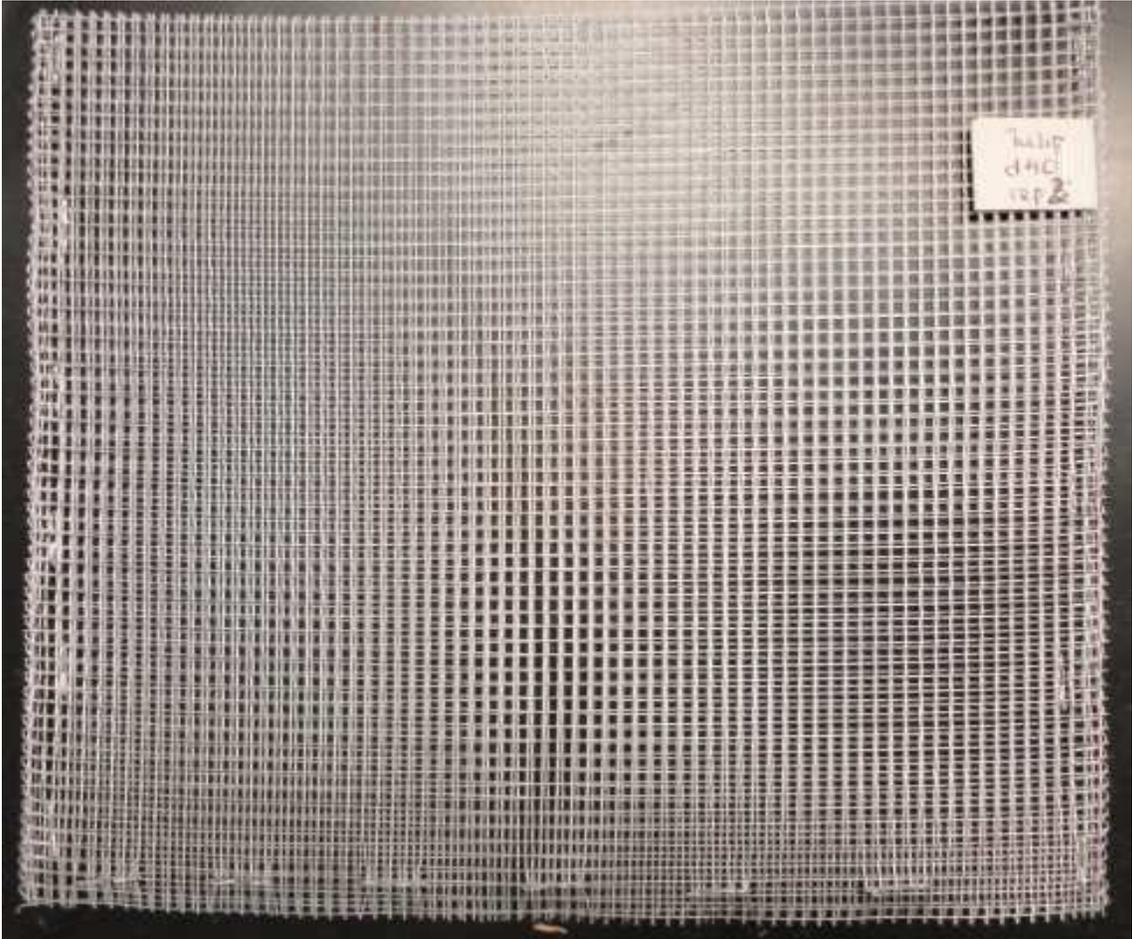
Figure 3.1. Prepared leaf bags using 8 mesh aluminum screening.
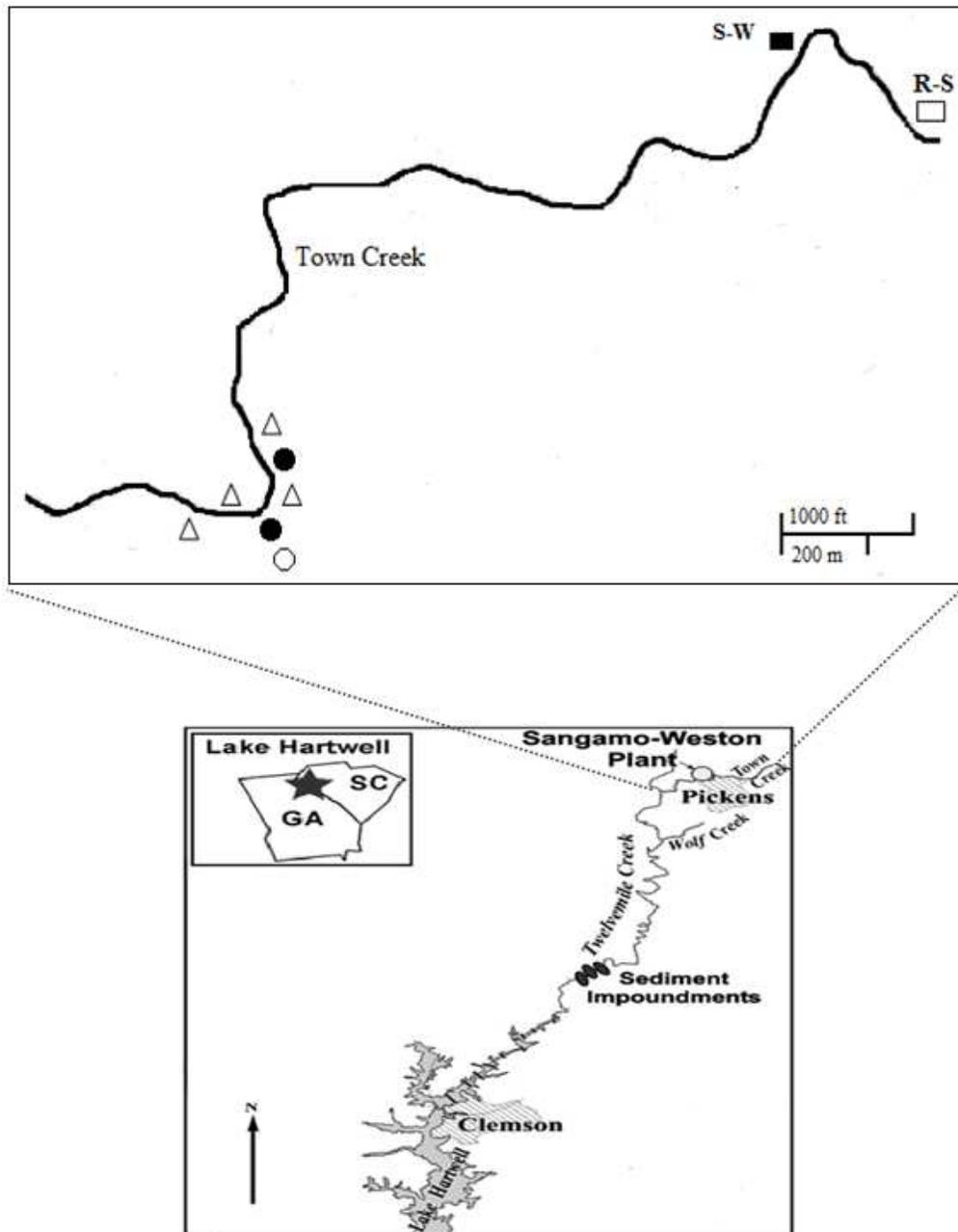
Figure 3.2. Map of leaves collection and deployment in Town Creek, SC. Solid circles represent sampling locations for deciduous species, while open circle indicates a sampling site for rhododendron. Open triangles represent four replicates of each species deployed. Solid rectangular indicates the former Sangamo Weston (S-W) plant site. Open rectangular indicates a reference site (R-S) where decomposing leaves were collected for quantification method.

Analytical methodology

Leaf materials were removed from the freezer, thawed, and air-dried in a fume hood for 48 hours. The litter materials were then chopped into small particle sizes using a blender. PCBs extraction was performed by homogenizing and mixing the samples with an amount of baked sodium sulfate in a beaker so that the total mass of the mixture was approximately 15 g. PCBs 14 and 169 were added to the mixture as surrogate standards prior to extraction. PCBs were extracted with a Dionex Accelerated Solvent Extractor (ASE-200) using hexane: acetone (1:1 v/v) (USEPA, 2005). Lipid content was measured by a gravimetrical method. Concentrated extracts were cleaned up on an alumina (7% deactivated) column, followed by lipid removal using concentrated sulfuric acid, and solvent exchanged with isooctane (USEPA, 2005). Reagent, check standard, blank and spiked blank leaves were always run with eight samples per batch. Total PCB concentrations and congener-specific analysis, and statistical methods were carried out as described in Chapter 2.

Results and discussion

Four replicate samples of individual species were successfully retrieved at day 20, and 50, while only three replicates were retrieved at day 90 due to the loss of one replicate. Detection limits for total PCB concentrations ($\sum$PCBs) were 5.4 ($\pm$ 2.6 SE) ng/g dry wt for sample mass ranging from 3.5 to 5 g. PCBs were always greater than detection limits in all deployed leaf samples (Appendix A2). Recovery of the surrogate standards averaged 71 $\pm$ 22% and 48 $\pm$ 11% for PCBs 14 and 169, respectively. Previous

research also indicated a low factor of recovery of PCB 169 (< 60% recovery) in riparian samples (Dang, 2007). Low recovery of PCB 169 could be attributed to the fact that PCB 169 favorably accumulated in lipid and was partially removed during lipid quantification and sulfuric acid processes.

∑PCBs were below detection limit for deciduous species while the concentration in rhododendron was measured at 60.6 ± 12.3 ($\pm$1SD, ng/g dry wt) at day 0 (Figure 3.3). An elevated concentration of PCBs detected in rhododendron at day 0 was likely from uptake of volatilized PCBs from the stream (Chapter 4). Several studies also detected PCBs in conifers, which have been widely used for biomonitoring of airborne organic pollutants (Kylin et al., 1994; Hellstron, 2003; Loganathan et al., 2007). Because of long-lived foliage and a lipophilic outer layer, evergreen plants can be a significant sink for deposition of gaseous pollutants.

On a dry mass basis, the total PCB concentration in tulip poplar was four times higher than concentrations in the other three species after 20 days, but was significantly decreased after 50 days, and remained after 90 days (Figure 3.3). Maple and oak showed an increase in uptake rates throughout the 90 day period, except for a slight decrease in maple after 90 days (Figure 3.3). PCB concentrations in rhododendron were not changed significantly after 50 days, and increased after 90 days (Figure 3.3). On a lipid basis, uptake rates of PCB by oak and tulip poplar were higher than maple and rhododendron (Figure 3.4). An exception was that the tulip poplar showed a gradual decrease in concentrations after 50 days. Total PCBs were not statistically changed with time in the rhododendron when normalized to lipid concentrations (Figure 3.4). Among-replicate

variation in total PCBs was low at day 0 and 20, but was high at day 50 and 90 when concentrations were normalized to both dry mass and lipid concentration.
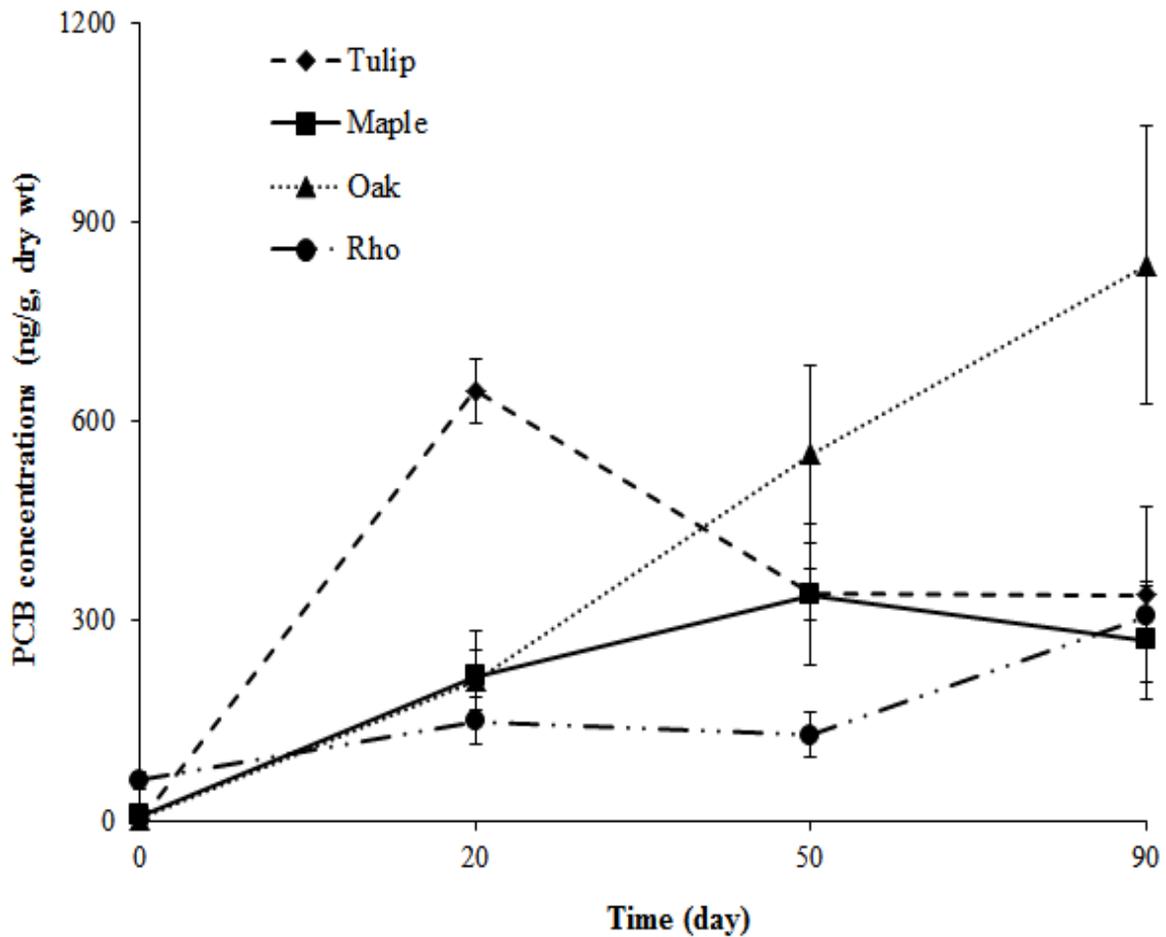
Figure 3.3. PCB concentrations (± SD, ng/g dry wt) for four individual leaf species at day 0, 20, 50, and 90. Values were averages of four replicates at day 0, 20, and 50, and average of three replicates at day 90. Error bars indicate standard deviations.

Figure 3.4. Lipid-normalized concentrations for four individual species (± SD, ng/g lipid) at day 0, 20, 50, and 90. Values were averages of four replicates at day 0, 20, and 50, and average of three replicates at day 90. Error bars indicate standard deviations.
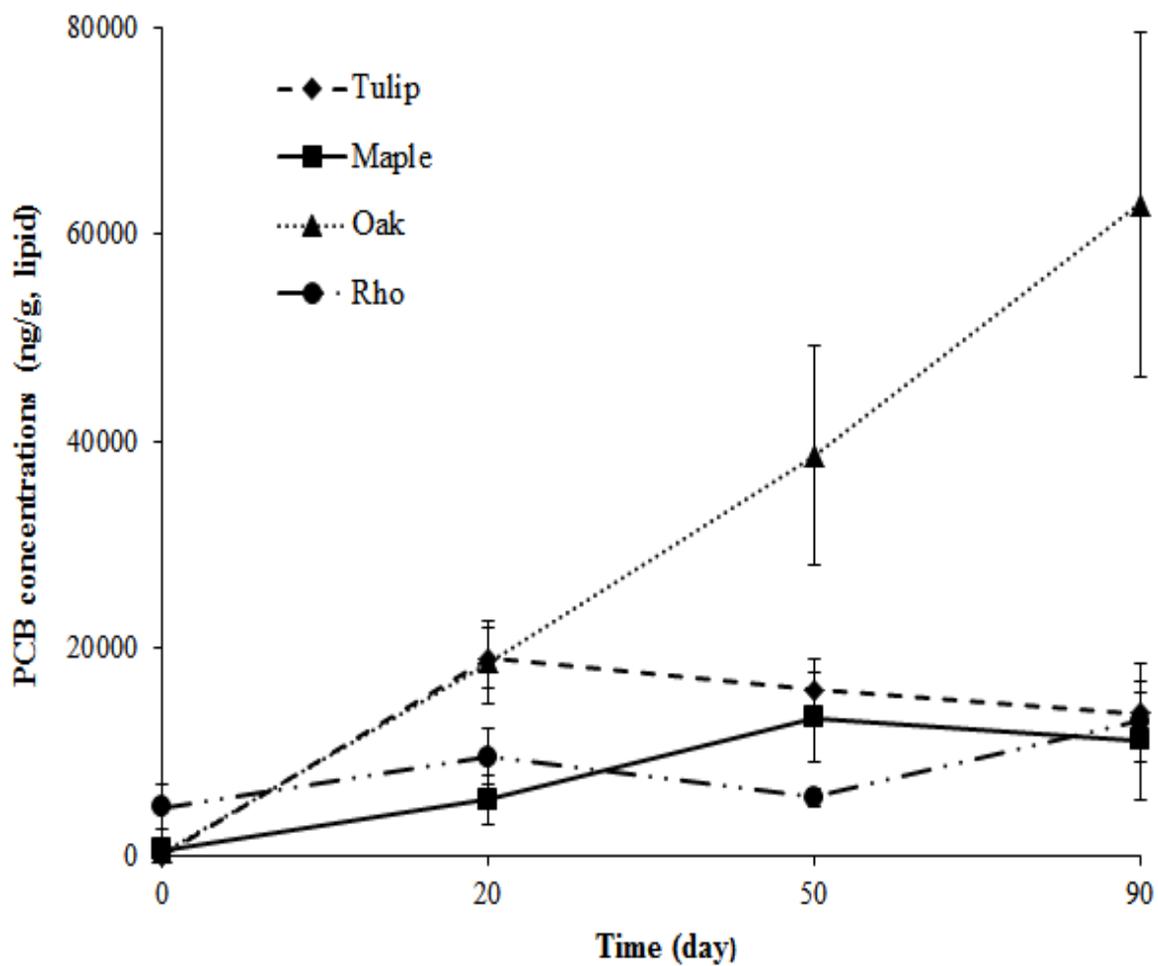
Among-species leaf quality

Uptake rates and PCB concentrations in leaves were potentially related to nutritional quality of leaves (e.g., C:N, and lignin). I adapted the substrate quality for individual leaf species from Kominoski et al. (2007) due to the similarity of species examined between my study and their study (Table 3.1). The observed patterns of PCBs accumulation by the deciduous versus evergreen species were in good agreement with leaf quality. For instance, the rhododendron, which contains the highest C:N, would be subject to a slower decomposition rate because it is less favorable to microbial conditioning on its surface and; therefore, subject to a slower uptake of PCBs than higher quality leaves (e.g., oak, maple, and tulip poplar) (Figures 3.3 and 3.4). Paul and Meyer (1996) also indicated a slower decay for rhododendron than tulip poplar and maple in a 1st-order headwater stream and a 4th-order stream. The effect of other substrate like lignin on decomposition rates was also critical due to the fact that lignin is an inhibiting factor in the enzymatic degradation of cellulose and other carbohydrates, as well as proteins. It has been shown that high initial levels of lignin may slow decomposition rates (Alexander, 1977). Cromack (1973) concluded that "lignin content of a given tree species' leaf litter at the time of litter-fall is an important biological property that influences species' rate of decomposition". It is thus necessary to take into account both C:N and lignin content when referring to leaf breakdown process.

Table 3.1. Fresh leaf quality (mean ± SE) adapted from Kominoski et al. (2007). Values represent concentrations as a percentage of Ash Free Dry Mass (AFDM).

Species	C:N	Lignin
Tulip poplar	56.3 ± 0.4	12.5 ± 0.4
Maple	93.7 ± 1.8	9.8 ± 0.2
Oak	61.6 ± 1.3	14.4 ± 0.5
Rhododendron	162.7 ± 4.8	10.6 ± 0.1

Microbial colonization and leaf quality, which drives decomposition rates, will affect the uptake rates and PCB concentrations as shown in rhododendron. Another example is that the tulip poplar was expected to decompose quickly (Kominoski et al., 2007), and thus I predicted that the tulip poplar could favor microbial conditioning and further accumulate PCBs to a greater extent than the maple and oak, which was the case in the first 20 days (Figure 3.4). However, the ∑PCB concentration decreased in the tulip poplar after 50 days. It could be due to the fact that snails were present in the tulip poplar leaf bags, but not in either the oak or maple bags at the time of collection at day 50 and 90. The snails were likely feeding on the biofilm growing on the tulip leaf surfaces. Moreover, the literature indicated that the foliar calcium concentration of the tulip poplar is much higher than that of the maple and oak (Jenkins et al., 2007). The snails would likely prefer the tulip poplar leaves to acquire calcium for reproduction and, most notably, for shell production (Fournie and Chetail, 1984; Hickman et al., 2003).

Lipid content (total extractable hydrophobic materials) might also be related to PCB concentrations in the leaves. Due to their degree of hydrophobicity, PCBs are favorably associated with lipid tissues in plants and organisms. I found a positive correlation between PCB concentrations and lipid content in the leaves, but this correlation was not strong (r^2 <0.5 for all four species) (Figure 3.5). Measured lipid content can be assumed as the lipid in both the attached microorganisms and particle substrates (lignin, cellulose, and hemicelluloses). This lipid content will change unpredictably with time and become less correlated with PCB concentrations. I hypothesize that fungal biomass or ergosterol would be a better representative for PCB uptake and concentrations than lipid in the leaves (Figure 3.6). Ergosterol is a lipid that occurs in the membranes of most fungi, but not in plants or animals. Because it breakdowns rapidly after cell death, ergosterol can be used as an indicator of living fungal biomass. Additional study is recommended to monitor the typical pattern of change of ergosterol concentrations with time as well as its effect on PCB uptake and concentrations in the microbially conditioned leaves.
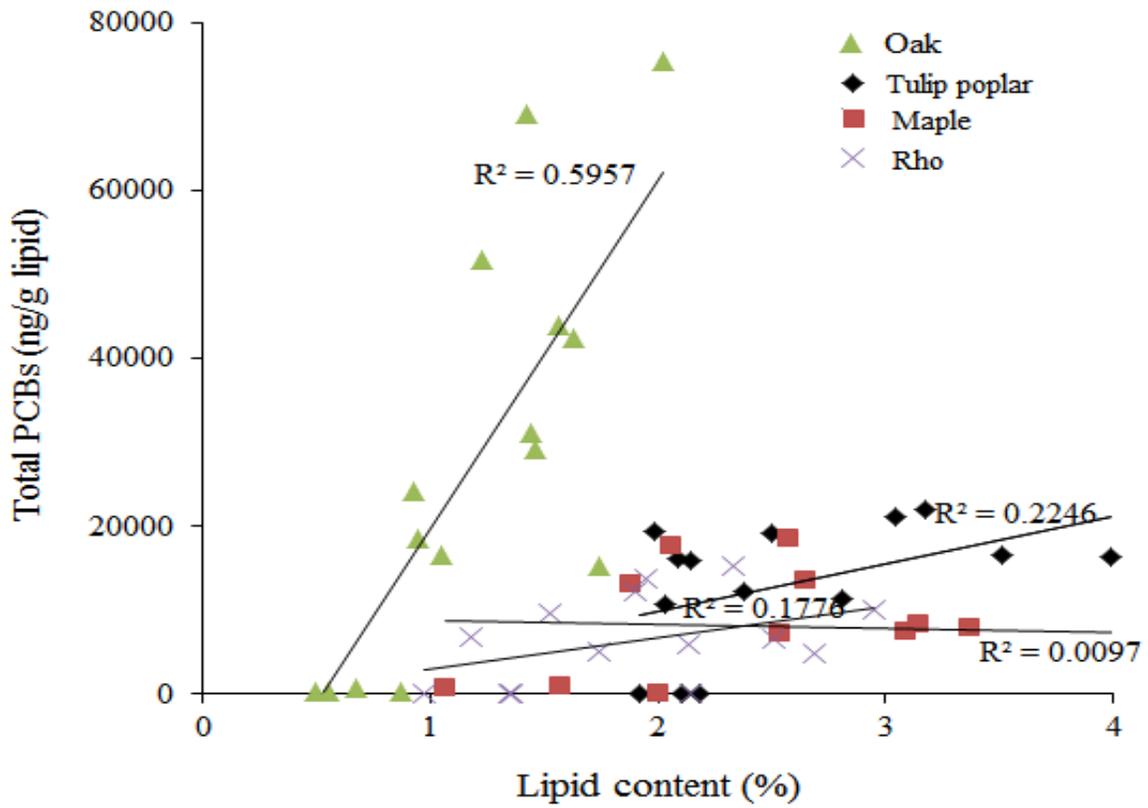
Figure 3.5. Correlation between total PCB concentrations (ng/g lipid) and lipid content (%) for four leaf species.
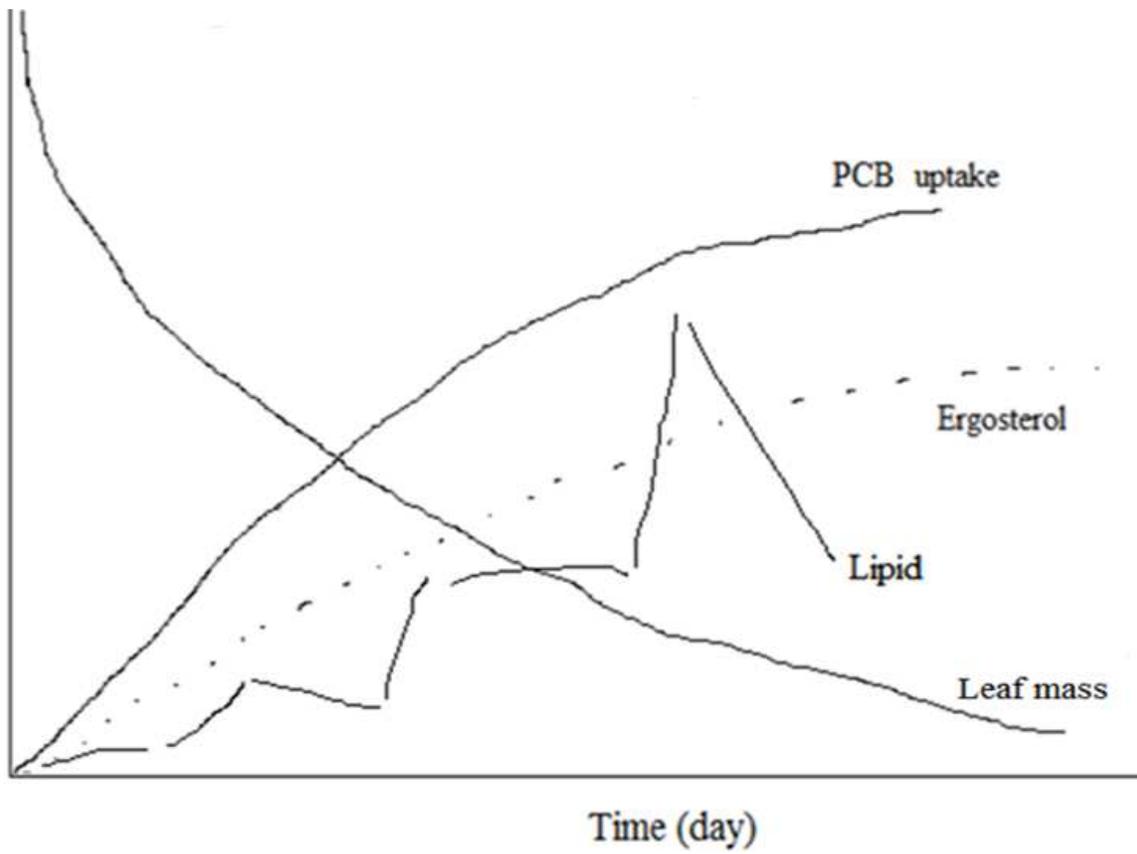
Figure 3.6. Proposed patterns of PCB uptake, ergosterol, lipid, and mass loss of microbial decomposing leaves with time.

Congener-specific pattern

Analysis of PCB congeners showed a consistent pattern in all studied leaf species at day 20, 50, and 90 (Figures 3.7, 3.8, and 3.9) suggesting that leaves were contaminated with PCBs from the same source. Low chlorinated congeners (di+tri-, and tetra-CBs) accounted for >70% of total PCBs, while moderate-weight congeners (penta-CBs) accounted for <30% of total PCBs. The homolog distributions were consistent with time suggesting that leaf materials accumulated PCBs from the same source. NMS analysis indicated a high proportion of di-+tri- and tetra-CBs in the leaves and PEs, while sediment resembled a predominant pattern of high chlorinated congeners (e.g., hexa-, and hepta-CBs) (Figure 3.10). Therefore, dissolved PCBs appear to be the main sources because the pattern of PCB congeners in the deployed leaves was similar to the dissolved PCBs pattern found in polyethylene (PE) passive samplers. Because of the sorptive nature and a large surface area, biofilm associated with the decaying leaves could sequester considerable quantities of dissolved PCBs from the water.
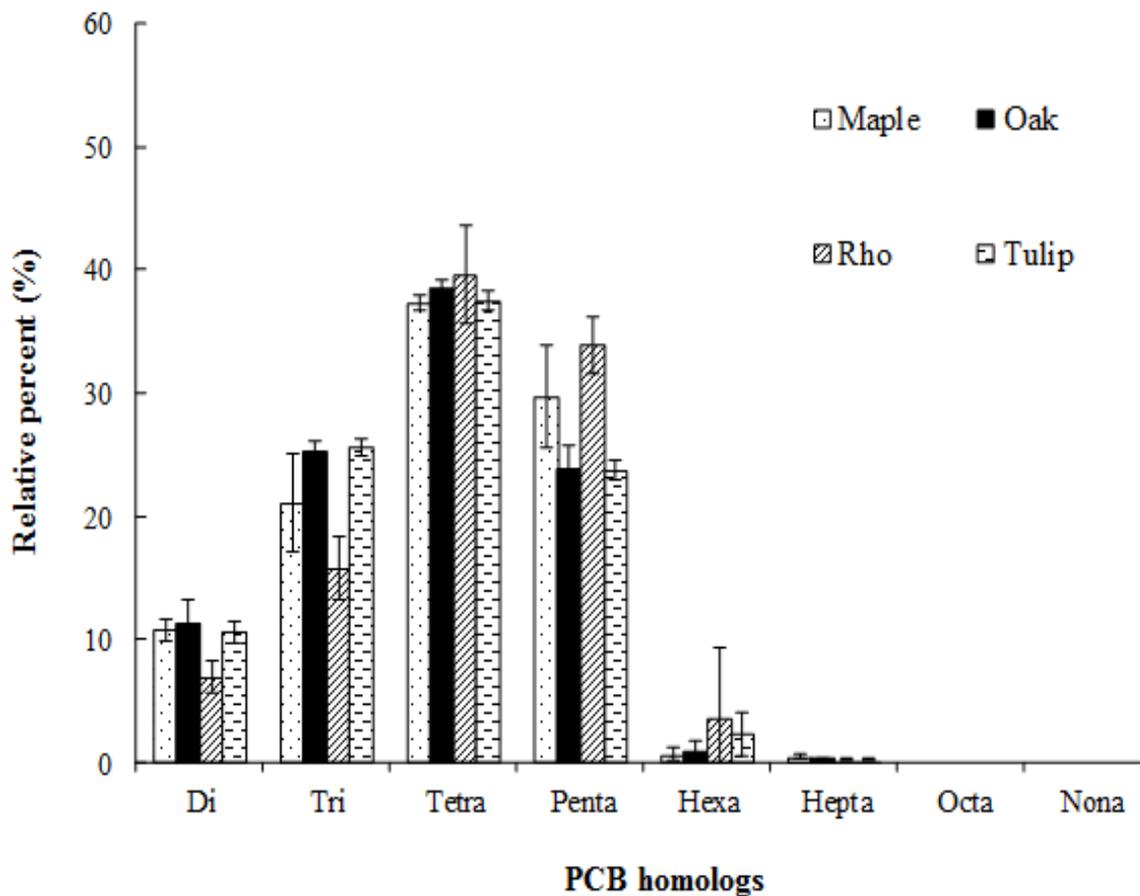
Figure 3.7. PCB homologs distribution (wt %) in individual leaf-species retrieved at day 20. Concentrations of most PCB homologs in the deciduous leaves were below the detection limit at day 0. The relative amounts of each homolog were computed as the sum of recovered amounts of congeners of a homolog divided by the total amount of PCB from the sample. Error bars indicate standard deviations.

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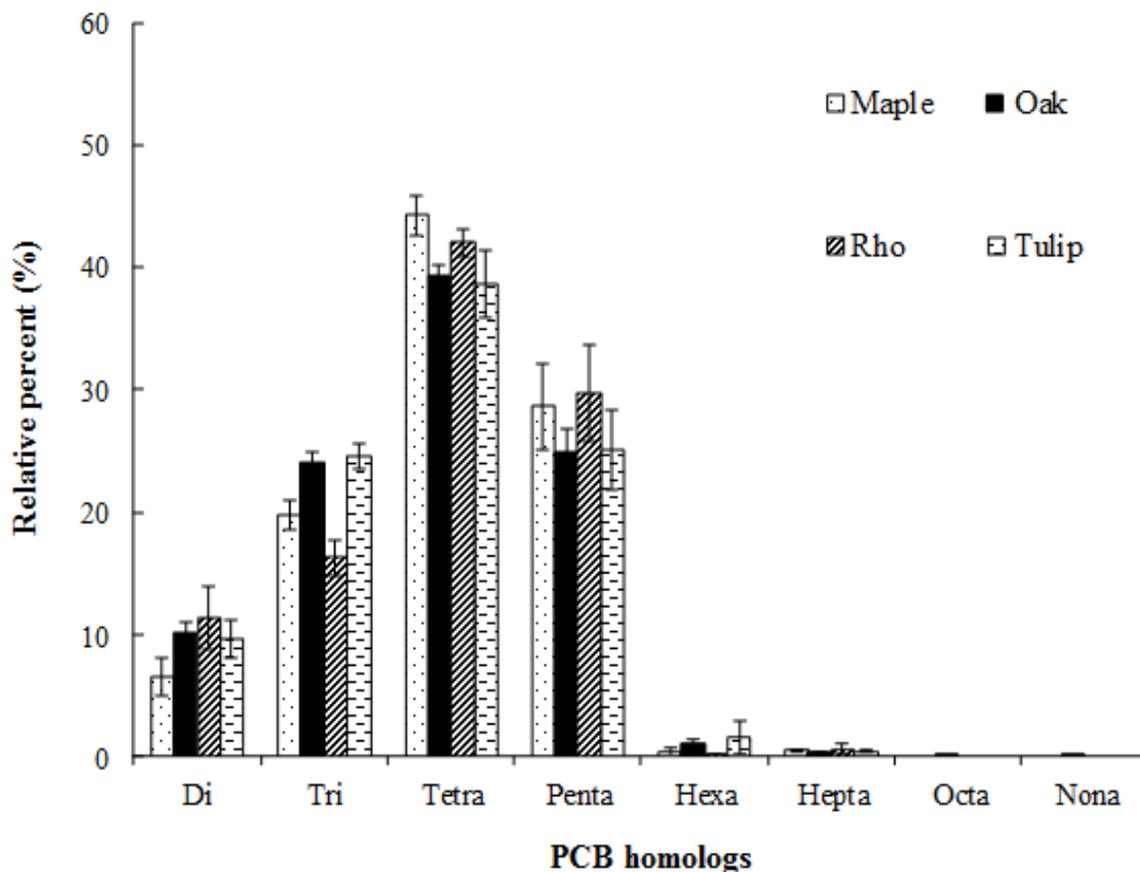
Figure 3.8. PCB homologs distribution (wt %) in individual leaf-species retrieved at day 50. Concentrations of most PCB homologs in the deciduous leaves were below the detection limit at day 0. The relative amounts of each homolog were computed as the sum of recovered amounts of congeners of a homolog divided by the total amount of PCB from the sample. Error bars indicate standard deviations.

60
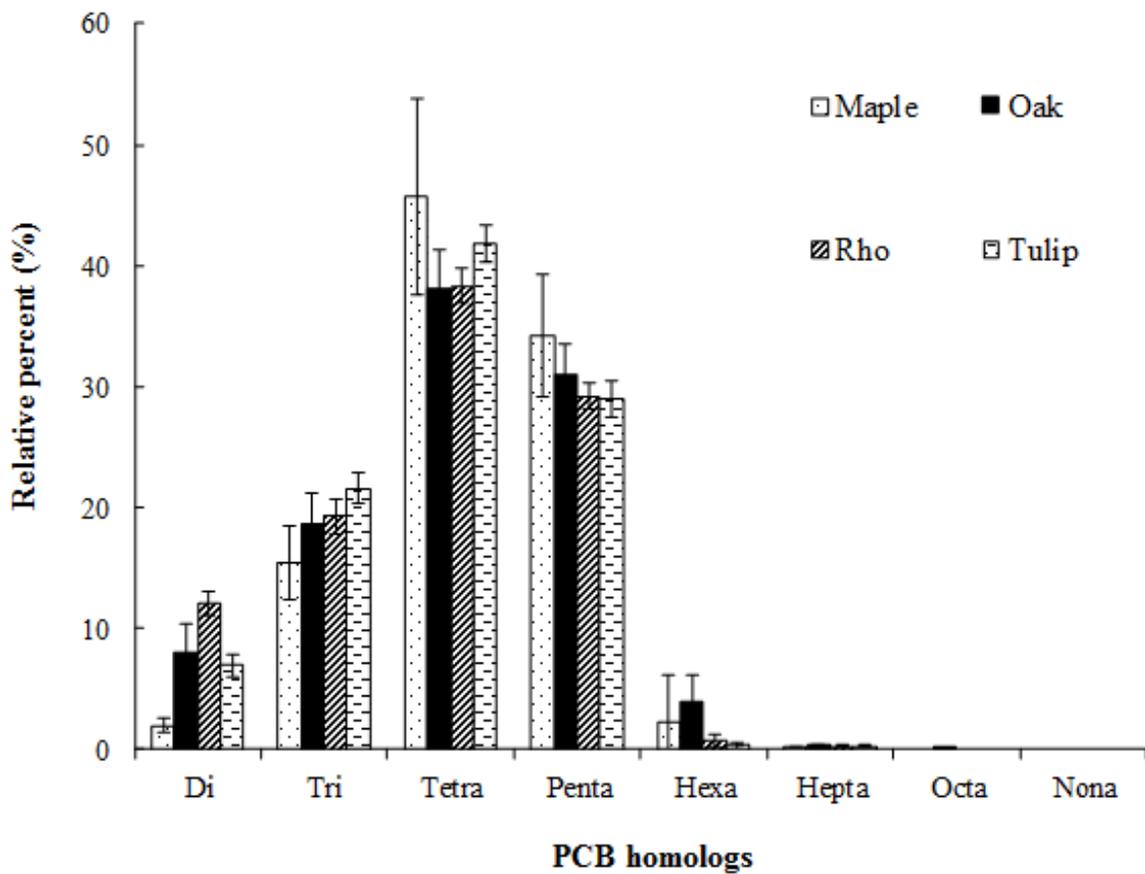
Figure 3.9. PCB homologs distribution (wt %) in individual leaf-species retrieved at day 90. Concentrations of most PCB homologs in the deciduous leaves were below the detection limit at day 0. The relative amounts of each homolog were computed as the sum of recovered amounts of congeners of a homolog divided by the total amount of PCB from the sample. Error bars indicate standard deviations.
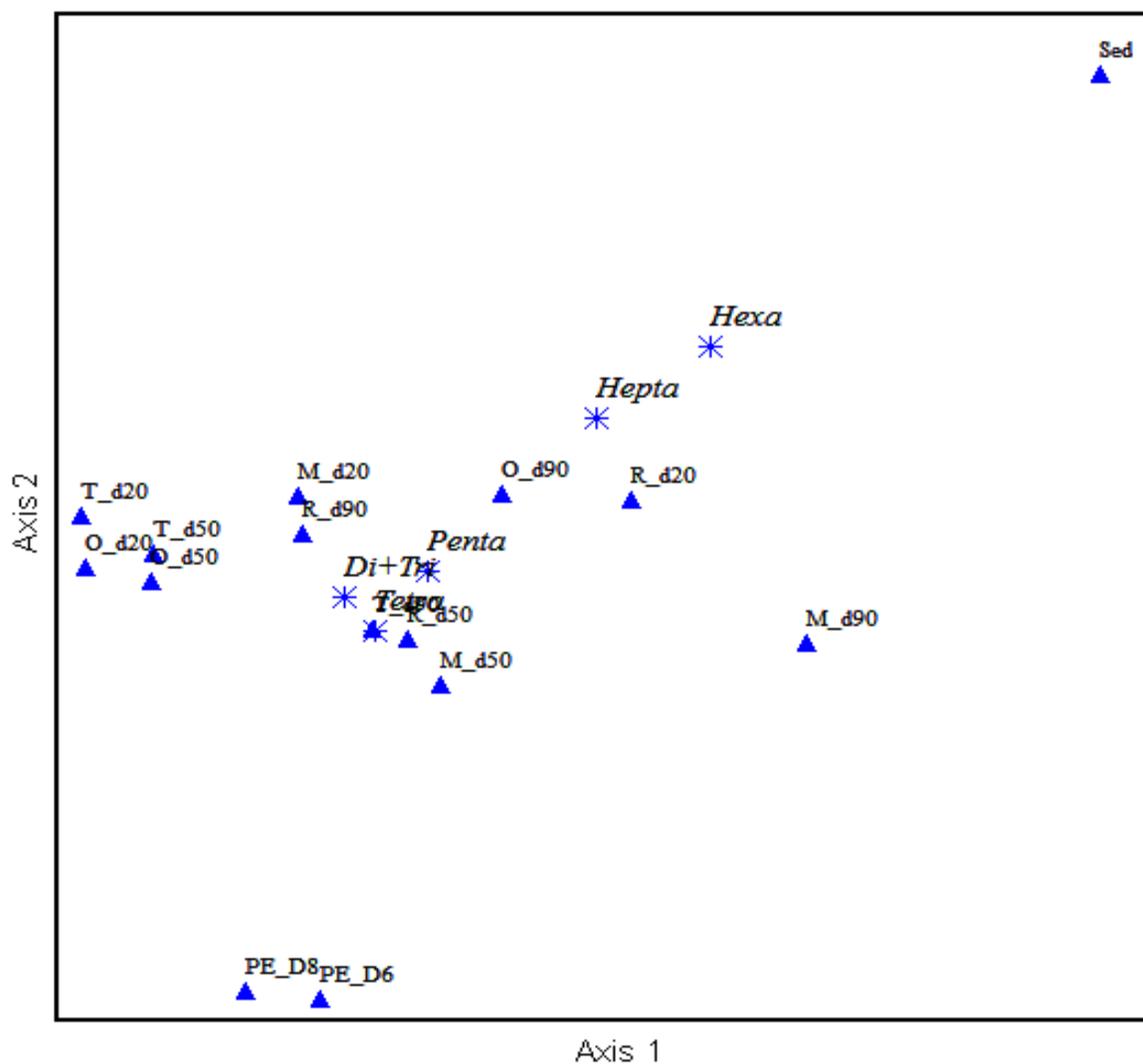
Figure 3.10. Nonmetric multidimensional scaling (NMS) plot of PCB homolog patterns in the leaves, PEs, and sediment. R_d20, for example, represents the rhododendron collected after 20 days.

Conclusion

Contributions of riparian vegetation as energy inputs are significant to stream food webs. Dissolved PCBs in the water can be absorbed by microbially conditioned leaves, which serve as a basal resource for aquatic primary consumers in the stream. This study has provided insight into the effect of leaf quality on uptake rate and PCB concentrations in the water column and how they varied among species. The study found that leaves with highly recalcitrant substrates were less subject to microbial colonization, which drives breakdown rate, resulting in slower uptake rates and lower PCB concentrations. Results of this study will provide information on the behavior of contaminants at the base of the food web and support further study on contaminant transfer to higher trophic levels that drive risk to human health.

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CHAPTER 4

SOURCES AND MAGNITUDE OF PCBs IN EVEGREEN *RHODODENDRON*

MAXIMUM NEXT TO A CONTAMINATED STREAM

Abstract

Accumulation of semi-volatile organic contaminants (SVOCs) such as PCBs by vegetation is crucial because plants are the major vector of these compounds into terrestrial food chains. Long rang atmospheric deposition, root uptake from soil, and sorption of volatilized PCBs from the water column are potential sources of contaminant in the evergreen leaves. A study was conducted to monitor the source and magnitude of PCB levels in an evergreen *Rhododendron maximum* next to a contaminated water body. Rhododendron leaves and soil samples were collected for three seasons (fall 2010, winter and spring 2011) in Town Creek. Evergreen leaves of blue spruce and American holly were also sampled upstream of the contamination in Town Creek. PCB concentrations declined with season and corresponded to flow discharge, precipitation, and environmental temperature. High PCB levels in the fall were likely related to high discharge of the stream, while revolatilization to the atmosphere at high temperature could result in decreased PCB concentrations in the spring. PCB congener pattern was similar throughout three seasons indicating a similar contamination source. NMS analysis showed a consistent pattern of increasing tetra- and penta-CBs, which reflected closely the congener pattern in passive samplers deployed in the water column. Difference in homolog distribution between the rhododendron leaves and soil samples suggested that

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volatilized PCBs from the stream was primarily the source of contaminants in the rhododendron leaves rather than long-range atmospheric transport or uptake from contaminated soil.

Introduction

Current studies of PCB influences have focused on human beings and other animals, but studies on vegetation are less intensive and widespread. Vegetation is an important, dynamic, and active environmental compartment which can take up semivolatile organic contaminants (SVOCs) from the atmosphere given their large surface area covered with waxes (McCrady et al., 1990; Riederer, 1995). Terrestrial vegetation is thus the first step of multiple pathways to transfer contaminants from aquatic systems to land-based food webs (McLachlan, 1996; Gouin et al., 2002; Barber et al., 2004). The pathway of organic pollutants entering the vegetation is a function of chemical and physical properties of the pollutant (e.g., vapor pressure, hydrophobicity, and aqueous solubility) as well as local environmental conditions (e.g., temperature, soil organic carbon content, and plant species) (Hellstrom, 2003). Root uptake and atmospheric deposition are the only two mechanisms that regulate the uptake of contaminants in vegetation. Franzaring and Eerden (2000) suggested that uptake of persistent organic pollutants (POPs) and their metabolites via plant roots can be neglected and may occur only when natural attenuation or degradation makes molecules available to plants. The root uptake for PCBs has been illustrated for only some specific plants (e.g., carrot, beet, and turnip) that contain lipid-rich peels (O'Connor et al., 1990; Fries

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and Marrow, 1981; Sawhney and Hankin, 1984; Webber et al., 1994). Because of their moderate vapor pressure, low solubility, and low reactivity, a number of SVOCs compounds including PCBs, PAHs, and pesticides have been widely distributed in the atmosphere (Hoff et al., 1992). Many of these compounds can further lead to long-range transport in the gas phase and long atmospheric residence times. Therefore, plant uptake of SVOCs has been reported to occur primarily from the atmosphere (O'Connor et al., 1991; Fries, 1995). The accumulation of SVOCs in plants is determined by one of three following processes: equilibrium partitioning between the vegetation and the gas phase, dry gaseous deposition, or particle-bound deposition to the vegetation (Welsch-Pausch et al., 1995; Paterson et al., 1991).

Application of plants as "passive samplers" of atmospheric contamination levels has been reviewed in some papers (Paterson et al., 1990; Kylin, 1994; Smith and Jones, 2000). Vegetation can sequester contaminants over time and vegetation sampling is much cheaper and easier than air sampling (Kylin, 1994). Different species have been used for biomonitoring POPs at local, regional, and global scales. Conifers are well suited for biomonitoring in part because of their long-lived foliage which may last several years (Hellstrom, 2003). For examples, evergreen needles have been used to monitor pesticides in agricultural landscapes and compounds like PCBs in urban-industrial landscapes (Jensen et al., 1992; Tremolada et al., 1996). Research concluded that higher concentrations of DDT and lindane in needles are found closer to spraying areas, while PCBs in needles are indicative of industrial activities.

Rhododendron maximum, known to gardeners as great laurel or rhododendron, is a common evergreen shrub widely distributed in eastern North American. Rhododendrons grow mainly in temperate climates and in moist but well-drained sites near streams (Irving and Hebda, 1993). Rhododendron retains its waxy layer, and deep-green leaves for up to eight years (Clinton, 2004), and these characteristics suggest that the rhododendron may be a useful biomonitor for the uptake of atmospheric organic pollutants. Previous studies used rhododendron leaves to study bioconcentration of organic chemical vapors including PCBs in closed-laboratory systems (Bacci et al., 1990; Bacci et al., 1992). In open systems, sources of PCBs to the rhododendron can be from long-range atmospheric transport of chemical bound particulate matter. Given high Henry's law constant (H_k) for some congeners, PCBs can also partition from the aqueous to the gas phase, and subsequently sorb to the rhododendron leaves while they are still on the plants. Environmental conditions such as precipitation, water flow discharge, and air temperature might affect the magnitude of PCBs in the rhododendron. Therefore, this research aimed to characterize the temporal extent and source of PCBs in the rhododendron leaves next to a contaminated water body. I hypothesized that 1) high accumulation of PCBs in the rhododendron leaves was primarily related to high precipitation and flow discharge that resulted in high volatilization of dissolved low-weight congeners and deposition of gaseous PCBs, 2) high air temperatures affected revolatilization of low chlorinated congeners from leaves, and 3) volatilization of dissolved PCBs in the stream was the predominant source to PCB in rhododendron leaves rather than root uptake from soil solution or through long-range atmospheric transport.

Materials and methods

Samples collection

Samples collection was conducted at Shady Grove along Town Creek (Figure 4.1). Seasonal variation in accumulation patterns of PCBs were addressed in a short-term study (< 1 year). Three replicates of ten leaves were collected from several shrubs in late October (fall) 2010, January (winter) and early May (spring) 2011. Rhododendron leaves were collected from the same shrub each time so that sample collection would be representative only for temporal changes. Because rhododendron plants were absent from a control site (about 1 km upstream of the S-W plant site), evergreen American holly (*Ilex opaca* Aiton) leaves and blue spruce (*Picea pungens*) needles were sampled. The American holly leaves and blue spruce needles were collected only in winter 2011 and spring 2011. The evergreens were all mature having their main growth at least one year prior to sampling. Surficial soils (top 5-cm layers) were collected only in spring 2011 from multiple areas near the shrub using a stainless steel spoon and pooled to make one sample. The leaf samples were wrapped in aluminum foil, while the soil samples were stored in an amber glass jar. All samples were stored in the freezer at -4^0C prior to extraction.
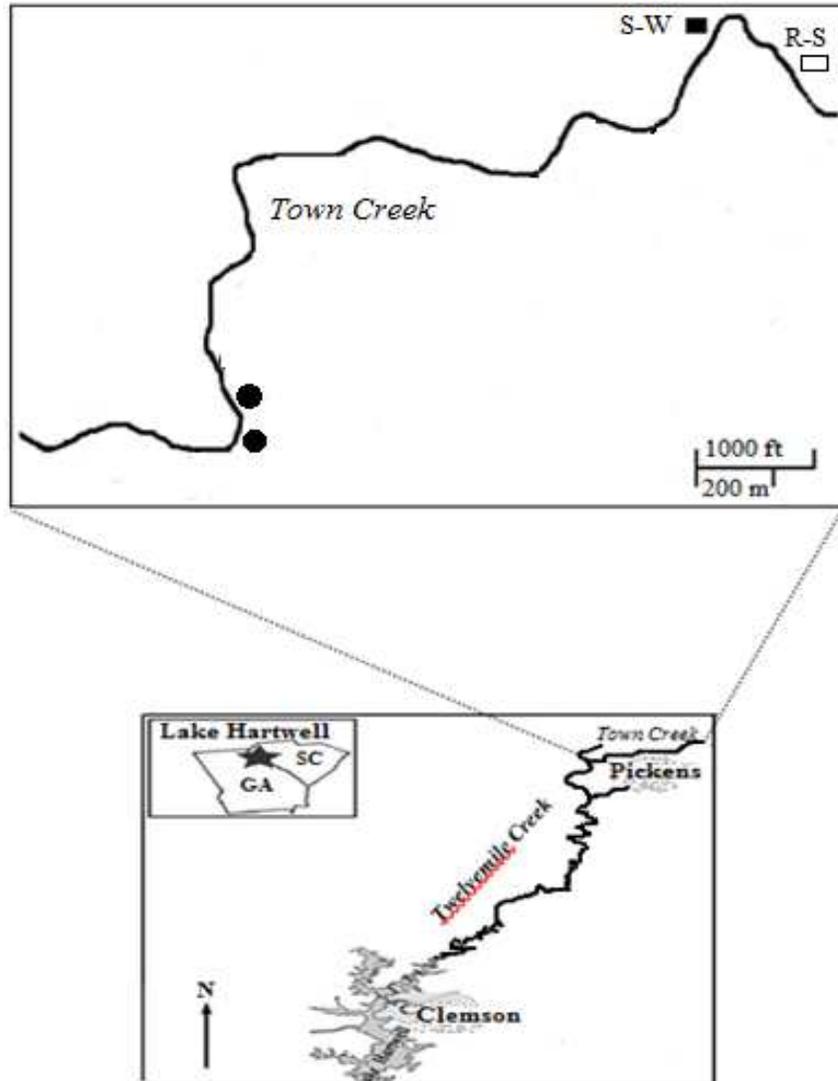
Figure 4.1. Map of evergreen rhododendron sampling locations in Town Creek, SC. Solid circles represent sampling location for rhododendron and soil samples. Open rectangular indicates the reference site (R-S). Solid rectangular indicates the former Sangamo-Weston (S-W) plant.

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Analytical methodology

PCB extraction from leaf materials were performed as described in Chapter 3. In brief, the leaf materials were chopped into small particle sizes using a blender. PCBs extraction was performed by homogenizing and mixing about 4 g of the samples with 10 g baked sodium sulfate in a beaker. Soil samples were air-dried for 48 hours in a fume hood. Approximately 15 g of the soil samples (three replicates from the pooled sample) were used for extraction and spiked with surrogate standards (PCBs 14 and 169) prior to extraction. PCB extraction from the leaf and soil samples was both performed on a Dionex ASE-200 using 1:1 hexane and acetone (1:1 v/v) (USEPA, 2005). The extracts were cleaned up on an alumina (level III-7% activated) column, followed by lipid removal using concentrated sulfuric acid, and solvent exchanged with isooctane. The soil extracts required an additional cleanup using copper powder for sulfur removal (USEPA, 2005). Reagent standards, check standards, blank leaves, and spiked blank leaves were always run with three samples per batch. Total PCB concentrations and congener-specific analysis were carried out as described in Chapter 2.

Statistical analysis

PCB congener patterns in rhododendron leaves, soil, and water column (PE as an alternative) were analyzed by NMS as described in Chapter 2. Analysis of significant correlations among total PCB, flow discharge, precipitation, and temperature variables were carried out using SAS 9.1 computer package. The distance metric used was Pearson and deletions were made pairwise.

<u>Results and discussion</u>

Recovery of surrogate standards for leaf samples was $57 \pm 10\%$ and $53 \pm 12\%$ for PCBs 14 and 169, respectively. Recoveries for soil samples were $64 \pm 14\%$ and $68 \pm 17\%$ for PCBs 14 and 169, respectively. As indicated in Chapter 3, a low percentage of recovery of PCB 169 was presumably attributed to a loss during lipid quantification and sulfuric acid processes. However, a low factor of recovery of both PCBs 14 and 169 in this study would indicate sample loss during extraction. Total PCB concentrations in rhododendron leaves collected at three seasons are presented in Figure 4.2. Mean concentrations measured at 4153.4 ± 601.6, 3722.2 ± 93.3, and 727.4 ± 199.5 ($\pm$SD, ng/g lipid) in fall, winter, and spring, respectively. Leaf PCB concentrations were not different between fall and winter, but significantly decreased in spring. PCB concentrations in the American holly leaves and blue spruce needles collected from the reference site were both below the detection limit. PCB concentration means in soil samples were 1527.8 ± 207.1 ng/g dry wt.
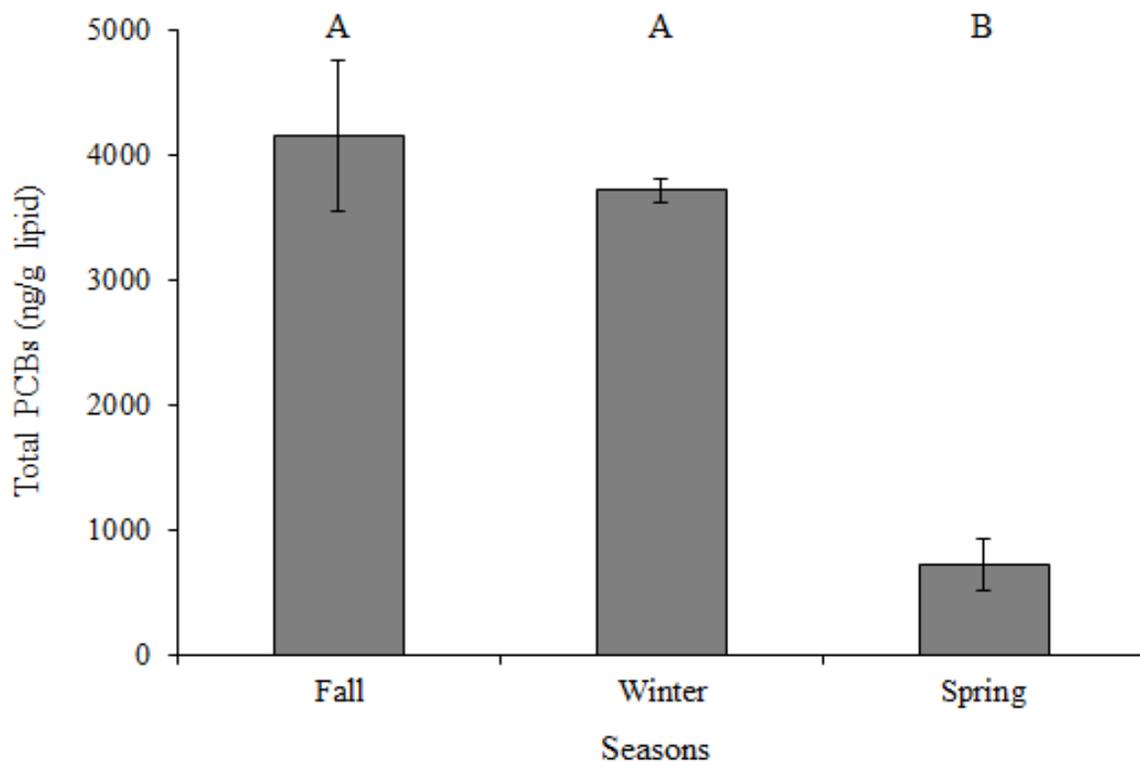
Figure 4.2. Total PCB concentrations (±SD, ng/g lipid) in rhododendron leaves collected at three seasons along Town Creek, SC. Concentrations were lipid normalized. Error bars indicate standard deviations. Sites with different letters are significantly different (α = 0.05, Tukey posthoc test).

Temporal pattern of PCBs

Measurable PCBs in the rhododendron leaves indicate that the rhododendron can be used as a potential biomonitor of airborne PCBs. Temporal trends in the rhododendron PCB concentrations might be affected by seasonal air temperature, and levels of atmospheric pollution, which depend on the magnitude of the emission source.

Changes in air temperature, which were calculated as a mean over three months prior to sampling, had an expressed effect on the accumulation of PCBs in the rhododendron leaves, except for fall (Figure 4.3). A negative correlation between mean PCB concentrations and temperature suggest that more sorption occurred at low temperature (winter) and more volatilization from the plant surface at high temperature (spring). This temperature effect was apparent as concentrations for more volatile congeners (di-+tri, and tetra-CBs) in winter were much higher than in spring due to a greater extent of revolatilization (loss) of these components back to the atmosphere at high temperature (Table 4.1). Simonich and Hites (1994) suggest that there exists an annual cycle of partitioning of SVOCs into the waxy layer at low temperature, which might contribute to more sorption occurring in the winter than in the spring. This temperature mediated effect, however, were not expressed in the case of fall samples. Perhaps, the sorption rate or emission source was much greater than the loss of PCBs in the fall. High PCB concentrations in the fall were likely related to high stream flow and precipitation due to storm events (Figure 4.4). As the discharge increases, the dissolution rate of PCBs from sediment to water increases resulting in rapid volatilization from water. Airborne pollutants can reach plant surface as free gas molecules, dissolved in

water droplets, or sorbed to particles. Both gaseous and particle-bound PCBs are known to be effectively removed from the atmosphere via precipitation (Eisenreich et al., 1981; Bidleman, 1988; Poster and Baker, 1996). Although PCBs in the fall were significantly higher than in the spring, the precipitation between the fall and spring was slightly different. Perhaps, precipitation is not strongly related to PCB concentrations in the rhododendron. It is also evidence that less than 3% of PCBs is truly dissolved in rain while the remaining fraction is associated with particles (Atlas and Giam, 1988; Poster and Baker, 1996). In addition, Umlauf et al. (1994) reported that the uptake of semi-volatile organic pollutants by plants is much more important from the gas phase than particle deposition onto plant surfaces.
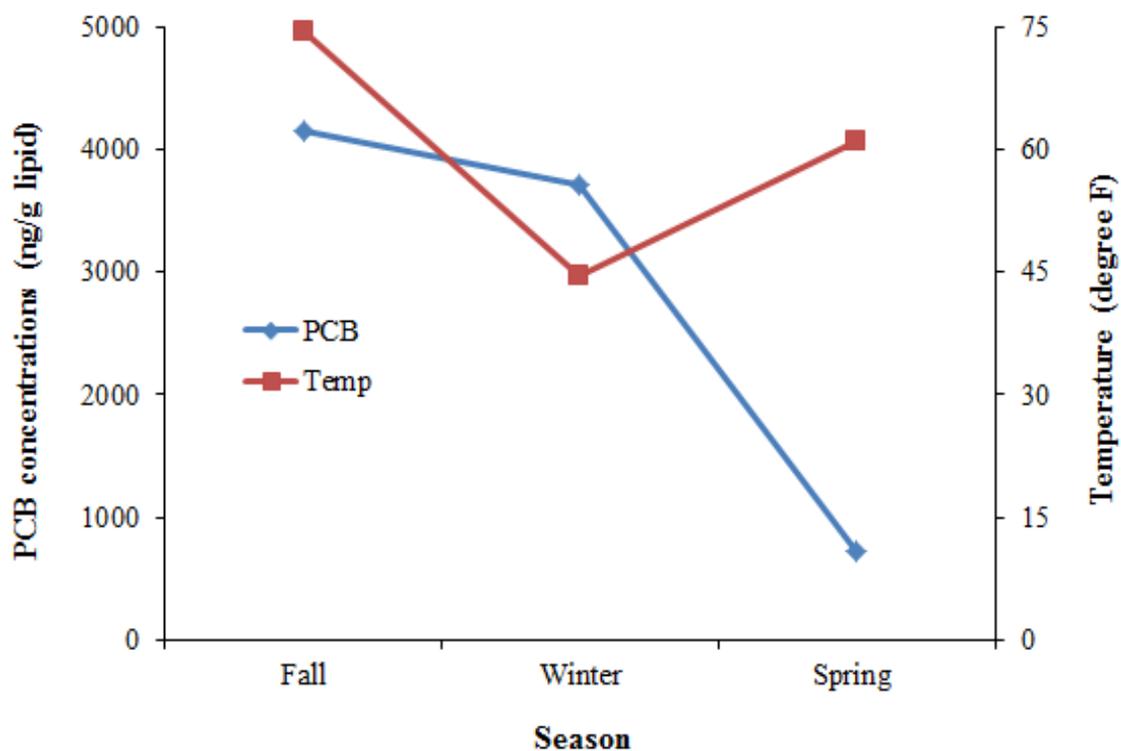
Figure 4.3. Correlation between mean PCB concentrations and air temperature in winter and spring. Air temperatures are a mean over a total three month prior to sampling.

Table 4.1. Concentrations (mean ± 1 SD, ng/g lipid) of PCB homologs in the rhododendron.

	Di- + Tri-CBs	Tetra-CBs	Penta-CBs	Hexa-CBs	Hepta-CBs
Fall 2010	4.9 ± 1.0	25.2 ± 4.4	28.3 ± 8.2	1.1 ± 1.2	0.5 ± 0.1
Winter 2011	6.2 ± 0.5	27.3 ± 1.2	27.7 ± 2.1	0.2 ± 0.1	0.4 ± 0.1
Spring 2011	0.7 ± 0.6	6.4 ± 1.6	8.5 ± 2.5	ND	0.7 ± 0.1

ND: below detection limit.

Figure 4.4. Mean stream flow (ft^3/s) and precipitation (inches) data for USGS stream gaging station (station number 0218600) at Twelvemile Creek, a recipient of Town Creek, near Liberty, SC. Values are means over the three months prior to sampling.

Source of PCBs in rhododendron

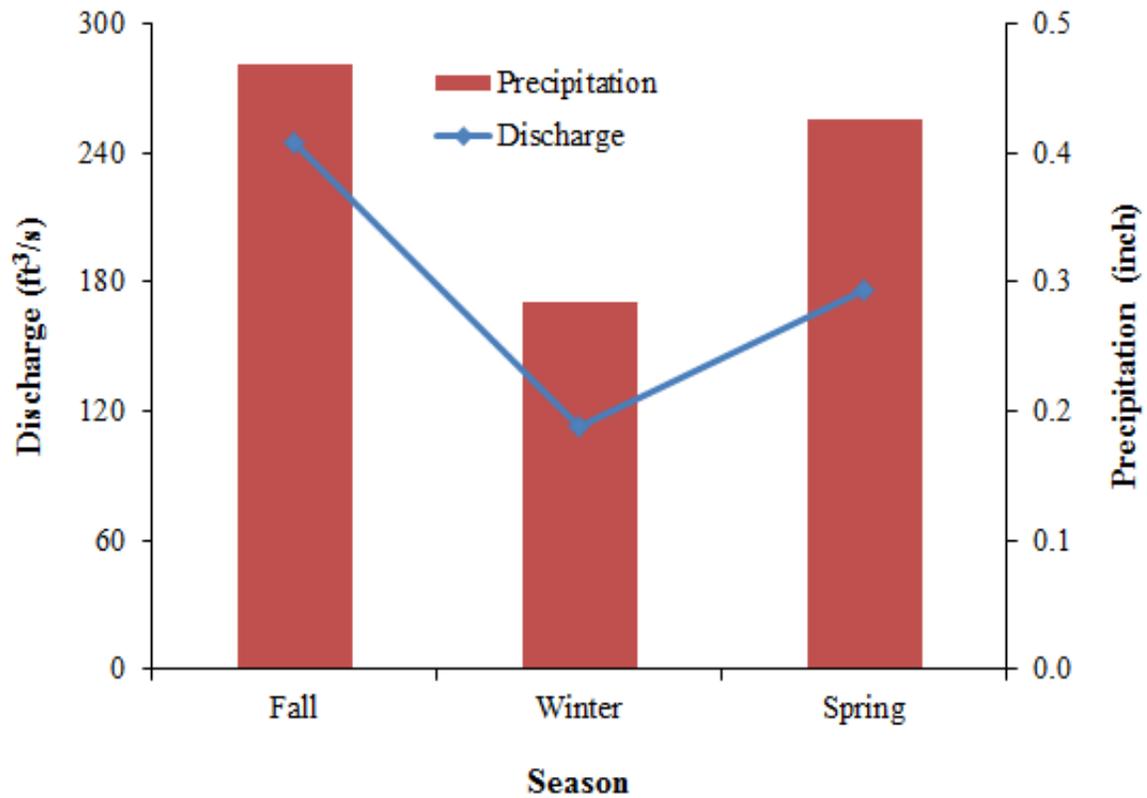The similarity of homolog pattern in leaves collected for three seasons indicates a similar source of PCBs in the rhododendron (Figure 4.5). The total PCBs burden of all the samples was dominated by moderately chlorinated congeners with tetra- and penta-CBs always making up >90% of the total PCBs load. Other homologs such as di- + tri-CBs accounted for approximately 10% and congeners with more than five chlorine atoms contributed insignificantly to the total PCBs. There appeared a gradual shift in the relative proportions of the homologs from fall and winter to spring, where the homolog distributions in the fall and winter peaked at both tetra- and penta-CBs while the distribution peaked at only penta-CBs in the spring.

The emission source is likely an important factor that ultimately determines the accumulation. The emission source could be a volatilization from the contaminated stream, soil, or long-range transport in the atmosphere. Since PCBs were below detection limit in the American holly leaves and blue spruce needles, the long-range transport was not the source of PCBs in the rhododendron. A previous study observed that the level of PCBs found in foliage is mainly due to vapor transport from soil, rather than to translocation through the plant (Buckey, 1982). High PCB concentrations in Town Creek soil samples would suggest a potential contamination source of PCBs from the surficial soil via volatilization to the atmosphere. Furthermore, volatilization of dissolved PCBs as a loss mechanism from the water column may be another source of PCBs in the rhododendron leaves. NMS ordination indicates that rhododendron and PEs were not separated along axes 1 or 2 and close to lower chlorinated congeners (e.g., di-+tri-, tetra-

CBs), while soil was separated from the rhododendron along axis 1 and represented a close ordination to higher chlorinated PCBs (e.g., hexa, and hepta) (Figure 4.6). PCBs in the rhododendron are thus primarily from the stream volatilization rather than from the soil surface.
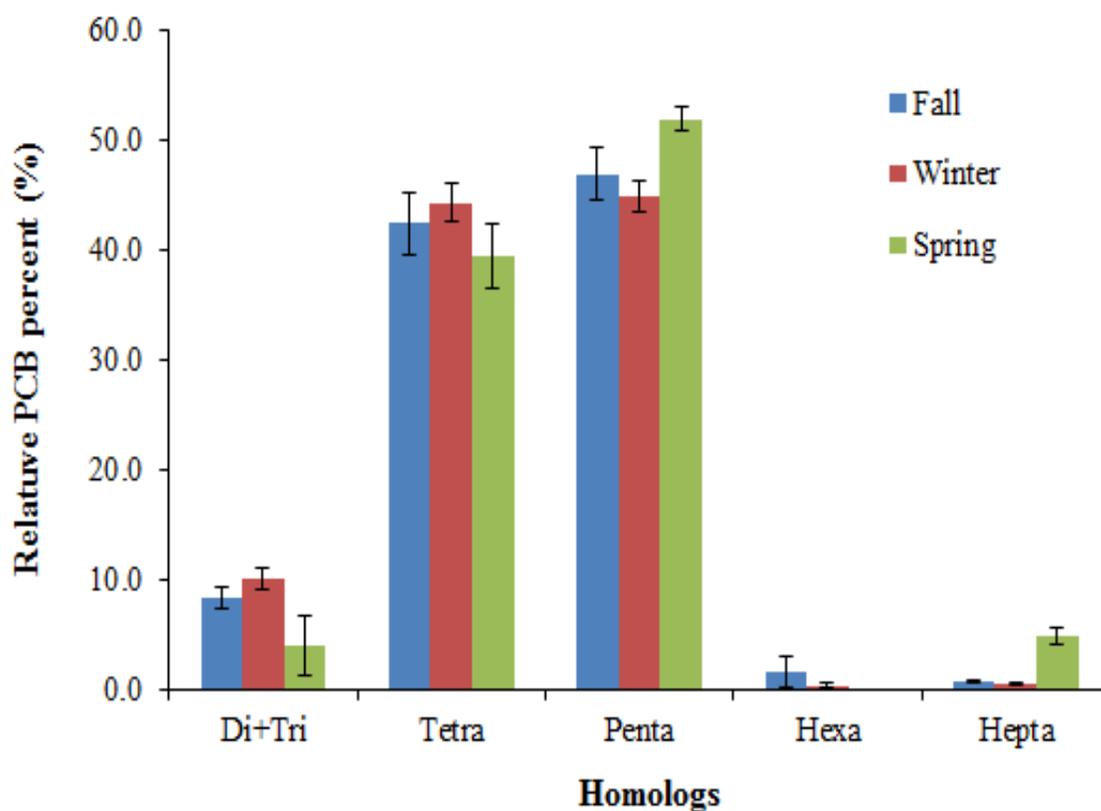
Figure 4.5. PCB homologue distribution (wt %) in the rhododendron leaves collected at three seasons along Town Creek, SC. The relative amounts of each homologs were computed as the sum of recovered amounts of congeners of a homolog divided by the total amount of PCB from the sample. Error bars indicate standard deviations.
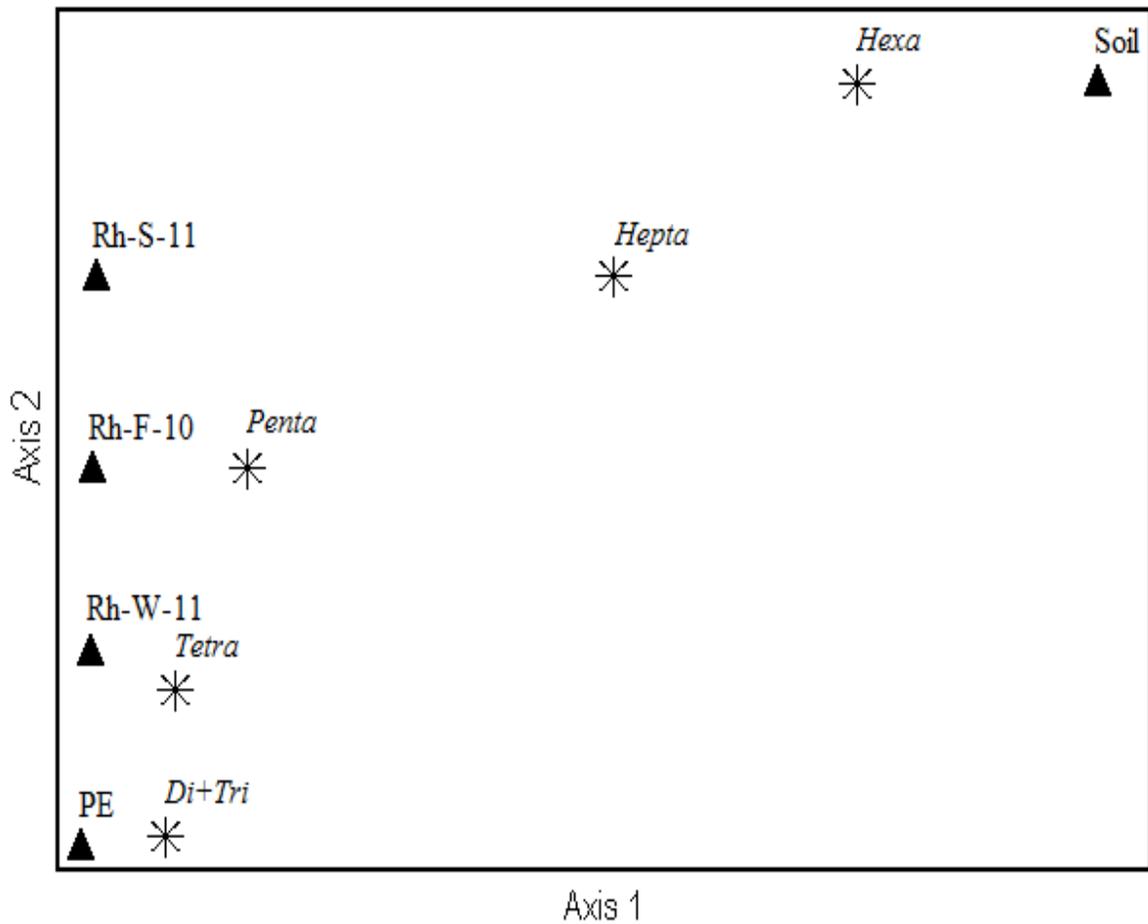
Figure 4.6. Nonmetric multidimensional scaling plot of PCB homologue pattern in the rhododendron, PEs, and soil. Rh-S-11, for example, represents rhododendron collected in spring 2011.

Conclusion

The temporal magnitude and source of PCBs in the evergreen rhododendron next to a PCB-contaminated stream has been examined. PCBs were similar from the fall to the winter, while concentration was significantly decreased in the spring. PCB concentrations were temperature dependent only in the winter and the spring showing a negative correlation between PCB concentrations and mean temperature over three months prior to sampling. Conversely, high PCBs in the fall were likely associated with high emissions of PCBs, which is driven by flow discharge and precipitation. PCBs detected in the rhododendron leaves corresponded closely to pattern of dissolved PCBs in the water column suggesting that volatilization of PCBs from the stream is more likely a major source of PCBs than the uptake from the soil or long-range transport. Results from this study draw attention to the global cycling of PCBs as they can be exported from aquatic to terrestrial system via volatilization and subsequently pose risks to agriculture land and human health. Findings in this study can guide the next application of indicator development, which involves some estimate of temporal/spatial scale.

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CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This dissertation has provided more in-depth knowledge of the source and fate of dissolved PCBs in a stream. Research directly addressed the potential ongoing sources of PCBs to the water column and their pathways to the base of food webs. Some main conclusions are summarized below.

1) Multiple lines of evidence were used to assess the potential for ongoing sources of dissolved PCBs (e.g., ongoing sources, groundwater inputs, and surface runoff) in a contaminated stream. I observed 1) an increase in dissolved PCB concentrations with increasing downstream distance, 2) the congener pattern in passive samplers at contamination source was similar to the historical mixture of Aroclors, and 3) EF values for chiral PCBs remained relatively constant throughout the system. Evidence from total PCBs and congener specific analysis, but not chiral signatures in passive samplers, thus suggests that PCBs are still leaking from the original S-W plant site.

2) Decomposing leaf material is an important source of carbon and nutrients to stream food webs and further an important media to transfer organic pollutants to food webs. I observed that dissolved PCBs in the water could be absorbed by microbial decomposing leaves, and the leaves with different nutritional quality would accumulate PCBs to a different extent. From the observations, I developed the hypothesis that uptake

rates and PCB concentrations in the leaves found in the stream are closely related to their decomposition rates and fungal biomass.

3) Evergreen plants next to a contaminated water body can act as a bioindicator for volatilization of dissolved PCBs from the water column. I observed that PCB concentrations in rhododendron leaves were highest in the fall and winter, but lowest in the spring. Temporal variations in concentration were likely related to temperature, precipitation, and stream flow. While uptake via root and long-range transport were negligible, volatilized PCBs from stream was likely the main mechanism of PCB uptake by the rhododendron leaves.

Given a long half-life in the environment and toxic effects, PCBs potentially pose a long-term risk to aquatic ecosystems and human beings. Findings in this research will add to the body of knowledge of pathways of contaminants in the environment, which are crucial for ecosystem risk assessment. In addition, observations from this research will be beneficial to other contaminated sites to better understand the pathways of contaminants to food webs and solve questions of source control.

Recommendations for future work

The PE study suggests that the abandoned wastewater treatment plant is also a potential for an ongoing source of PCBs to the system. Therefore, a fine-scale survey of total PCBs and congener pattern is recommended to monitor this potential input. In addition, the sources of dissolved PCBs can be from sediment resuspension, atmospheric deposition, and potentially colloidal transport in groundwater and/or erosion of particles

from land. Information on groundwater input is still missing given that many streams maintain their stream baseflow by gaining a large volume of water from underground, which will then contaminate the surface water. More research is thus needed to evaluate the extent of groundwater contributing dissolved PCBs to the surface water in a stream system.

Microbially conditioned leaves are an important source of carbon and nutrient to food webs, and also an important vector of contaminants to primary consumers and the rest of the food web. Knowledge about the leaf decomposition is abundant, but the effect of leaf quality on the uptake of contaminants is still limited. Future studies are needed to determine what mechanisms stimulate the uptake rates of PCB in microbial decomposing leaf materials and their pathways to transfer contaminants to primary consumers.

Evergreen rhododendron next to contaminated water bodies can be contaminated with PCBs via volatilization from the water. However, there is still a lack of evidence for the use evergreen leaves to monitor volatilized PCBs as a source of air pollution. Further development work which involves some estimate of spatial/temporal scale will guarantee the potential of rhododendron as a bioindicator. A study can be set up by deploying rhododendron at sites to determine spatial and temporal uptake of PCBs and then returning the rhododendron to greenhouses to examine volatilization rates and temperature effect.

APPENDICES

Appendix A

PCB concentrations in the passive samplers (PEs) at 7 locations.

Locations	PCB concentrations (mean ± 1SD, ng/g PE)
D1	748.9 ± 191.5
D2	2110.0 ± 103.7
D3	2557.7 ± 263.3
D4	2130.0 ± 562.8
D6	3568.6 ± 139.1
D7[*]	3333.3 ± 1008.5
D8	3547.6 ± 476.0

*: samplers were analyzed, but excluded from the results since the basket containing the samplers had settled to the streambed.

Appendix B

PCB concentrations in individual leaf species at different collection time

Sample	PCB concentrations (ng/g dry wt)	PCB concentrations (ng/g lipid)
T_day 0	1.6 ± 1.2	78.9 ± 65.7
T_day 20	645.4 ± 47.8	19055.5 ± 2941.7
T_day 50	339.6 ± 38.4	15980.2 ± 2941.9
T_day 90	339.6 ± 132.3	13736.7 ± 4729.1
M_day 0	7.7 ± 6.3	575.7 ± 454.6
M_day 20	216.7 ± 68.3	5405.2 ± 2297.3
M_day 50	338.3 ± 106.0	13314.7 ± 4364.3
M_day 90	271.2 ± 88.7	11136.3 ± 5668.9
O_day 0	1.4 ± 1.6	365.8 ± 225.6
O_day 20	210.8 ± 44.5	18687.5 ± 3930.9
O_day 50	550.4 ± 134.2	38668.8 ± 10568.8
O_day 90	835.0 ± 208.4	62919.3 ± 16655.2
R_day 0	60.4 ± 12.4	4710.6 ± 2200.6
R_day 20	149.5 ± 35.4	9592.9 ± 2644.8
R_day 50	128.6 ± 32.9	5638.3 ± 817.5
R_day 90	307.3 ± 46.4	13041.4 ± 2671.7

T: Tulip poplar; M: Maple; O: Oak; R: Rhododendron.