Fall 2012

Rhizosphere biotransformation of selected polychlorinated biphenyl (PCB) congeners by switchgrass and poplar

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RHIZOSPHERE BIOTRANSFORMATION OF SELECTED POLYCHLORINATED BIPHENYL (PCB) CONGENERS BY SWITCHGRASS AND POPLAR

by

Richard Edward Meggo

An Abstract

Of a thesis submitted in partial fufillment of the
requirements for the Doctor of Philosophy degree
in Civil and Environmental Engineering in
the Graduate College of
The University of Iowa

December 2012

Thesis Supervisor: Professor Jerald L. Schnoor

ABSTRACT

Selected PCB congeners (PCB 52, 77, and 153) singly and in mixtures were spiked and aged in soil microcosms and subsequently planted with switchgrass (*Panicum virgatum)* or poplar (*Populus deltoids x nigra* DN34). The planted reactors showed significantly greater reductions in PCB parent compounds when compared to unplanted systems after 32 weeks, both in single congener exposures and when all three congeners were present in a mixture. There was evidence of reductive dechlorination in both planted and unplanted systems, but higher concentrations of transformation products were observed in the planted systems than the unplanted. Although planted systems resulted in greater biotransformation, this improvement in PCB-reduction was not the result of plant uptake but rather was due to transformations occurring in the root rhizosphere. Parent PCB congeners were transformed by reductive dechlorination resulting in successively less chlorinated PCB congeners. These dechlorination products accounted for approximately all of the molar mass of parent compound lost. Based on the transformation products, reductive dechlorination pathways are proposed for rhizospheric biotransformation of PCB 52, 77, and 153. Results suggest that PCB 52 transformation proceeds through PCBs 18 and 9 down to monochlorinated PCB 1. Biotransformation of PCB 77 occurs through the intermediaries PCB 35 and 37. The pathway for the rhizospheric transformation of PCB 153 is through PCB 101 and PCB 99. This study provides insight into rhizosphere biotransformation pathways for reductive dechlorination in marginally aerobic,intermittently flooded soil as evidenced by a mass balance on transformation products. Despite the marginally aerobic conditions it is likely that highly reduced microzones existed in the soil particles during flooding and provided the opportunity for reductive dechlorination. In these experiments, planted microcosms with fully developed roots and rhizospheres showed significant reductive dechlorination and

greater biotransformation than unplanted reactors. In addition, planted systems that were intermittently flooded had greater transformation of the parent PCB compounds than systems that were not.

A poplar planted system resulted in the complete removal of 26 of the 29 PCB congeners detected in a commercial garden soil, while the unplanted soil only had 2 congeners completely removed after 96 days. In addition, the most recalcitrant congener, PCB 52, only decreased by 0.1% in the unplanted reactors while declining by 22.3% in the planted system. There was also greater removal of a PCB 77 spike in the planted system when compared to the unplanted system, 17.2% in the planted system versus 2.8% in the unplanted system. The results suggest that phytoremediation may be an effective tool in cleaning commercially available garden soils that are lightly contaminated with PCBs.

Abstract Approved: _____

Thesis Supervisor

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Title and Department

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Date

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The University of Iowa
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CERTIFICATE OF APPROVAL

PH.D. THESIS

This is to certify that the Ph.D.thesis of

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has been approved by the Examining Committee for the thesis requirement for the Doctor of Philosophy degree in Civil and Environmental Engineering at the December 2012 graduation.

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To my family, especially Carol, Daniel and Micha'el

You have heard the law that says, ‘Love your neighbor’and hate your enemy. But I say, love your enemies! Pray for those who persecute you! In that way, you will be acting as true children of your Father in heaven. For He gives his sunlight to both the evil and the good, and He sends rain on the just and the unjust alike. If you love only those who love you, what reward is there for that? Even corrupt tax collectors do that much. If you are kind only to your friends, how are you different from anyone else? Even pagans do that. But you are to be perfect, even as your Father in heaven is perfect. Dear friends, I am not writing a new commandment for you; rather it is an old one you have had from the very beginning. This old commandment—to love one another—is the same message you heard before.

Jesus Cristo and John, Matthew 5: 43-48; 1John 2:7

ACKNOWLEDGEMENTS

The support and direction given by my supervisor Jerry Schnoor is deeply appreciated. Jerry, thank you for your steady guidance and the latitude afforded to grow and have ownership of this research. You have been a tower of strength, a reservoir of knowledge, a fountain of wisdom and patient beyond understanding. You have been an excellent advisor, but more than that, you are a superb and wonderful person. The advice and thought provokings questions from my committee members have allowed me to gain deeper insight and added rigor to my thought process. Dingfei Hu and Andres Martinez from the Keri Hornbuckle group have taught me all I know about PCB quantification, and for that I am eternally grateful. Their analytical support was invaluable. A special thank you to Collin Just who handled and the procurement of supplies and instrument trouble shooting for the research. Dr. Hans Lehmler got me started and going by providing high quality PCB congeners. Many thanks to the Iowa Superfund project for providing funding to undertake this research. Judy Holland, Angela Schenkel, Jennifer Rumping and Laura Myers provided excellent administrative services, a big thank you ladies. The faculty, staff and student of the EES program made the department a joyful place to work. Thanks to the Schnoor group for the many suggestions to improve my presentations and research, particularly Guangshu Zhai. A special thank you to Scott and the rest of the staff at the UI Water Plant for accommodating me and allowing me to utilize their space. I could not have done this without the loving support of my wife Carol and sons Daniel and Micha'el. I know that no matter what sort of day I had, going home to you guys was always the high point of my day. I love you. Last but not least, I thank the Lord Jesus Christ and God the Father whose grace and mercy carried me through, through the highs and the lows and through the good and bad. I am fully persuaded that He who started the good work is faithful to complete it to the end.

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CHAPTER 1 INTRODUCTION AND OBJECTIVES

1.1 Introduction

Polychlorinated biphenyls (PCBs) consist of a family of 209 compounds called congeners, which have a molecular weight ranging from 188 to 439.7. They have a chemical formula of $C_{12}H_{10-n}Cl_n$ and their structure consists of two phenyl (C_6H_5) rings single-bonded together, with between 1 to 10 chlorine atoms, substituted at different positions in the biphenyl rings. Depending on their position, the chlorine atoms in the biphenyl rings of PCBs can be classified as ortho (2,2' and 6,6' positions), meta (3,3' and 5,5' positions) or para (4) substituted. The notation can be seen in Figure 1.1.

The full chemical notation can be cumbersome especially for highly chlorinated congeners, and this has led to the development of alternative systems of nomenclature. One that has been popular among PCB researchers, and has been recognized by the International Union of Pure and Applied Chemistry (IUPAC) is the Ballschmiter and Zell (BZ) nomenclature system. The BZ system arranges congeners in ascending numerical order based on the number of chlorine atoms and their substitution pattern on the biphenyl base structure (Ballschmiter and Zell, 1980, Maervoet et al., 2004). Both the full chemical notation and the BZ number designation will be used throughout this dissertation.

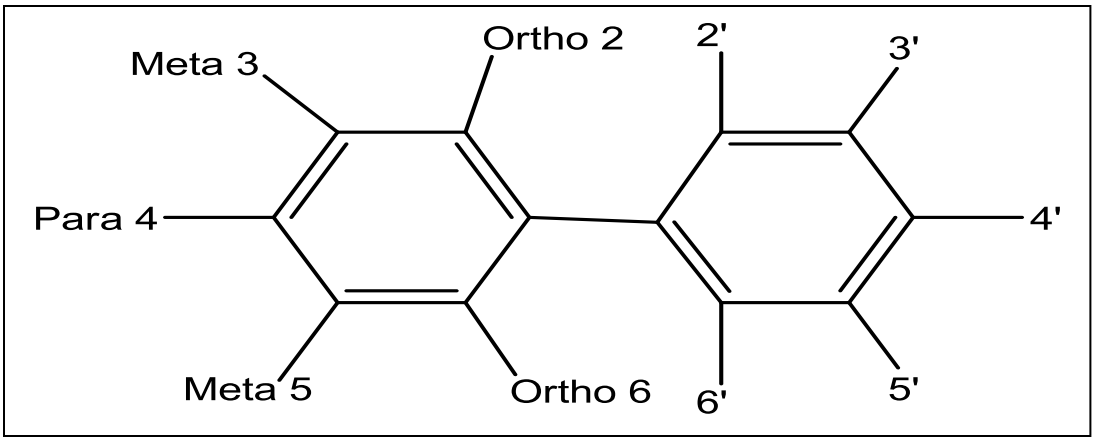

Figure1.1: Nomenclature for PCBs. The 12 carbon positions in the biphenyl are numbered using carbon 1 for the phenyl- phenyl bond and then numbers 2-6 for the first ring and 2'6' for the second ring. Thus, there are four ortho (i.e. 2, 2', 6 and 6'), four meta (i.e. 3, 3', 5 and 5') and two para (i.e. 4 and 4') positions. Short notations used for halogen-substituted biphenyls use either the notation pictured above, giving the substituted position in order of increasing numbers and using the prime notations for the second ring (e.g. for a given hexa-chlorobiphenyl = 2,2',3,3',5,6'-CB), or give first the positions of chlorines on the most chlorinated ring followed by the chlorinated positions on the second ring, separated by a hyphen without using the primes (e.g. 2,3,5- 2,3,6-CB or 235-236-CB) (Wiegel and Wu 2000).

PCBs are toxic pollutants which persist in the environment and are among the most persistent organic pollutants in the environment today. Since there are no known natural sources of PCBs, their presence in the environment is solely a result of anthropogenic factors. Though manufactured over a span of about 40- 50 years, their impact in the environment is predicted to last for several multiples of their manufacturing and usage span. This is because one of the qualities that made them desirable for industrial use, chemical inertness, is the same one that is facilitating their recalcitrance. The lack of reactivity makes it difficult for natural biota to degrade them at any significant rates, thereby driving their persistence (Meggo and Schnoor,2011). In addition to their chemical inertness, there is no single redox environment that is conducive to the degradation of the whole suite of congeners. Lower chlorinated congeners (1-4 chlorines) usually are more amenable to degradation under aerobic conditions while the converse is true for highly chlorinated congeners, that is, they are more readily degraded under

anaerobic conditions. Given the fact that PCBs were usually manufactured as mixtures with a range of lower and higher chlorinated congeners, this further aggravates the problems associated with their persistence.

Since their use in industry was widespread, it means that they have a correspondingly large footprint. Due to the painstakingly slow rate of degradation in the environment, remediation efforts have focused mainly on physical methods such as containment and incineration. Notwithstanding their defiance to natural degradation, there is evidence that microorganisms are capable of degrading PCBs, albeit rather slowly. Consequently, the next major focus of PCB research has been on maximizing the potential of these natural degraders in order to accelerate the degradation process.

For this research, two plant species and three PCB congeners were selected. The plant species were hybrid poplar (*Populus deltoids x nigra* DN34) and switchgrass (*Panicum virgatum*)**.** The congeners utilized were PCB 52 (2,2'5,5' tetrachlorobiphenyl) PCB 77 (3,3'4,4' tetrachlorobiphenyl), and PCB 153 (2,2',4,4'5,5' hexachlorobiphenyl). Switchgrass is a hardy, deep rooted perennial plant which is widespread, and possesses a C4 mechanism for photosynthesis. This makes it suitable for use in a wide range of environments. Poplar is also deep rooted, a model plant genetically and widely used in phytoremediation applications, and it has been shown to effectively stimulate the biotransformation of several xenobiotic compounds (Kacalkova and Tlustos, 2011,Liu et al., 2009, Zhai et al., 2010). PCB 52 transformation is thought to be particularly difficult by the most common route of PCB degradation for lower chlorinated PCBs, i.e. 2,3 (5,6) dioxygenase attack and aerobic oxidation. This is because in PCB 52, the normal sites of attack are occupied by chlorine substituents. Ortho substituents are particularly difficult to remove due to steric hindrances which mitigate against an enzymatic approach to those sites. However, it is possible that PCB 52 can be degraded by a 3, 4 (4,5) dioxygenase attack or even a monoxygenase mechanism (Bedard et al., 1987, Komancova et al.,2003). Therefore, PCB 52 (2, 2’5, 5’ tetrachlorobiphenyl) was selected because it is difficult to

degrade, due to the chlorine substituents in the ortho positions on both rings. PCB 77 was selected because it is one of the most toxic, lower chlorinated PCB congeners (with coplanar properties and exhibiting dioxin-like properties). However, it should be readily degraded because it is devoid of chlorine substituents in the ortho position. PCB 153 has a tendency to bioaccumulate in humans. In addition, it has ortho substituents on both rings, making it more recalcitrant to microbial degradation. This recalcitrance means that it will likely persist in the environment. However, microbial degradation of PCB 153 has been observed previously with *Burkholderia* LB 400 and *Alcaligenes eutrophus (now Ralstonia eutropha)* H850 (Bedard et al., 1986, Leigh et al. 2006).

To avoid ambiguity, the following definitions are pertinent to the discussion that follows.

Rhizosphere – soil in the root zone which is under the influence of the roots, usually defined as within1mm of the root. The reactors used in this study were shallow enough, for all of the soil column to be considered the rhizosphere.

Experimental system – consists of experimental apparatus during exposure and includes planted and unplanted setups. Two different reactor systems were employed in these experiments. The first was populated with a single plant per reactor, while multiple plants were planted in each of the reactors in the second reactor system.

Cycled treatment - planted or unplanted system that undergoes moisture content cycling and redox (ORP) variations.

Uncycled treatment - planted or unplanted system that does not undergo moisture content cycling.

This research is undertaken as a component of a larger initiative, the Iowa Superfund Research Program, which is geared at investigating the ramifications of the proposed dredging of PCB contaminated Indiana Harbor and canal in East Chicago, and the impact of the more volatile congeners that are primarily airborne.

1.2 Objectives

The main objective of this research is to evaluate the potential for enhancing degradation and transformation of selected volatile polychlorinated biphenyl (PCB) mixtures and single congener systems by planting contaminated soil with poplar and switchgrass. Furthermore, the impact of cyclic manipulation of moisture content in the root zone (rhizosphere) will be investigated. Another objective is to determine the PCB fate pathways for such a system.

1.2.1 Specific Objective 1 (SO1)

To determine if low redox in the root zone will impact total PCB degradation.

Hypothesis 1 (SO1/H1)

Manipulation of moisture content in a switchgrass (*Panicum virgatum**)*** or poplar (*Populus deltoids x nigra* DN34) planted rhizophere will produce changes in the redox potential in the rhizophere. Measurements of oxidation reduction potential (ORP) can be used to monitor this effect.

Hypothesis 2 (SO1/H2)

A poplar (*Populus deltoids x nigra* DN34) or switchgrass (*Panicum virgatum**)*** planted rhizosphere will enhance degradation of the PCB mixture and single congeners tested. Measurement of soil PCB concentrations in planted and unplanted systems will verify or disprove this hypothesis.

Hypothesis 3 (SO1/H3)

Significant transformation of the PCB mixture tested will occur in the moisture content cycled treatment. To verify this, transformation products will be measured and a mass balance performed.

1.2.2 Specific Objective 2 (SO2)

Evaluation of the role of plants.

Hypothesis 1 (SO2/H1)

PCBs will mostly be sorbed to soil and plant roots and not be significantly taken up or translocated by the plant. Analysis of plant material above and below ground will provide evidence that PCBs are not translocated.

1.3 Dissertation Organization

Chapter 2 will present results for an experiment with garden soil that had trace amounts of PCBs other than the spike. It shows that the planted treatments removed these trace contaminants, while the unplanted treatments did not. Chapters 3, 4 and 5 utilize data from one experiment, but with each chapter emphasizing a major outcome of the experiment. Chapter 3 looks at the impact of moisture content and redox cycling on the transformation of PCBs. The role of plants in the transformation of PCBs in mixture and as single congeners is outlined in Chapter 4, and Chapter 5 highlights pathways for the rhizopheric transformation of PCB 52, PCB 77 and PCB 153. Chapters 2, 3 and 5 are manuscripts that have been submitted to peer review journals.

CHAPTER 2 CLEANING PCB CONTAMINATED GARDEN SOIL BY PHYTOREMEDIATION

2.1 Introduction

The wide spread use of polychlorinated biphenyls (PCBs) in a variety of products from the 1930s – 1970's allowed for the ubiquitous presence of PCBs in various environmental matrices. Low, but measurable amounts can be found in most environments. Both urban and rural soils have been impacted by atmospheric transport of PCBs (Aichner et al., 2007, Wilcke and Zech, 1998). It has been reported that, urban garden soils had elevated level of PCBs, while agricultural soils had lower median concentrations than garden soil (Krauss and Wilcke, 2003). Although below the critical level of 200 ngg^{-1} for agricultural use, total PCBs ranging from 8.4 -59.5 ng/g were detected in rural soils (Wilcke and Zech, 1998). Measurable levels of PCBs in yard waste are not uncommon. This may be because of PCB partitioning in the bark of trees (Hermanson and Hites, 1990). Therefore, compost made from yard waste is also likely to contain measurable levels of PCBs. Elevated PCB levels have been found in many municipal solid waste (MSW) and sewage sludge (SS) compost (Malloy et al 1993). In one study it was shown that maternal gardening contributed to PCB levels in prepubescent boys' serum. Prepubescent Russian boys whose mothers gardened locally, had higher serum levels of PCBs, compared to those whose mothers didn't (Burns et al., 2009).

Given the global presence of PCBs, PCB degraders are found in many environments (Hiraishi, 2008, Bedard, et al. 2007, 2008, Magar et al., 2005, Pakdeesusuk et al., 2005, Tiedje et al., 1993). However, microbial degradation of PCBs in the natural environment is extremely slow and this accounts for their persistence in the environment.

Left alone, PCB compounds degrade slowly in soil so the risk of exposure is longer in unplanted soil. A five year simulation showed that even in planted systems, PCBs remained in the root zone and slowly degraded (Hsu et al. 1993).

The ability of plants to stimulate microbial activity in the rhizosphere is known (Mackova et al., 2007). Poplar is a model plant for phytoremediation, and hydroponics studies involving PCB and poplar have been undertaken (Liu et al. 2008, Liu et al., 2009). However, most field-scale phytoremediation interventions involve planting in a soil matrix which is more complex than hydroponics, yet few studies have been undertaken with real garden or commercial soil.

Given the ubiquity of PCBs, soil amendments and soil sold commercially may often be contaminated with PCBs. It is hypothesized that by planting PCB contaminated soil with poplar, the degradation of PCBs will be enhanced. The plants will provide a copious supply of electron donors for the microbes through the production of various exudates. Therefore, the objective of this experiment is to enhance PCB degradation and transformation by planting hybrid poplar trees in commercial potting soil contaminated with PCBs. If successful, such a technique could be recommended for "cleaning" garden soil. Spiked additions of PCB 77 were also utilized to understand the rhizosphere degradation of higher concentrations of a common detected and toxic congener.

2.2 Materials and Methods

Seven (7.0) kg of Scott's lawn soil from Menards (total nitrogen 0.08%, available phosphate 0.03%, soluble potash 0.02%) was thoroughly mixed with 4.9 mg of 3,3',4,4' tetrachlorobiphenyl (PCB 77) dissolved in hexane to achieve artificial contamination. PCB 77 is one of the most toxic PCB congeners and exhibits dioxin like toxicity. The targeted initial PCB 77 soil concentration was 700 ng g^{-1}. The contaminated soil was

placed in a fume hood and the solvent allowed to evaporate in a sealed container for 3 days. At the end of 3 days, 300 g portions of soil were transferred to 473 ml mason jars (22 in total), with a 1.9 cm diameter hole drilled in the covers. The hole was used for planting of trees and addition of water to the reactors. Sixteen (16) jars were planted with 22.9 cm hybrid poplar cuttings (*P. deltoids x nigra* DN34) obtained from Segal Ranch Hybrid Poplars Nursery (Grand View, WA), while six were unplanted. The poplar cuttings were inserted into the soil medium through the hole in the cover (Figure 2.1).

Plants were grown under a 16 hr light/ 8 hr dark photoperiod with a light intensity of 200 µmol $m^{-2}s^{-1}$ and at a temperature of 25^{o}C. After planting, the excess space around the trees was sealed with silicone to reduce losses by volatilization. The experimental set up consisted of poplar trees planted in 300 g contaminated soil, controls of trees planted in uncontaminated soil and unplanted contaminated soil with native bacteria only.

At the end of exposure (96 days), soil and plant material were analyzed for PCB content using GC/MS/MS triple quadropole mass spectrometry (Agilent Technologies 6890N GC with an Agilent 7683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA)). PCBs 14, 65, and 166 were used as surrogate standards and PCB 204 was the internal standard. The experimental set up and analyses were carried out in triplicate.

2.2.1 Extraction and Analysis of PCB

Denaturation and extraction of PCB in soil and plant material was conducted by adding 3 milliliters per gram of a 1:1 hexane: acetone mixture to 5 grams of grounded homogenized soil and sonicating for 1 hour. Prior to sonification, the samples were spiked with 50 ng of PCB14 (3,5 dichlorobiphenyl), PCB65 (2,3,5,6 tetrachlorobiphenyl) and PCB166 (2,3,4,4′,5,6 hexachlorobiphenyl) (Cambridge Isotope Laboratories, Inc.),

which were used as surrogate standards. These congeners are not normally found in environmental samples, are not degradation products of the congener tested and can be used as a surrogate for the whole suite of 209 PCB congeners based on their physico chemical properties. The sonicated material was centrifuged at 3000 rpm for 5 minutes, after which the supernatant was transferred to a fresh vial. A second extraction was performed and the supernatants combined. The combined supernatant was evaporated to dryness using rotary evaporation and the solvent changed to hexane. Any loss from evaporation was corrected using the surrogate recoveries. Surrogate recoveries ranged from 85.2±5.3 % to 98.6±3.8% for PCB 14, 90.6±2.9% to 99.8±5.2% for PCB 65 and 98.4±5.3% to 102.9±3.8% for PCB 166.

Removal of lipids and other polar substances was achieved by double extraction with concentrated sulfuric acid and hexane. This hexane extract was concentrated to approximately 0.5 ml and eluted with 10 ml of hexane through a filter consisting of 0.1g of silica, 0.1g of sodium sulfite and 0.9 g acidified silica gel. The eluent was concentrated and PCB204 (2,2′,3,4,4′,5,6,6′ octachlorobiphenyl) was added as an internal standard before analysis by GC/MS/MS triple quadropole mass spectrometry (Agilent Technologies 6890N GC with an Agilent 7683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA)). The gas chromatogram (GC) was fitted with a Supelco SBP-Octyl capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) with helium as carrier gas at a constant flow rate of 0.8 ml min^{-1}. The GC operating conditions were as follows: injector temperature 270 °C, interface temperature 290 °C, initial temperature 75 °C, initial time 2 min. The GC temperature program was 75 to 150 °C at 15 °C min^{-1}, 150 to 290 °C at 2.5 °C min^{-1}, and final time 1 min. Identification and quantification of PCB congeners in the samples was performed using a calibration standard consisting of all 209 congeners and is described elsewhere (Hu et al. 2010).

2.2.2 Statistical Analysis

Microsoft Excel Analysis Toolpak's ANOVA analysis and Students' t-test were used for statistical testing. The significance level of 0.05 was utilized to indicate whether the treatments were significantly different than the controls.

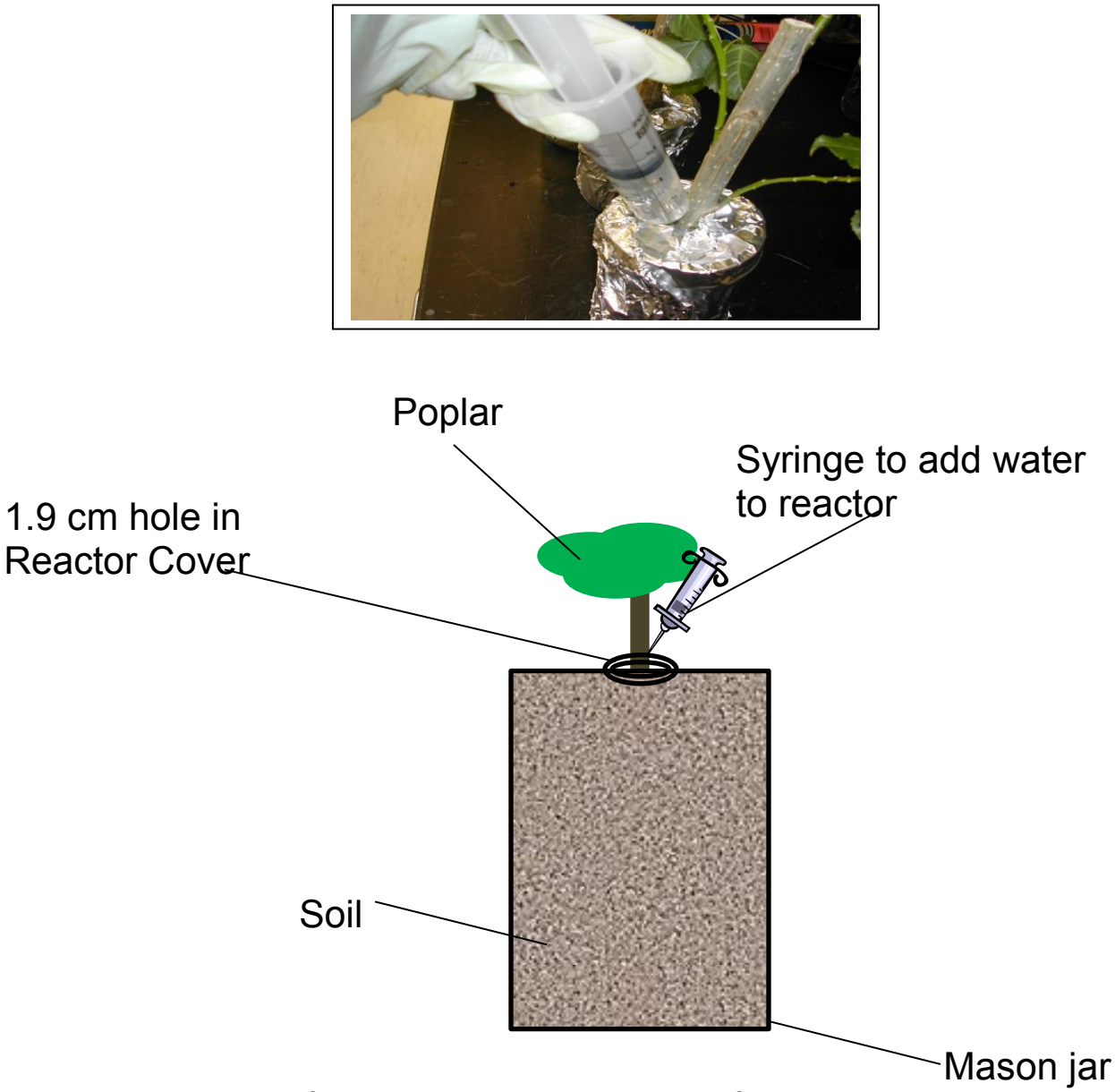

Figure 2.1: Schematic of reactors. Photo shows addition of water to reactor with a syringe. Bottom diagram shows schematic of reactor.

2.3 Results and Discussion

At the onset, it was expected that the only detectable amounts of PCB congeners in the soil would be the congener with which the soil was spiked. However, the analytical results indicated that there were clearly some other PCB congeners (here-after referred to as contaminants) in the soil at the beginning of the experiment. The contaminants are shown in Figure 1.2. They consisted of several congeners ranging from tetra chlorinated to mono chlorinated congeners. The predominant contaminant was the tetra chlorinated, PCB 52 (2,2', 5,5'tetrachlorobiphenyl).

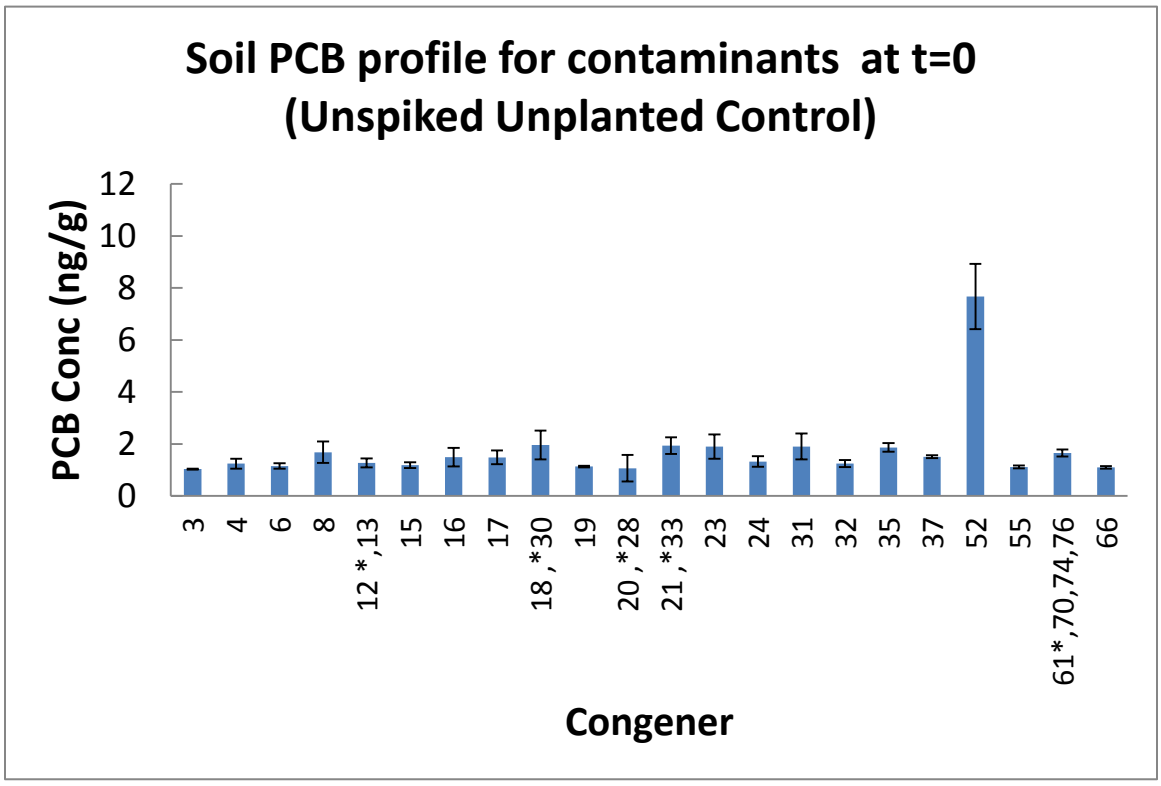

Figure 2.2: PCB congener profile of contaminants present in unspiked garden soil at t=0. Error bars are 1 standard deviation and are based on triplicate measurements.

The congener profile of the unplanted control after 96 days is shown in Figure 2.3. The results show that the congener profile was similar to the contaminant profile of the unspiked soil at the start of the experiment. In the unplanted control only 2 congeners (PCB 32 and PCB 55) were completely removed (Table 2.1 and Figure 2.2). Degradation of the other contaminants in the unplanted control ranged from 0.1% (PCB 52) to 41.1% (PCB 35) (Table 2.1). However degradation or transformation was not sufficient enough to change the contaminant congener profile in the unplanted control after 96 days when compared to the congener profile at the start of the experiment.

Table 2.1:Reduction in "contaminant" PCB congeners in unspiked garden soil after 96 days.

IUPAC PCB Congener	Decrease in Unspiked Unplanted Control	Decrease in Unspiked Planted Control
3	10.1%	100.0%
4	11.7%	100.0%
6	7.3%	100.0%
8	23.5%	100.0%
12 *,13	13.6%	100.0%
15	19.7%	100.0%
16	14.9%	100.0%
17	13.7%	100.0%
18 ,*30	24.4%	100.0%
19	15.7%	100.0%
20 ,*28	18.1%	100.0%
21 ,*33	24.4%	100.0%
23	16.1%	79.7%
24	28.0%	100.0%
31	17.3%	100.0%
32	100.0%	100.0%
35	41.1%	100.0%
37	31.0%	91.8%
52	0.1%	22.3%
55	100.0%	100.0%
61*,70,74,76	27.6%	100.0%
66	15.8%	100.0%

Figure 2.4 depicts the congener profile for the planted control after 96 days. It is evident that the congener profile of the planted control after 96 days is starkly different from the profile of the unspiked soil at the start of the experiment. After 96 days , the soil of the planted control was devoid of several congeners that were present at the beginning (Figure 2.4 and Table 2.1). PCB 52 showed some amount of recalcitrance and maintained a similar concentration to that at the beginning of the experiment, while recording a 22.3% decline (Table 2.1). The other two congeners that were not completely degraded in the planted control were PCB 23 and PCB 37. These decreased by 79.7% and 91.8% respectively.

The ability of the plants to effect PCB removal in a spiked system was tested by spiking the garden soil with PCB 77. The contaminants accounted for 4 % of the total PCB mass content in the spiked system. Initially, it was thought that the PCB standard used to spike the soil could have been the source of the contaminants detected in the spiked soil at the start of the experiment. However, analysis of the PCB 77 standard revealed that impurities in the PCB 77 standard were not the source of contamination. This, coupled with the fact that the contaminants were also detected in the unspiked soil confirmed that the source of the contaminants was the garden soil. In terms of removal of the contaminants detected in the soil at the beginning of the experiment, a result similar to what was obtained with unspiked planted system was seen with the planted spiked system (Figure 2.5). Several of the congeners were removed, with only nine of the twenty eight contaminants detected at the start of the experiment remaining. Of the remaining congeners, PCB 52 had the least decrease in concentration registering a 14.8% decline (Table 2.2). The other PCB congeners remaining (PCB 3, PCB 12/13 which were co-eluted, PCB 21/33 co-eluted, PCB 23, PCB35 and PCB37) recorded declines of between 70.9% and 98.9% (Table 2.2).

A comparison of the PCB 77 concentration in the soil of the planted and unplanted spiked systems at the end of exposure with the concentration at the beginning

of incubation is shown in Figure 2.6. This illustrates that the unplanted spiked system did not have any significant reduction in PCB 77 at the end of exposure ($p<0.05$), only registering a 2.8% decrease. On the other hand, the planted system showed a decrease of 17.2%. So, the planted system was not only able to remove the trace contaminants present in the garden soil, but also record a modest(significant) decrease in the concentration of the PCB 77 spike ($p<0.05$).

Table 2.2:Reduction in "contaminant" PCB congeners in spiked garden soil after 96 days.

IUPAC PCB Congener	Decrease in Spiked Unplanted System	Decrease in Spiked Planted System
3	22.2%	90.2%
4	5.4%	100.0%
6	6.6%	100.0%
8	12.5%	100.0%
12 *,13	23.6%	80.2%
15	38.8%	100.0%
16	19.7%	100.0%
17	11.3%	100.0%
18 ,*30	14.2%	100.0%
19	13.9%	100.0%
20 ,*28	27.5%	100.0%
21 ,*33	17.0%	98.9%
23	18.4%	81.5%
24	22.7%	100.0%
31	19.4%	100.0%
32	100.0%	100.0%
35	28.2%	70.9%
37	19.7%	87.9%
52	0.1%	14.8%
55	100.0%	100.0%
61*,70,74,76	33.5%	100.0%
66	6.7%	100.0%
77 (Spike)	2.8%	17.2%

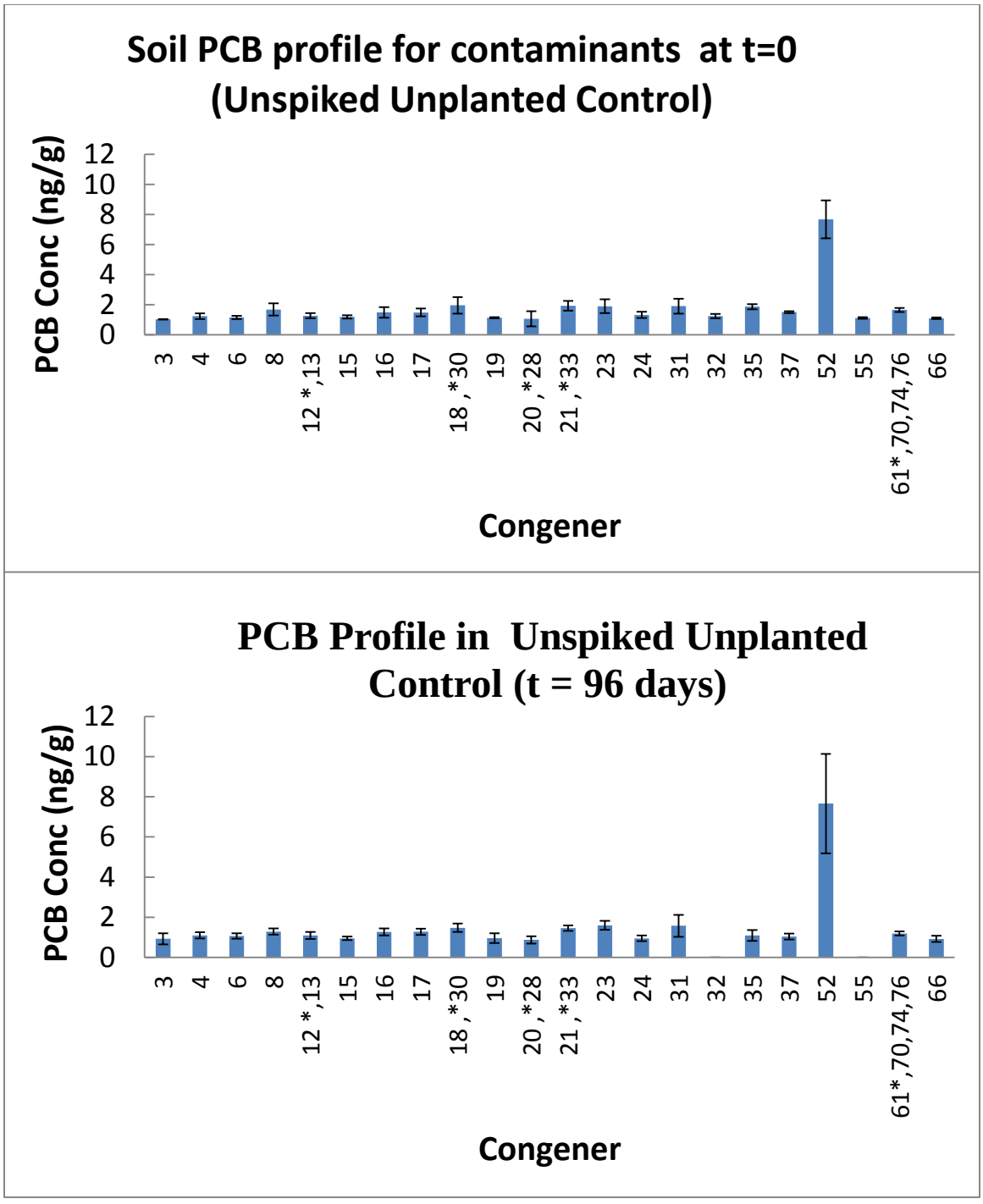

Figure 2.3: Contaminant congener profile in unplanted control at t = 96 days. This shows that the PCB congener profile in the unplanted control was similar to the unspiked unplanted profile at the t=0 days. Error bars are 1 standard deviation and are based on triplicate measurements.

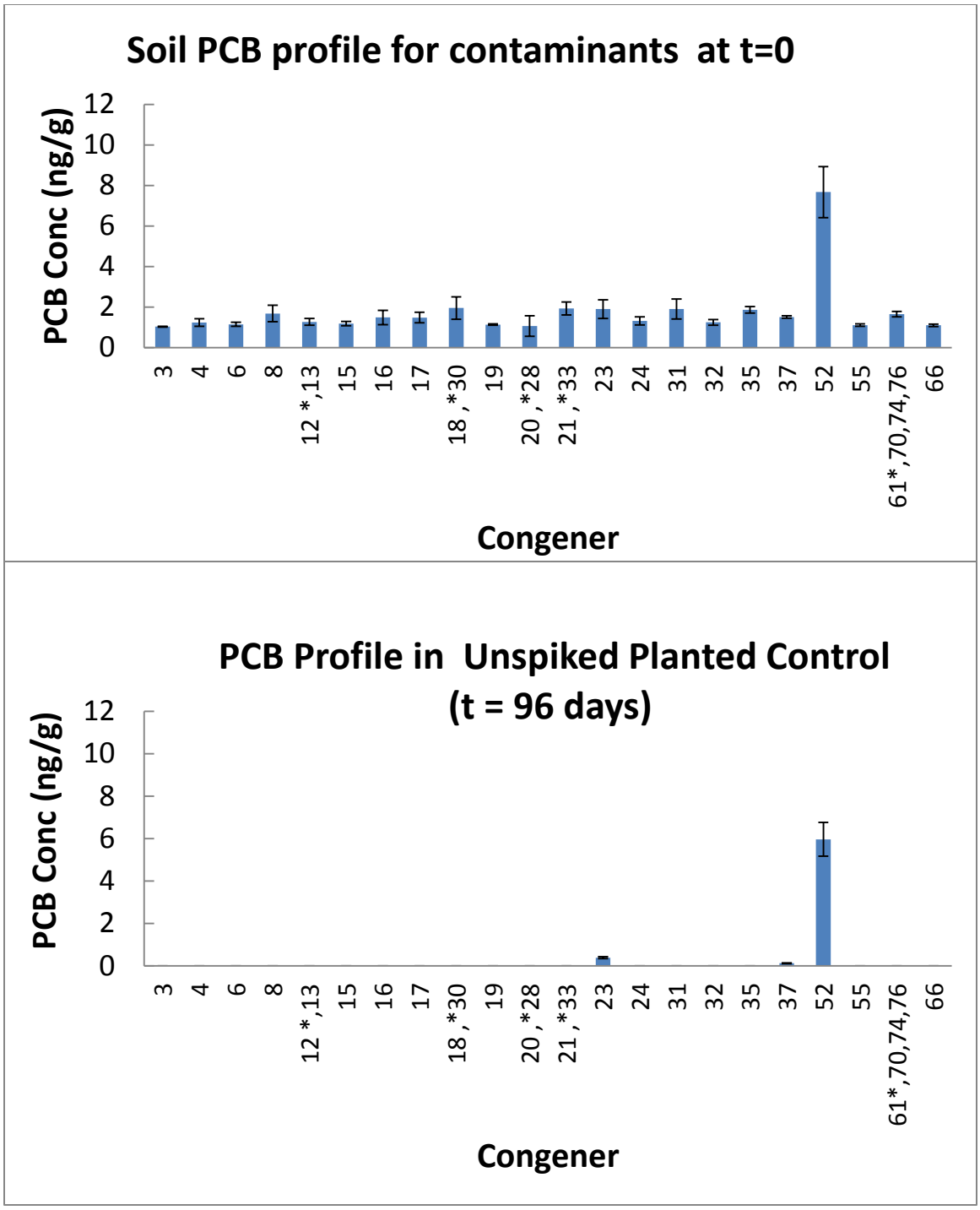

Figure 2.4: Congener profile of planted control at t = 96 days showing removal of some lower chlorinated congeners seen in the garden soil at t = 0 days. Error bars are 1 standard deviation and are based on triplicate measurements.

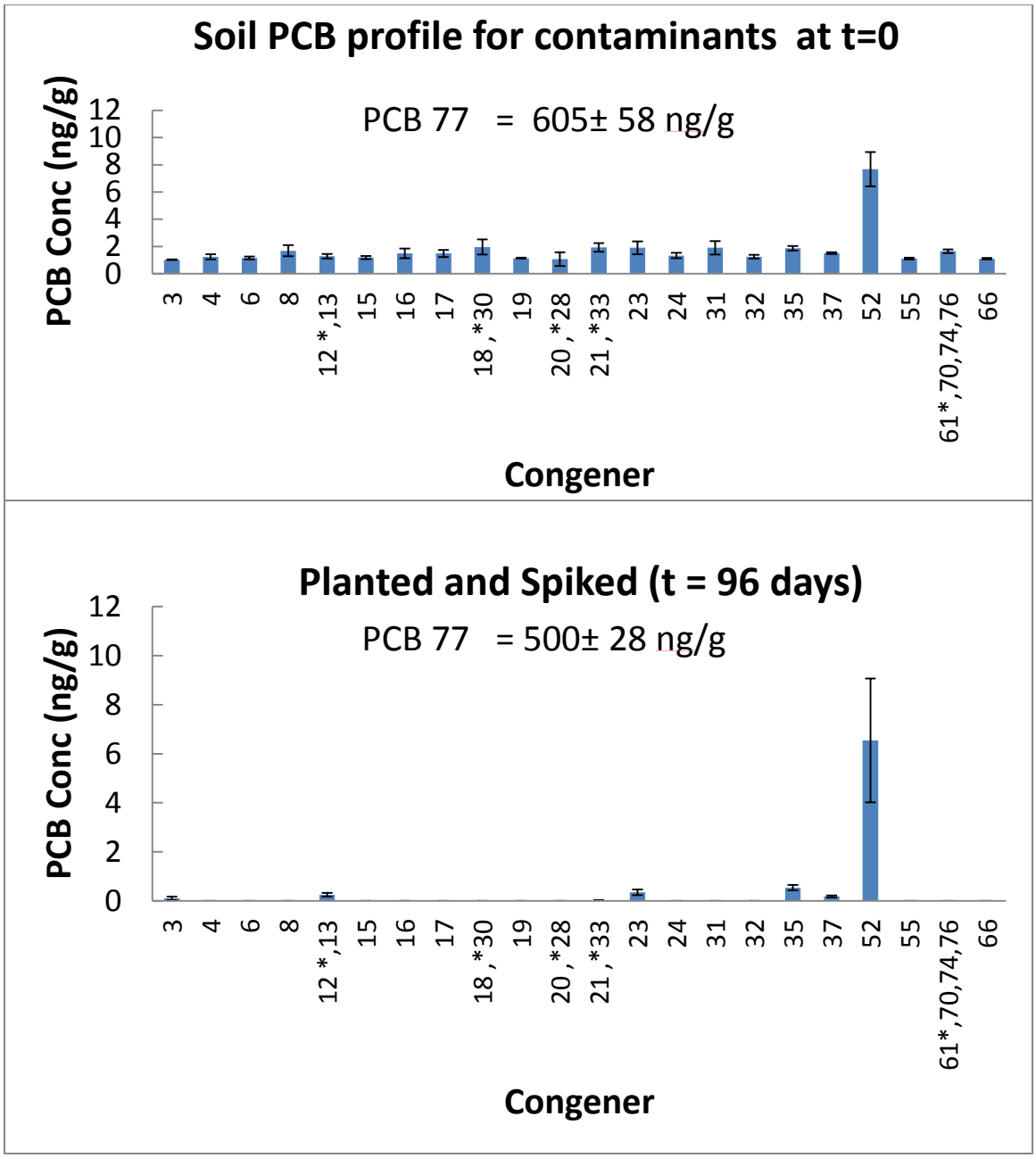

Figure 2.5: Congener profile of planted system spiked with PCB at t = 96 days showing removal of some lower chlorinated congeners seen in the garden soil at t = 0 days. The spiked PCB 77 concentration at t=0 and t = 96 days are shown in word form on the top and bottom panels respectively, because of the dwarfing effect they would have had on the "contaminants" due to large concentration differences. Error bars are 1 standard deviation and are based on triplicate measurements.

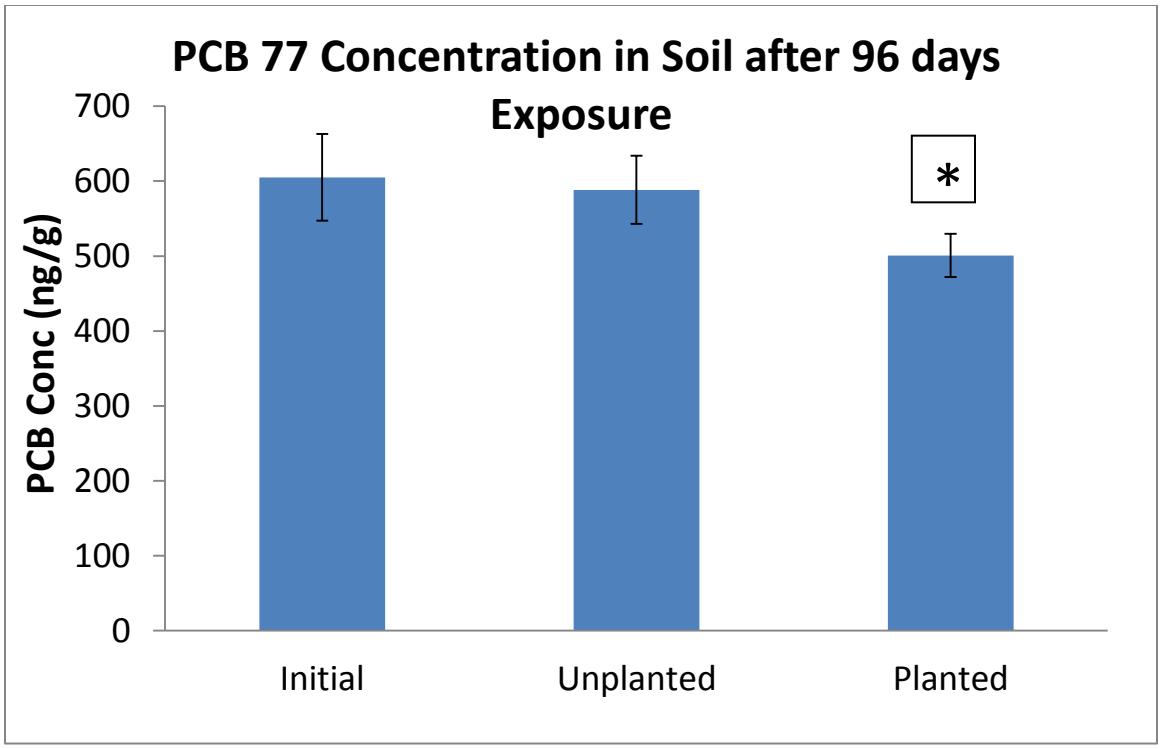

Figure 2.6: PCB 77 concentration in planted and unplanted soil after 96 days exposure. *= significant reduction in comparison to starting concentration ($p<0.05$). PCB 77 was not detected in the unspiked controls. Error bars are 1 standard deviation and are based on triplicate measurements.

After exposure, most of the PCB remained in the soil with only a small portion (<2%) detected in the plant tissue (Figure 2.7). The root accounted for most of the PCB detected in plant material, with very little in the above ground material (Figure 2.7). It was quite difficult, even after multiple rinses, to dislodge all the particles containing PCBs from the roots. The stem material was separated in bottom (below ground) and upper (above ground) portions, to differentiate between effects caused by sorption versus translocation. Furthermore, the bottom and upper stems were divided between inner and outer sections, to definitively determine what was due translocation versus diffusion.

The trend observed in the planted and unplanted controls was also observed in the spiked systems. That is, a comparison of congener profile in the unplanted spiked system after 96 days, with the congener profile of the planted control at the start of the

experiment, shows that the soil congener profile for the unplanted spiked system was similar to the congener profile of the planted control at the beginning of the exposure. This indicates that similar to the unplanted control, the removal of the contaminants in the unplanted spiked system after 96 days, was not sufficient to alter the congener profile when compared to the congener profile at the start of the experiment (Table 2.2). Similar to the unspiked unplanted control PCB 52 was only removed by 0.1% in the unplanted spiked system (Table 2.2). The congeners remaining in the unplanted spiked treatment decreased by between 5.4% (PCB 4) and 38.8% (PCB15) (Table 2.2). The same two congeners (PCB 55 and PCB 32) that were completely removed in the unplanted unspiked system were completely removed in the unplanted unspiked treatment (Table 2.2).

On the other hand, the spiked treatments that were planted with poplar showed disappearance of several congeners with only 9 congeners remaining (Figure 2.5). Of the remaining congeners, PCB 35 (3,3',4 trichlorobiphenyl), PCB 37 (3,3,4' trichlorobiphenyl), PCB 12(3,4 dichlorobiphenyl), PCB 13(3,4'dichlorobiphenyl) and PCB 3 (4chlorobiphenyl) are potential degradation products of PCB 77 (3,3'4,4'tetrachlorobiphenyl). Volatilization as a source of the loss was ruled out because, at the end of exposure, the congener profile of the unplanted system was similar to the congener profile of the contaminants profile at the beginning. Volatilization would have occurred in both the unplanted and planted systems after 96 days if this was the source of the loss. A mass balance on the spiked reactors resulted in resulted in a 99.5% recovery in the unplanted reactors, and 88.0% of the PCBs were recovered in the planted reactors. This indicates that sorption to the reactor walls was not a major factor in the loss of PCB from the reactors. In the planted reactors, the lack of a complete mass balance could have been due to aerobic bio-oxidation.

An indication of PCB degradation is the accumulation of less chlorinated congeners, particularly mono chlorinated ones (Bedard et al., 1987), but this was not

evident in the spiked treatment. One possible reason could be the occurrence of ring cleavage under the aerobic conditions resulting in the lack of accumulation of mono chlorinated congeners. Another possibility is that there could have been mineralization, but that is highly unlikely as typically organisms that degrade PCBs are only able to dechlorinate, but not mineralize the congeners that they reduce (Abraham et al., 2002). Mineralization generally requires a different consortium of organisms. The aerobic degraders preferentially dechlorinate the least chlorinated ring, and release the other ring as chlorobenzoic acids (Abraham et al., 2002). Since the chlorobenzoates require a different consortium of aerobic microorganisms to facilitate further reduction they are a potential bottleneck in the mineralization process.

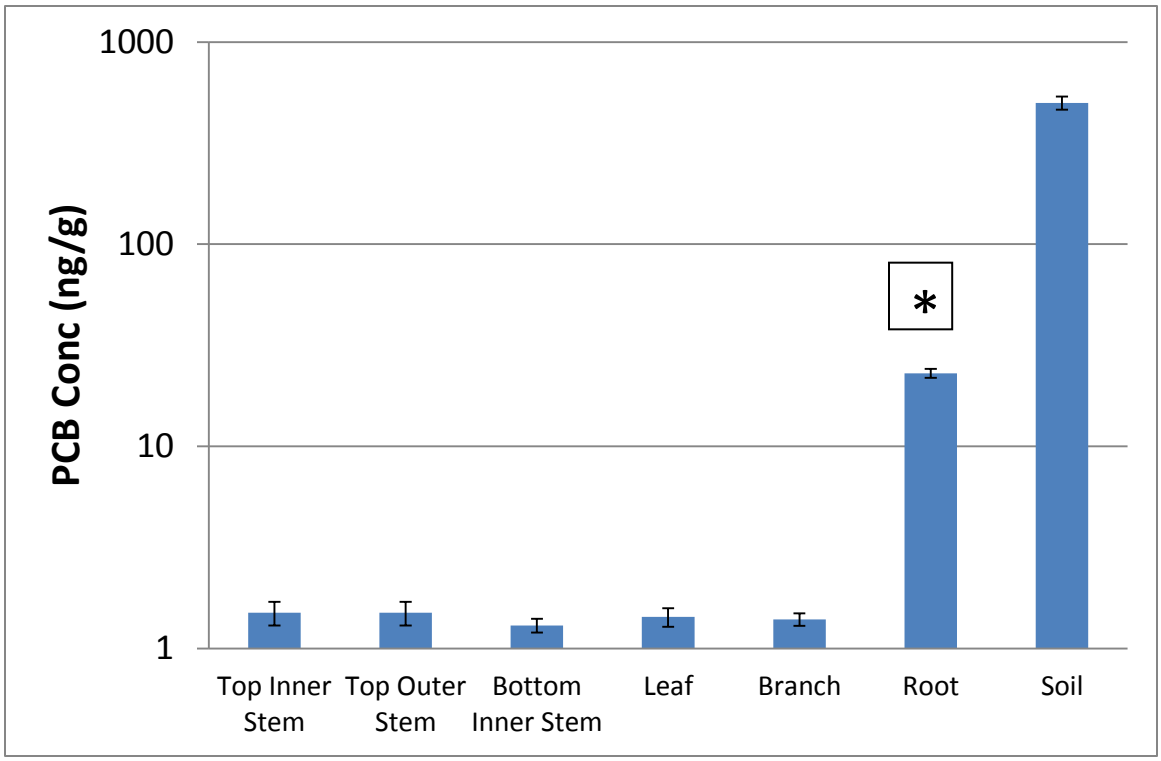

Figure 2.7: Distribution of PCB in Soil and Plant material at t=96 days. * = significantly higher concentration than in other plant parts ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements

The overarching conclusion here, as shown by the results, is that the presence of planted material had a positive impact on the removal of PCBs and the cleaning of the garden soil except for PCB 52. Another conclusion is that this method (phytoremediation) can clean up lightly contaminated commercial soils in only 96 days for most congeners. The mechanism by which this was achieved was probably due to microbial activity which was stimulated by the presence of the plant. There are several instances in which microbial activity and numbers have been stimulated in the rhizophere (Chekol et al., 2004, Jordahl et al., 1997). For example, Jordahl et al. (1997), reported significantly higher concentration of denitrifiers, pseudomonads, monoaromatic petroleum degraders and atrazine degraders in soil samples from the rhizophere of poplar trees than in surrounding adjacent agricultural soil. They also found poplar secreted a substantial amount of organic carbon into the rhizophere with the poplar producing up 0.25% of their biomass as soluble exudates. They speculated that the significant increase in microbial numbers in the rhizophere was a direct result of the exudates because the BOD decay coefficient of the exudates suggest that they would be easily degraded, thereby making them good substrates for the microbes.

Similarly, changes in the bacterial community structure and dioxygenase activity, and bacterial number have been observed in the rhizosphere of willow planted deliberately, and willow growing naturally, in PCB contaminated soil when compared to controls and other tree species (Ionescu et al. 2009, de Carcer et al., 2007; Leigh et al. 2006, Slater et al.2011). *Burkholderia sp.* LB 400, a well-known PCB degrader has been shown to exhibit the same growth rate when propagated on flavonoidal compounds derived from mulberry roots as the sole carbon source as with biphenyl as the carbon source, suggesting that these compounds have similar efficacy to biphenyl which is normally used to culture PCB degraders in the laboratory (Leigh et al., 2002).

The importance of plants to ensuring that contaminants were removed from the soil is illustrated by the observation that, even planted systems, in which the plants died

after 75 days into the experiment, but continued to be incubated for 96 days, showed removal of the contaminants that were present in the potting soil at the beginning of the experiment. This suggests that dead roots can provide a source of substrate for PCB degrading bacteria as postulated by Leigh et al.(2002). In their research, which investigated the effect of root turnover on PCB degradation, they observed that the increase in phenolic compounds in the fine mulberry roots increased 2 fold during the latter parts of the growing season, when most roots were dying.

Donnelly and Fletcher (1994) in investigating the ability of different plant species to release phenolic compounds found that all 17 species released phenols in the root matrix. Not surprisingly, there was variation in production among the plant species. A comparison of the released concentration, with a known concentration that supported PCB degraders, revealed that while the concentration in the entire rhizosphere was lower than the known substrate level, the concentration at the root surface was 3-5 times higher than composite leachate throughout the rhizosphere. This, they suggested, may indicate that at the root surface there would be production of substrate level phenolic compounds for PCB degraders.

While poplar plays an important role in the apparent reduction of PCB 77 and the other contaminants detected at the beginning of the exposure period, there appears to be little if any uptake of PCBs. This is illlustrated in Figure 2.7, which shows that most of the PCB detected in the plant material resided in the roots, possibly as a result of sorption. This is consistent with the fact that most PCBs are hydrophobic with hydropobicity increasing with an increase in the degree of chlorination. Schnoor et al. (1995) posited that given their hydrophobicity PCBs are unlikely to enter the transpiration stream. In addition, their octanol water (K_{ow}) coefficient would suggest that they would be strongly adsorbed to organic material. However, it is possible that some lower chlorinated PCBs may be able to be taken up by plants given their greater solubilty compared to their higher chlorinated counterparts (Liu et al, 2008). Roots were washed

with hexane to desorb any PCB that was reversibly sorbed, and explicity identify what was irreversibly bound or taken up by the plants.

2.4 Conclusion

The results have provided evidence of poplar assisted rhizosphere removal of PCBs in contaminated commercially available garden soil. The importance of poplar in the overall scheme is highlighted by the fact, that even when the plants died, the planted system outperformed the unplanted systems. The mechanism for the better performance of the planted systems could be a combination of factors, inclusive of increased microbial activity in the poplar rhizosphere, because of the secretion of exudates and secondary compounds by the plants. However, there was a clear demonstration that the systems planted with poplar resulted in the removal of the PCB contaminants detected in the garden soil at the beginning of exposure, while the unplanted sytems did not.This suggests that phytoremediation may be an important tool in cleaning commercial soils lightly contaminated with PCBs.

CHAPTER 3 IMPACT OF REDOX CYCLING ON RHIZOSPHERE BIOTRANSFORMATION OF SELECTED PCB CONGENERS

3.1 Introduction

The persistence of polychlorinated biphenyls (PCBs) in the environment is driven by their relative inertness and this is exacerbated by the fact that there is no single redox environment that is conducive to their complete degradation. Higher chlorinated PCBs i.e. those with 4 or more chlorine substituents are highly oxidized and as such are more amenable to degradation under highly reducing conditions. On the other hand, the more reduced congeners (with less than 4 chlorine substituents are more readily transformed under highly oxidized conditions. At PCB contaminated sites it is highly unlikely that both of these condition coexist, so one process will always predominate leaving behind the less favored PCB fraction. Therefore, in order to have complete PCB degradation both redox environments will have to be present. Having recognized this, a few researchers have undertaken studies designed to exploit the utilization of sequential cycles of anaerobic and aerobic conditions to enhance PCB degradation with the intent of adapting this to engineered systems (Master et al. 2002, Fathepure and Vogel, 1991, Evans et al., 1996, Anid et al., 1993). For example, Anid and colleagues attempted to induce aerobic conditions into anaerobic sediments from the Hudson River which showed evidence of natural in situ reductive dechlorination occurring. They further subjected the sediments to anaerobic conditions for 76 weeks after which they added hydrogen peroxide (H_2O_2) and allowed the system to incubate for 96 days. There was a positive correlation between an increase in H_2O_2 concentration in the previously anaerobic sediments and reduction of total PCB concentration. They also indicated that there was a shift in the PCB congener profile to lower chlorinated congeners after anaerobic

treatment and subsequent aerobic treatment. Similarly they observed an increase in bacterial count on addition of H_2O_2 , and postulated that the aerobes are naturally present at these sites and just need to be stimulated. However, it is likely that the increase in bacterial count may have been due to the presence of facultative organisms rather than obligate aerobes. Some of the microbes from the sediments were isolated and were exposed to a specially formulated PCB mixture and two of the isolates showed good versatility in reducing both higher and lower chlorinated PCB congeners.

These studies have either utilized bioaugmentation or added chemicals that may be inimical to the wellbeing of microbes. For example, H_2O_2 can be toxic to subsurface microorganisms at concentrations as low as 0.003%. Bioaugmentation has not had a lot of success in the field because sometimes the added organisms are outcompeted by natural biota.

It is widely held that plants can play an indirect role in the degradation of PCBs by providing the right environment in the root zone of the plant (rhizosphere) (Dzantor 2007; Mackova et al. 2007; Dzantor et al. 2000). Leigh et al. (2002) posited that flavonoidal compounds produced by plant roots could stimulate the growth of PCB degrading bacteria. They subjected *Burkholderia sp.* LB400, to three mulberry root flavones (morusin, morusinal and kuwanon C), which were the sole carbon source. They observed the same growth rate as when biphenyl was the carbon source, whereas there was no growth in the substrate free control. They also observed a 2 fold increase in phenolic compounds in the fine roots during the latter parts of the growing season, when most roots were dying. Thus they concluded that the dead fine roots of mulberry can provide a source of substrate for PCB degrading bacteria. This has significant implication for rhizoremediation because if this is the case for a number of plants, it means that if root growth can be stimulated during the growing season then PCB degradation may be able to take place even as the roots die. In addition, if plants are able to take up and transform the more soluble lower chlorinated PCBs, which should accumulate if there is

microbial degradation of higher chlorinated PCBs, then the symbiotic relationship between plant and microbes in aiding PCB degradation would be greatly enhanced. Bacteria could transform the higher chlorinated PCBs anaerobically with the roots providing additional electron donors. However, what is not known is the extent of the effect that plants will have on the redox conditions in the rhizosphere, which could negatively impact the anaerobic degradation of the higher chlorinated PCBs by bacteria.

Given that these recent studies have indicated that planted systems are able to stimulate PCB degradation, the objective of the current study was to harness the ability of plants to enhance PCB degradation and couple that to the manipulation of redox conditions to further enhance PCB transformation since all previous studies examining the redox manipulation phenomenon have been bacteria based. Redox conditions were manipulated by creating alternate cycles of flooding and no flooding in one set of reactors (hereafter referred to as moisture content cycling). In addition, all previous studies redox manipulation experiments utilized multiple reactors/multi-compartment systems or had single cycles. Therefore, another objective was to investigate the impact of multiple cycles in a single reactor.

3.2 Materials and Methods

3.2.1 Experimental Set up

Soil from Amana colonies IA, was passed through a 60 mesh sieve and artificially contaminated with tetra chlorinated PCB 52, PCB77 and hexa chlorinated PCB 153 separately and as a mixture of the three congeners. The soil was homogenized using the quartering technique. Quartering consists of dividing the soil into four quadrants on a quartering canvas and making several diagonal trajectories to mix the soil components

together. Twenty passes (diagonal trajectories) were made to homogenize the soil thoroughly. To account for heterogeneity, 20 subsets of soil were collected from different locations, homogenized and analyzed in triplicate. Triplicate concentration measurements were conducted to establish the initial concentration, and it was compared to the target concentration for the purpose of quality assurance. The contaminated soil was aged for two months to reduce bioavailability and to ensure conditions more representative of field sites (Alexander et al., 2000, Thompson et al., 1998, White et al., 2006). The aged, contaminated soils were planted separately with switchgrass (*Panicum virgatum)* seeds from Adams-Briscoe Seed Co. (Jackson, GA) and with 22.9 cm poplar (*Populus deltoids x nigra* DN34) cuttings from Segal Ranch Hybrid Poplars Nursery (Grand View, WA).

Treatments consisting of switchgrass and poplar planted in uncontaminated soil were used as blanks and functioned as volatilization controls. Plants were grown under a 16 hour light/8 hour dark photo period with a light intensity of 200 µmol $m^{-2}s^{-1}$. Rubber maid shoes boxes (33.8 cm x 21.6 cm x 11.9 cm (LxWxD)) with 1.9 cm holes drilled in the covers, lined with aluminum foil and containing 2500 g of soil were used as reactors. Holes (1.9 cm) were bored in covers of the reactors to facilitate measurement of redox potential and dissolved oxygen concentration during periods of flooding (low redox conditions). The plants were allowed to establish themselves for four (4) weeks. After 4 weeks, one set of plants were subjected to alternate cycles of seven (7) days flooding (to induce anaerobic conditions) and seven (7) days of no flooding (aerobic conditions). Another set had a normal watering regimen, with no flooding. To achieve flooding, three liters of deionized water was initially added to the reactors and supplemented as dictated by transpiration rates. Transpiration typically functioned as an efficient mechanism for water withdrawal, so there was no need to withdraw excess water at the end of the flooding cycle. The soil was sampled and analyzed 8 weeks after planting, and at regular intervals ranging from two to eight weeks thereafter, up to a period of thirty two (32)

weeks. Sampling was conducted after the completion of a full flooding and no flooding cycle.

Twelve (12), five (5) g samples of soil were collected randomly from each treatment using a Lock and Hold™ syringe (Ben Meadows Company WI, USA) and homogenized prior to analysis. At the end of exposure (32 weeks), soil and plant materials were sampled and analyzed for PCB content using GC/MS/MS triple quadropole mass spectrometry (Agilent Technologies 6890N GC with an Agilent 7683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA)). PCBs 14, d65, and 166 were used as surrogate standards and PCB 204 was the internal standard. The experimental set up and analyses were carried out in triplicate. A schematic representation of the reactors used, the procedure for sampling and adding water and the procedure for making redox measurements can be seen in Figure 3.1.

3.2.2 PCB Extraction

Denaturation and extraction of PCB in soil was conducted by adding three (3) milliliters per gram of a 1:1 hexane/acetone mixture to 5 grams of grounded homogenized soil and sonicating for 1 hour. Prior to sonification, the samples were spiked with 50 ng of PCB14 (3,5-dichlorobiphenyl), deuterated PCB65 (2,3,5,6-tetrachlorobiphenyl) and PCB166 (2,3,4,4′,5,6-hexachlorobiphenyl) (Cambridge Isotope Laboratories, Inc.), which were used as surrogate standards. Surrogate recoveries ranged from 87.2±3.1% to 96.4±3.8% for PCB 14, 92.8±4.7% to 105.1±4.2% for PCB 65 and 93.2±2.5% to 106.4±2.5% for PCB 166.

The sonicated material was centrifuged at 3000 rpm for 5 minutes, after which the supernatant was transferred to a fresh vial. A second extraction was performed and the

supernatants combined. The combined supernatant was evaporated to dryness using rotary evaporation and the solvent changed to hexane. Any loss by rotary evaporation was accounted for using the surrogate standard recoveries. Removal of lipids and other polar substances was achieved by double extraction with concentrated sulfuric acid and hexane. This hexane extract was concentrated to approximately 0.5 ml under a gentle stream of N_2. The concentrate was eluted with 10 ml of hexane through a filter consisting of 0.1g of silica (70−230 mesh, Fisher Scientific, Inc.), 0.1g of anhydrous sodium sulfate (Na_2SO_4) and 0.9 g silica gel acidified with H_2SO_4 (2:1). The eluent was concentrated and PCB204 (2,2′,3,4,4′,5,6,6′ octachlorobiphenyl) was added as an internal standard before analysis by GC/MS/MS triple quadropole mass spectrometry (Agilent Technologies 6890N GC with an Agilent 7683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA)). The gas chromatogram (GC) was fitted with a Supelco SBP-Octyl capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness) with helium as carrier gas at a constant flow rate of 0.8 ml min^{-1}. The GC operating conditions were as follows: injector temperature 270 °C, interface temperature 290 °C, initial temperature 75 °C, initial time 2 min. The GC temperature program is 75 to 150 °C at 15 °C min^{-1}, 150 to 290 °C at 2.5 °C min^{-1}, and final time 1 min. Hu et al. (2010) describes the procedure for identification and quantification of the PCBs.

3.2.3 Measurement of Redox Conditions

Redox measurements were obtained using a Hanna Instruments Redox/pH combination meter. For quality assurance purposes, the accuracy of the ORP meter was checked with 475 mv and 229 mv redox buffers (Aqua Solutions).

3.2.4 Statistical Analysis

Microsoft Excel Analysis Toolpak's ANOVA analysis and Students' t-test were used for statistical testing. The significance level of 0.05 was utilized to indicate whether the treatments were significantly different than the controls.

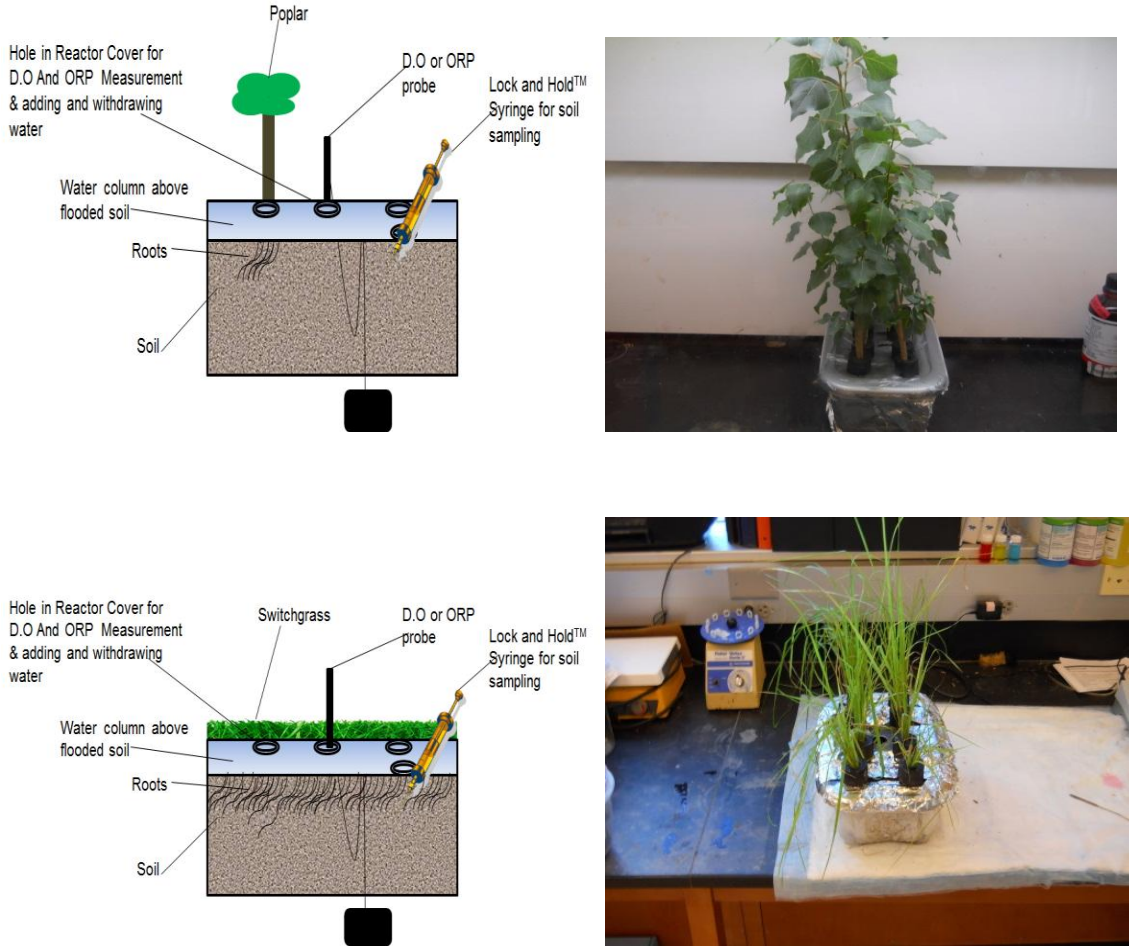

Figure 3.1 Schematic of reactors. Samples were collected using a Lock and Hold syringe to make a vertical core in the soil column D.O and ORP.measurements were taken by inserting the probe through a 1.9 cm hole drilled in the cover of the reactor.

3.3 Results and Discussion

3.3.1 Redox Measurement

The soil redox potential in the moisture content cycled treatments for the mixture and single congener exposure during a one week flooding cycle is illustrated in Figure 3.2. During periods of flooding, the planted systems showed evidence of hypoxia, but oxygen was not sufficiently depleted to produce anaerobic conditions. The inability to develop bulk anaerobic or anoxic conditions in the planted system was probably due to plant transfer of oxygen to the rhizosphere during photosynthesis. In the planted systems the redox potential became poised at about 20 millivolts (mV). The unplanted systems had lower redox potential than the planted systems and were anoxic (0-200 mV).

Figure 3.3 depicts a longer trend for the redox potential for the cycled treatment that contained the PCB mixture and shows that even though anaerobic conditions didn't develop throughout the soil column in the flooded reactors, there were variations in redox potential which mirrored the manipulation of the soil moisture content.

3.3.2 Measured versus Target Initial PCB Concentration

The targeted initial concentration for each congener in the mixture (PCB 52, PCB 77 and PCB 153) was 500 ng g^{-1}. In the single congener exposures, an initial concencentration of 1000 ng g^{-1} was targeted. However, the measured initial concentrations were different from the target in both the mixture and single congener treatments, probably due to the heterogeneity of the soil matrix. In the mixture, the measured initial concentrations were 573± 29 ng g^{-1}, 512 ± 24 ng g^{-1} and 499 ± 25 ng g^{-1} respectively for PCB 52, PCB 77 and PCB 153. For the single congener exposure, the

measured initial concentrations were 1120 ±58 ng g^{-1} (PCB 52), 1012 ±3 ng g^{-1} (PCB 77) and 1033 ±28 ng g^{-1} (PCB 53).

3.3.3 PCB Dissipation in Mixture: Switchgrass Planted Soil

Figure 3.4 compares the concentration of PCB 52, PCB 77 and PCB 153 in soil containing a mixture of the three congeners and planted with switchgrass after 32 weeks of incubation. A 53% decrease in soil PCB 52 concentration was obtained when the soil containing the mixture was planted with switchgrass, and did not undergo cycling of the water moisture content. On the other hand, there was a 56% reduction in the soil PCB 52 concentration for the cycled treatment. In both cases, this represented a significant decline when compared to the initial concentration ($p<0.05$). However there was no significant difference in reduction of PCB 52 concentration between the cycled and uncycled treatments ($p<0.05$).

With respect to PCB 77 dissipation in the mixture, the switchgrass planted uncycled treatment recorded a 44% decline, while the cycled treatment registered a 48% diminution (Figure 3.4). Both the cycled and uncycled treatments had significant reductions when compared to the initial PCB 77 concentration in the mixture ($p<0.05$). However, the cycled treatment had significantly higher decrease in soil PCB 77 concentration than the uncycled system ($p<0.05$).

Compared to the initial concentration in the mixture, the concentration of PCB 153 in soil planted with switchgrass diminished by 49% in the treatment that did not undergo moisture content cycling. The cycled treatment recorded a 55% decrease. This represented a significant difference in dissipation between the cycled and uncycled

treatments ($p<0.05$). Compared to the initial PCB 153 concentration, both the cycled and uncycled systems had significant declines ($p<0.05$).

3.3.4 PCB Dissipation in Single Congener Exposure: Switchgrass Planted Soil

The PCB reduction results for the single congener exposure with switchgrass as the plant species are shown in Figure 3.5. After 32 weeks of exposure, there was a 56% reduction in the soil PCB 52 concentration for the uncycled treatment, and 63% for the cycled system. This represented a significant decrease when compared to the initial concentration ($p<0.05$). Overall, both the uncycled and cycled treatments had significant declines in soil PCB 52 concentration ($p<0.05$).

When PCB 77 was used as the sole contaminant and the soil was planted with switchgrass, the cycled treatment showed a 54% decline (Figure 3.5). This diminution was significantly higher than the 48% decrease observed with the uncycled treatment ($p<0.05$), (Figure 3.5). With respect to the intial PCB concentration in the soil, both the uncycled and cycled treatments had significantly diminished PCB 77 concentrations at the end of exposure ($p<0.05$).

There was a 44% reduction in soil PCB 153 concentration for the planted cycled treatment while a 51% decline was recorded with the planted cycled treatment (Figure 3.5). In the single congener exposure experiment with PCB 153 and switchgrass, both the cycled and uncycled treatments had significantly reduced concentration in the soil after 32 weeks ($p<0.05$). However, the cycled treatment exhibited a significantly higher diminution than the uncycled treatment ($p<0.05$).

3.3.5 PCB Dissipation in Mixture: Poplar Planted Soil

The PCB 52 concentration in soil planted contaminated with the mixture of PCB 52, PCB 77 and PCB 153 and planted with poplar after 32 weeks of incubation is shown in Figure 3.6. Both the moisture content cycled and uncycled treatments recorded significant declines in the PCB 52 concentration in the soil ($p<0.05$). A 57% decrease in PCB 52 concentration was obtained when the soil moisture content was cycled. A statistically analagous reduction of 54% was registered in the uncycled treatment($p<0.05$). Percentagewise, the results obtained with PCB 52 diminution and poplar as the plant species, were statistically similar to those obtained for PCB 52 decrease in the mixture when switchgrass was the plant species (Figure 3.4).

There was a 52% reduction in soil PCB 77 concentration in the mixture for the moisture content cycled treament that was planted with poplar. In contrast, a significantly lower decrease of 42% was recorded for the uncycled treatment ($p<0.05$). Compared to the initial PCB 77 concentration in the mixture, both the cycled and uncycled treatments recorded statistically significant declines ($p<0.05$). With regards to PCB77 dissipation in the mixture and plant species, the results obtained with the poplar planted soil were statistically similar to those achieved with switchgrass planted soil ($p<0.05$).

Using poplar as the plant species, the PCB 153 concentration in the planted soil contaminated with the mixture diminished by 48% in the uncycled treatment, and by 55% in the cycled treatment (Figure 3.6). The diminution recorded with the cycled treatment was significantly higher than that achieved with the uncycled treatment ($p<0.05$). At the end of the exposure period, both the cycled and uncycled treatments had significantly reduced concentrations of PCB 153 when compared to the initial PCB 153 concentration in the mixture. Like the other congeners in the mixture, the PCB 153

reduction achieved in poplar planted soil was statistically equivalent to that seen in switchgrass planted soil ($p<0.05$).

3.3.6 PCB Dissipation in Single Congener Exposure:

Poplar Planted Soil

The reduction of soil PCB 52 concentration in the single congener exposure with poplar as the planted species is shown in Figure 3.7. A 57% decrease was obtained for the treatment that underwent cyclic manipulation of soil moisture content whereas a 49% decline was observed with the uncycled treatment. Significant reductions in soil PCB 52 concentration were obtained in both the cycled and uncycled treatments when compared to the initial concentration($p<0.05$). However, the cycled treatment had a significantly greater loss than the uncyled treatment ($p<0.05$). The decreases in PCB 52 concentration observed with poplar planted soil were significantly lower than the declines seen with switchgrass in a similar single congener exposure experiment ($p<0.05$).

Figure 3.7 shows the reduction of soil PCB 77 concentration with poplar as the planted species and PCB 77 as the sole contaminant. The soil PCB concentration in the moisture content cycled treatment diminished by 47% reduction at the end of exposure. On the other hand, a significantly lower decrease of 39% was recorded for the uncyled treatment. Both the cycled and uncycled treatments had significant reductions in their PCB 77 concentration when compared to the initial concentration. Again, lower reductions were experienced with PCB 77 as the single congener and poplar as the plant species, when compared with the switchgrasss system and PCB 77 as the single congener.

When PCB 153 was the sole contaminant and poplar was the plant species, a reduction of 49% was registered with the moisture content cycled treatment. In

comparison, the decline recorded in the uncycled treatment was 41%. The decrease observed in the moisture content cycled treatment was significantly higher than the diminution in the uncyled treatment ($p<0.05$). Compared to the initial PCB 153 concentration, both the cycled and uncycled treatments had significant reductions in their soil PCB concentration at the end of incubation ($p<0.05$). Similar decreases in PCB 153 concentration were obtained with the poplar and switchgrass planted systems.($p<0.05$).

The decrease in parent compound concentration in the uncycled and cycled planted systems at most time points were similar for both the single congener exposures and the exposures containing the mixture ($p<0.05$) (Appendix Figures A3-A8). However at the end of exposure there were significant differences between the the cycled and uncyled treatments in all cases except one. This could be attributed to the fact that for the cycled systems, neither anaerobic nor sulfidogenic conditions developed at any time during the 7 days flooding period that was intended to create anaerobic conditions in the root zone. This necessitated the need for multiple cycles of redox manipulation to produce any significant difference between the cycled and uncycled treatment.

3.4 Conclusions

There was difficulty in lowering the redox potential to sulfidogenic conditions in the planted reactors as designed here. This suggests plant oxygen transfer downwards to the roots and soils. The soil reactors with plants became redox "poised" at about +20 mV, while the unplanted reactors became poised at about -150 mV. It is possible, that with a longer period of flooding (greater than 7 days) sulfidogenic conditions can be achieved in planted reactors. However, even under marginally positive bulk redox conditions, the cycled switchgrass and poplar planted systems showed significantly higher dissipation of parent PCB compounds. In addition, the cycled planted systems had a higher mass of

transformation products than uncycled planted systems. Multiple cycles were necessary to achieve significant differences between the cycled and uncycled treatments because of the short cycles needed to maintain plant health. For a short multiple cycle regime to be effective, it will have to be combined with planting because the impact of cycling on PCB dissipation and transformation in the switchgrass and poplar planted systems was not replicated in the unplanted system (Appendix Figures A1 and A2). Planted systems clearly showed greater reductive dechlorination than unplanted systems, and those where redox was cycled, showed significantly greater transformations than uncycled treatments.

While methanogenic conditions are desirable for anaerobic microbial reductive dechlorination to occur, it is possible to effect reductive dechlorination in a sulfidogenic environment (Fava et al. ,2003, Kuo et al. 1999). Both sulfate reducing and methanogenic bacteria have been hypothesized as mediating the reduction of single congeners similar to the one used in this study, and highly chlorinated congeners in Aroclor mixtures (Kuo et al., 1999, Fava et al., 2003, Zanarolli et al.,2006). These reductions resulted in the accumulation of less toxic lower chlorinated congeners.

The inability to develop anaerobic conditions in the root zone means that it is not tenable to achieve these conditions at the the 7 days time scale. The inability to induce anaerobic conditions in the root zone may have resulted from plant produced oxygen during photosynthesis. Plants can affect soil oxygen levels through radial oxygen transfer from the roots as evidenced by the diurnal fluctuation shown when *Carix nigra* was planted in sand (Adema et al. 2003). Similarly, Nzengung et al. (2004) working in hydroponic solution was able to see significant diurnal swings in redox potential in willow planted treatments. The diurnal fluctuations were of the order of 450 mV. Similar results, but with a lower swing were observed in soil by Struckhoff (2009).

There have been reports of attempting to use a combination of anaerobic and aerobic systems to enhance microbial degradation of PCB mixtures containing higher and lower chlorinated congeners. However, these have always been two compartment

systems whereby the contaminant is held for a long period of time in anaerobic conditions and then the effluent is moved to an aerobic environment (Fathepure and Vogel, 1991, Master et al. 2002) .

The novelty of this system is using a single reactor to accomplish the same purpose. The natural fluctuations in redox potential, if large enough and often enough, could act as a microzone for the bigger setup. Plant influenced root zone aeration is suggested by the fact there was a quicker drop in the redox potential in the unplanted cycled system than with the planted cycled system (Figure 3.2). Differences in the cycled and uncycled treatments were not readily apparent and multiple cycles were needed for the impact of cycling to be significant in a planted system (Appendix Figures A3-A8), possibly due to the short cycling periods which are necessary to maintain plant health.

In addition to dissipation of parent compounds, several transformation products of the parent PCBs were observed in the cycled and uncycled treatments. For PCB 153 the transformation products measured ranged from pentachlorinated to dichlorinated compounds. PCBs 52 and 77 produced dechlorinated compounds ranging from trichlorinated to monochlorinated congeners. A mass balance on the switchgrass and poplar planted systems indicated that there was a higher mass of transformation products in the cycled system in comparison to the uncycled system (Tables 3.1 and 3.2).

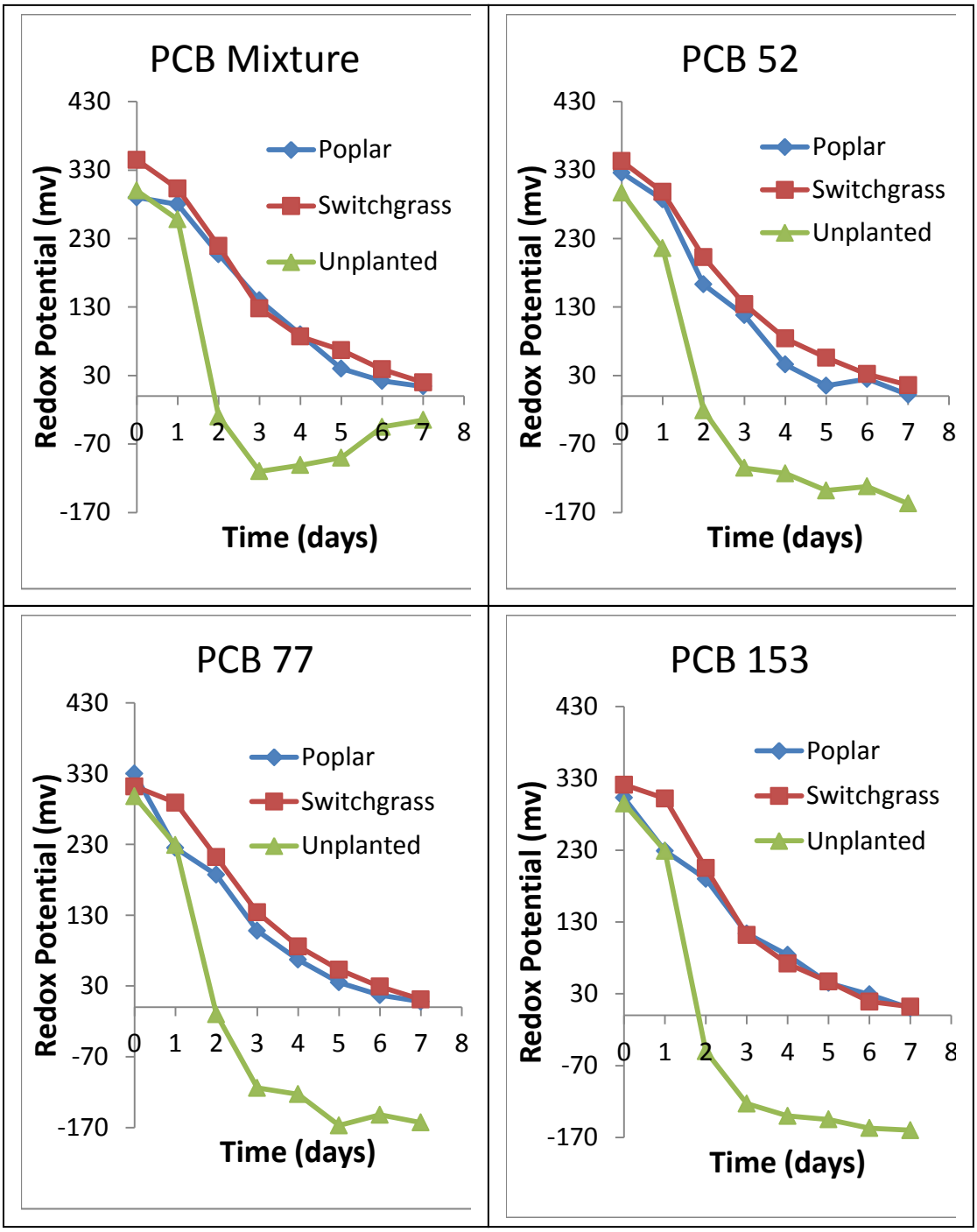

Figure 3.2: Representative redox potential in flooded soil containing a mixture of PCB 52, 77 and 153 and single congeners and planted with switchgrass or poplar. Also shown is the redox potential in the flooded unplanted system.

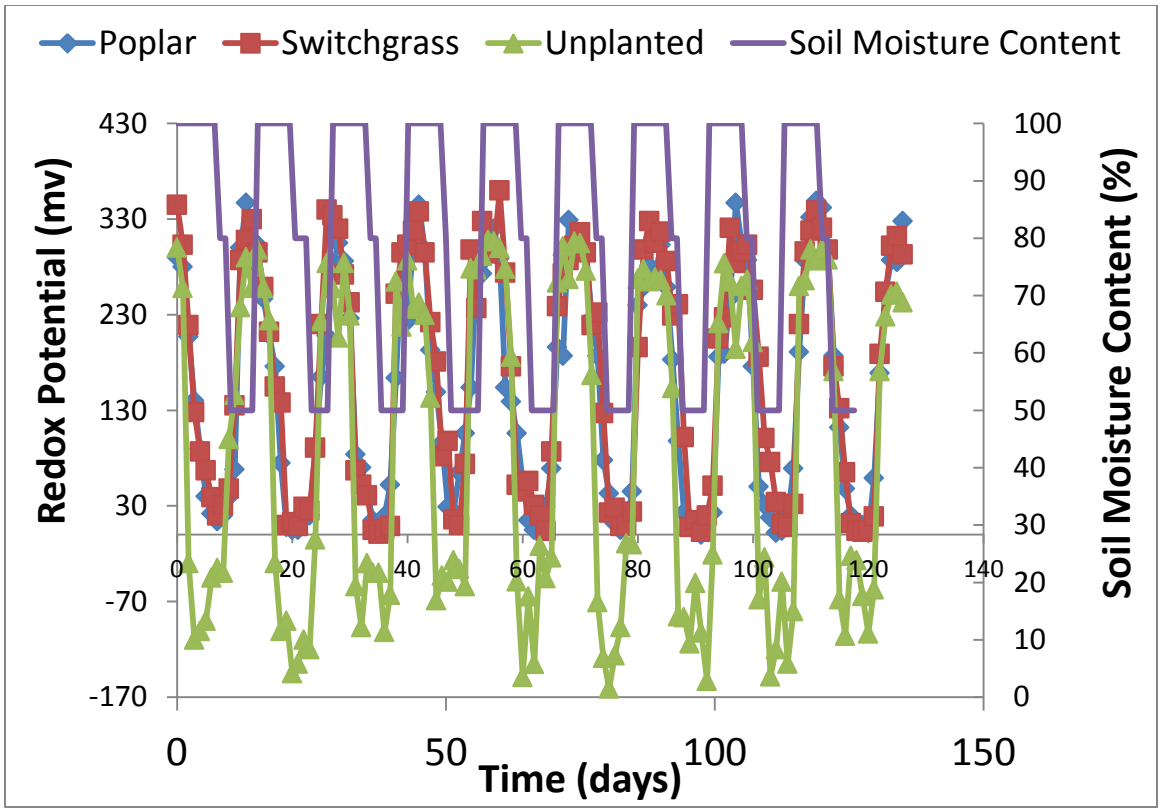

Figure 3.3: Redox potential in flooded soil containing a mixture of PCB 52, 77 and 153 and planted with switchgrass or poplar. Also shown is the redox potential in the flooded unplanted system.

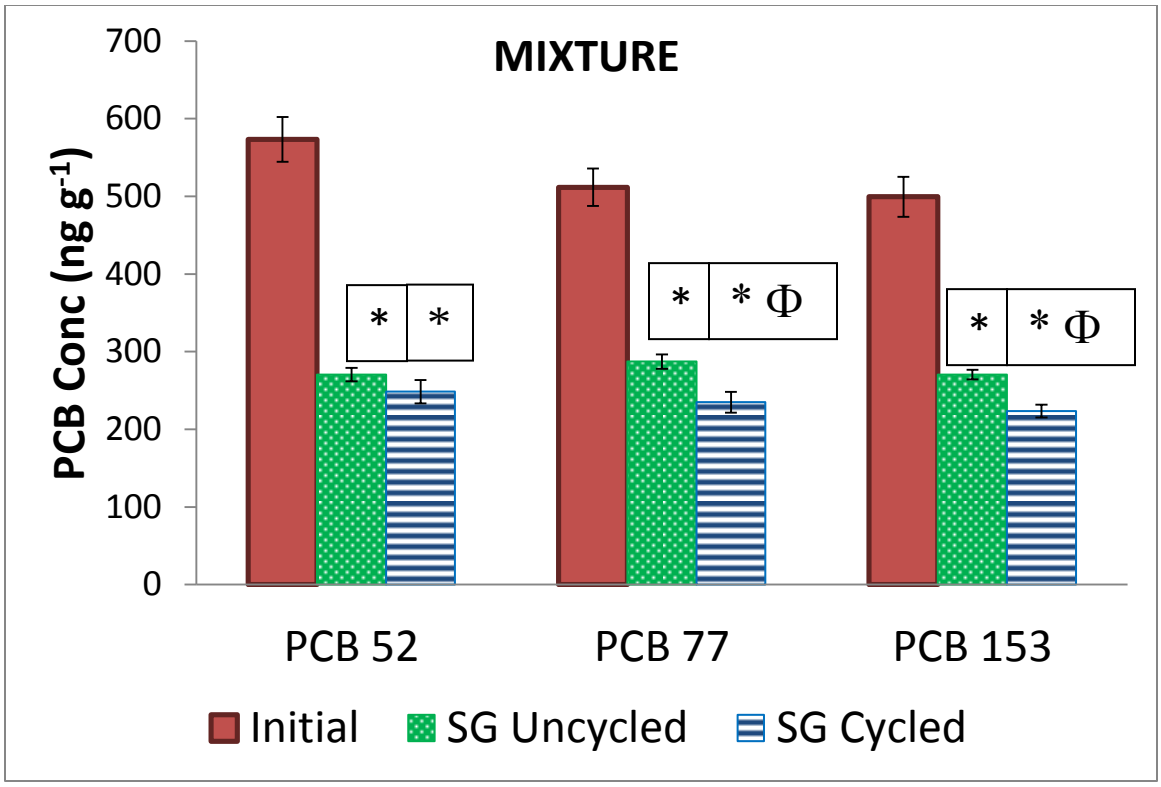

Figure 3.4: Dissipation of PCB in soil contaminated with mixture of PCB 52, PCB 77 and PCB 153 and planted with switchgrass. SG = switchgrass. The spike congeners were undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Φ = significantly higher reduction when compared to uncycled treatment ($p<0.05$). Error bars are 1 standard deviation and n=3.

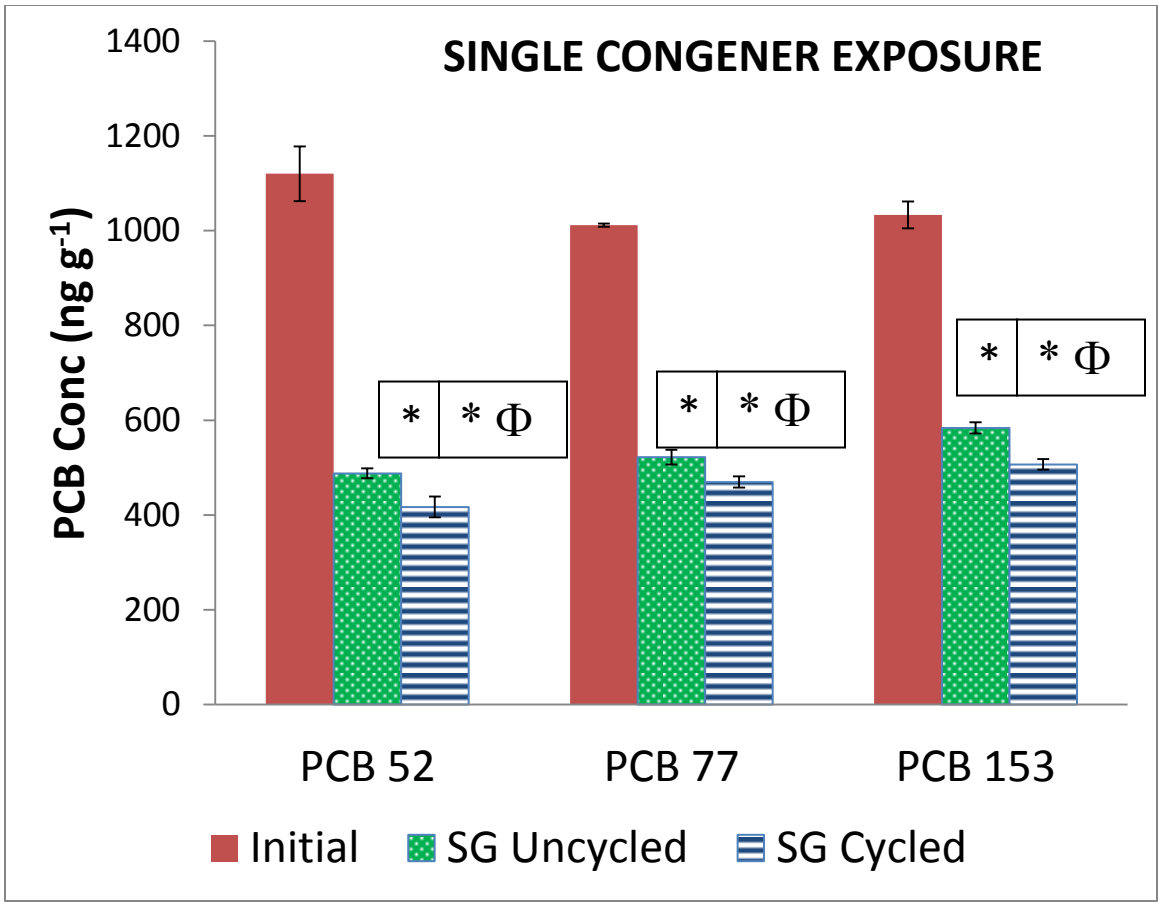

Figure 3.5: Dissipation of PCB in soil contaminated PCB 52, PCB 77 and PCB 153 as the sole contaminants and planted with switchgrass. SG = switchgrass. The spiked congeners were undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Φ = significantly higher reduction when compared to uncycled treatment ($p<0.05$). Error bars are 1 standard deviation and n=3

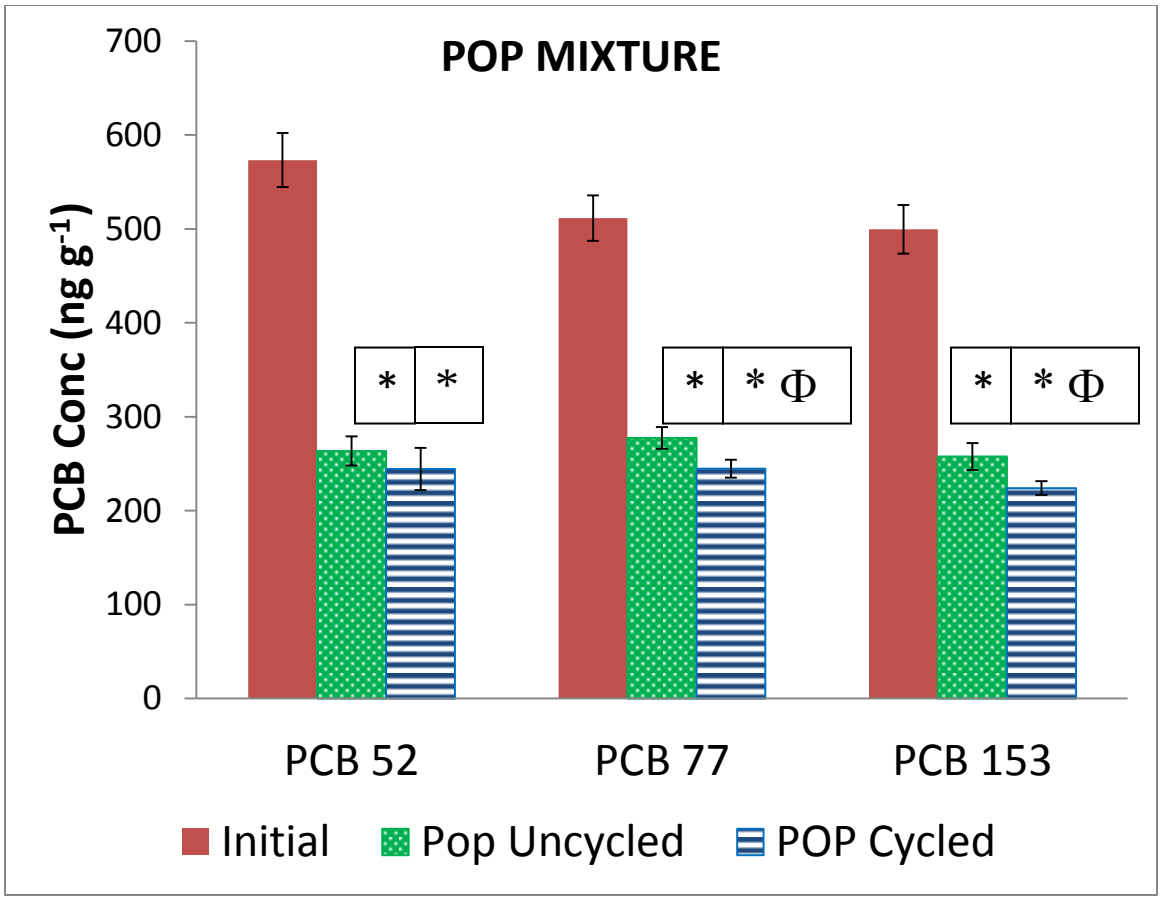

Figure 3.6: Dissipation of PCB in soil contaminated with mixture of PCB 52, PCB 77 and PCB 153 and planted with poplar. POP = Poplar. The spike congeners were undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Φ = significantly higher reduction when compared to uncycled treatment ($p<0.05$). Error bars are 1 standard deviation and n=3.

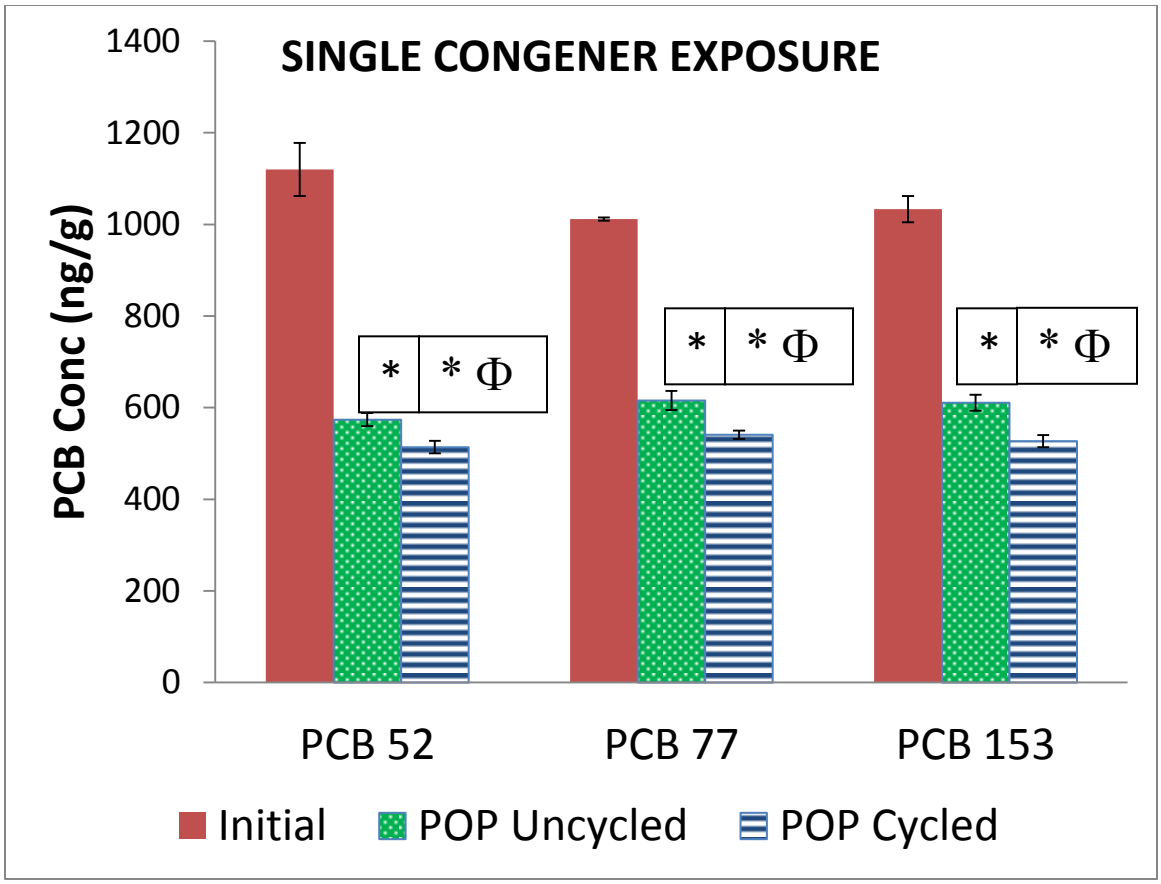

Figure 3.7: Dissipation of PCB in soil contaminated with PCB 52, PCB 77 and PCB 153 as the sole contaminants and planted with poplar. POP = poplar. The spiked congeners were undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Φ = significantly higher reduction when compared to uncycled treatment ($p<0.05$). Error bars are 1 standard deviation and n=3.

Table 3.1: Molar Mass Balance in Switchgrass Planted System with and without Moisture Content Cycling

	Parent Compound In Soil (mole %)	Transformation Products in Soil (mole %)	Roots (mole %)	Shoots (mole %)	Recovered (mole%)
PCB52 Uncycled	43.6	55.1	2.6	<0.1	101.3
PCB52 Cycled	37.2	63.0	3.5	<0.1	103.7
PCB77 Uncycled	52.0	42.1	4.4	<0.1	98.5
PCB77 Cycled	46.5	55.5	4.5	<0.1	106.5
PCB153 Uncycled	56.1	40.1	2.2	<0.1	98.4
PCB153 Cycled	49.3	46.9	2.2	<0.1	98.4

Table 3.2: Molar Mass Balance in Poplar Planted System with and without Moisture Content Cycling

	Parent Compound In Soil (mole %)	Transformation Products in Soil (mole %)	Roots (mole %)	Leaf (mole%)	Recovered (mole %)
PCB52 Uncycled	51.2	45.9	3.5	<0.1	100.6
PCB52 Cycled	46.4	56.2	3.4	<0.1	106.0
PCB77 Uncycled	61.1	35.7	2.5	<0.1	99.3
PCB77 Cycled	53.8	47.4	2.6	<0.1	103.8
PCB153 Uncycled	59.2	35.3	3.5	<0.1	98.0
PCB153 Cycled	51.0	47.7	4.3	<0.1	103.0

CHAPTER 4 ROLE OF PLANTS IN RHIZOSPHERE BIOTRANSFORMATION OF SELECTED PCB CONGENERS

4.1 Introduction

Polychlorinated biphenyls (PCBs) are recalcitrant organic compounds and persist in the environment. It is estimated that 300 million kg have been discharged to the environment since production began in the 1930's (Houlebek 2001). Remediation options are limited and the current modus operandi for remediation is confined to physical methods such as stabilization and containment on site, excavation and incineration and excavation and storage in secure confined disposal facilities (Abdul and Ang 1994, Johnson 1994, Bremle and Lawson 1998, Poland et al. 2001, de Carcer et al. 2007,). Excavation followed by treatment with a permeable reactive barrier has also been utilized (Kalinovich et al. 2008).

Biphenyl degraders are relatively ubiquitous in soil (Hernandez et al 1995, Focht,1995) and several PCB degrading microbes have been characterized and isolated (Sakai et al., 2005, Adebusoye et al., 2007, Ionescu , 2007). There is evidence of microbial mediated in situ PCB degradation in contaminated native environments (Bedard, 2008, Di Toro 2006, Fava 2003), although the process is a slow one which has contributed to the recalcitrance of PCBs. Plants also have the ability to remediate organic pollutants through direct uptake and accumulation or transformation in plant tissues (Kacalkova and Tlustos, 2011). In addition, plants may produce secondary compounds and exudates which stimulate microbial activity and transformation of xenobiotics in the rhizosphere. Many plant exudates are analogues of xenobiotics.

PCB degraders are found in many environments (Hiraishi, 2008, Bedard, et al. 2007, 2008, Magar et al., 2005, Pakdeesusuk et al., 2005, Tiedje et al., 1993) and the

ability of plants to stimulate microbial activity in the rhizosphere is known. (Mackova et al.,2007). In this research, soil was artificially contaminated with a mixture of PCBs and planted with poplar (*Populus deltoids x nigra* DN34) and switchgrass (*Panicum virgatum)*. Poplar has been shown to be effective in remediating many organic pollutants via uptake and transformation (Gordon 1998, Burken and Schnoor, 1997, Newman et al., 1997, Thompson et al. 1998, Van Aken et al. 2004, Liu et al. 2009, Zhai et al. 2011). Several investigators have used grasses to study plant PCB degradation because grasses seem to outperform other species in terms of resistance to toxicity,sensitivity, loss of biomass and performance in aiding PCB dissipation (Weber and Morzek,1979, Chekol et al., 2004). Leigh et al. (2006) found that the concentration of PCB degraders in grass rhizosphere was between 30- 60 times more than the concentration of the other plants at comparable depths. The higher concentration in the grass rhizosphere was attributed to the finer root system of the grass, which provides more surface area per gram.

Poplar is a model plant for phytoremediation, but studies involving PCB and poplar thus far, have relied on hydroponic exposure (Liu et al. 2008, Liu et al., 2009). Most field-scale phytoremediation interventions involve planting in a soil matrix which is more complex than hydroponics, yet few studies have been undertaken with real soil. Over the past decade, a few studies have looked at PCB amelioration utilizing plant based systems, but these results have been mixed and have mostly focused on reduction of parent compounds or mixtures. In addition to dissipation, this study also looks at transformation products to gain insight into the mechanisms that may potentially be at play.

Therefore, the broad objectives of this study involved investigating the potential to enhance the degradation and transformation of selected PCB mixtures and single congener systems by planting contaminated soil with poplar and switchgrass, and to understand the metabolic pathways involved in rhizosphere degradation of the selected PCB compounds. Three congeners were used in the study. These were used in single

congener studies, and also combined as a mixture. PCB 52 (2,2'5,5' tetrachlorobiphenyl) is thought to be difficult to degrade because there are chlorine substituents in the ortho positions on both rings, a position which is not readily amenable to microbial degradation because of steric hindrances. PCB 77 (3,3'4,4' tetrachlorobiphenyl) is coplanar, exhibits dioxin like properties, and is one of the most toxic PCB congeners. The other congener PCB 153 (2,2',4,4',5,5'hexachlorobiphenyl), has a high tendency to accumulate in humans and also has chlorine substituents in the ortho position on both rings.

4.2 Materials and Methods

4.2.1 Experimental Set Up

Soil collected from Amana colonies IA, was passed through a 60 mesh sieve and artificially contaminated with tetrachlorinated PCB 52, PCB77 and hexa chlorinated PCB 153 separately and as a mixture of the three congeners. The quartering technique was used to homogenize the soil. Four quadrants were created on a quartering canvas and several diagonal trajectories were made to mix the soil components together. Homogenization was achieved by making twenty passes (diagonal trajectories) to mix the soil thoroughly. To account for heterogeneity, 20 subsets of soil were collected from different locations, homogenized and analyzed in triplicate. Triplicate concentration measurements were conducted to establish the initial concentration, and it was compared to the target for the purpose of quality assurance. This contaminated soil was aged for two months to reduce bioavailability and to ensure conditions more representative of field sites (Alexander et al, 2000, Thompson et al. 1998, White et al. 2006). The aged, contaminated soils were then planted separately with switchgrass (*Panicum virgatum)*

seeds from Adams-Briscoe Seed Co. (Jackson, GA) and with 22.9 cm poplar (*Populus deltoids x nigra* DN34) cuttings from Segal Ranch Hybrid Poplars Nursery (Grand View, WA).

Treatments consisting of switchgrass and poplar planted in uncontaminated soil were used as blanks and functioned as volatilization controls. Plants were grown under a 16 hour light/8 hour dark photo period with a light intensity of 200 µmol $m^{-2}s^{-1}$. Rubber maid shoes boxes (33.8 cm x 21.6 cm x 11.9 cm (LxWxD)) with 1.9 cm holes drilled in the covers, lined with aluminum foil and containing 2500 g of soil were used as reactors. The plants were allowed to grow and establish themselves for eight weeks before soil samples were taken. Thereafter, the soil was sampled and analyzed at regular intervals ranging from two to eight weeks, up to a period of thirty two (32) weeks.

Twelve (12), five (5) g samples of soil were collected randomly from each treatment using a Lock and Hold™ syringe (Ben Meadows Company WI, USA) and homogenized prior to analysis. At the end of exposure (32 weeks) soil and plant materials were sampled and analyzed for PCB content using GC/MS/MS triple quadropole mass spectrometry (Agilent Technologies 6890N GC with an Agilent 7683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA)). PCBs 14, d65, and 166 were used as surrogate standards and PCB 204 was the internal standard. The experimental set up and analyses were carried out in triplicate.

4.2.2 PCB Extraction

Denaturation and extraction of PCB in soil and plant material was conducted by adding three (3) milliliters per gram of a 1:1 hexane/acetone mixture to 5 grams of grounded homogenized soil and sonicating for 1 hour. Prior to sonification, the samples were spiked with 50 ng of PCB14 (3,5-dichlorobiphenyl), deuterated PCB65 (2,3,5,6-

tetrachlorobiphenyl) and PCB166 (2,3,4,4′,5,6-hexachlorobiphenyl) (Cambridge Isotope Laboratories, Inc.), which were used as surrogate standards. Surrogate recoveries ranged from 89.5±4.3% to 96.4±3.8% for PCB 14, 92.8±4.7% to 99.6±5.3% for PCB 65 and 99.5±3.2% to 102.7±4.7% for PCB 166.

The sonicated material was centrifuged at 3000 rpm for 5 minutes, after which the supernatant was transferred to a fresh vial. A second extraction was performed and the supernatants combined. The combined supernatant was evaporated to dryness using rotary evaporation and the solvent changed to hexane (3 ml). Any loss by rotary evaporation was accounted for using the surrogate standard recoveries. Removal of lipids and other polar substances was achieved by double extraction with concentrated sulfuric acid and hexane. This hexane extract was concentrated to approximately 0.5 ml under a gentle stream of $N_{2.}$ The concentrate was eluted with 10 ml of hexane through a filter consisting of 0.1g of silica (70−230 mesh, Fisher Scientific, Inc.), 0.1g of anhydrous sodium sulfate (Na_2SO_4) and 0.9 g silica gel acidified with H_2SO_4 (2:1). The eluent was concentrated and PCB204 (2,2′,3,4,4′,5,6,6′ octachlorobiphenyl) was added as an internal standard before analysis by GC/MS/MS triple quadropole mass spectrometry (Agilent Technologies 6890N GC with an Agilent 7683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA)). The gas chromatogram (GC) was fitted with a Supelco SBP-Octyl capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) with helium as carrier gas at a constant flow rate of 0.8 ml min^{-1}. The GC operating conditions were as follows: injector temperature 270 °C, interface temperature 290 °C, initial temperature 75 °C, initial time 2 min. The GC temperature program was 75 to 150 °C at 15 °C min^{-1}, 150 to 290 °C at 2.5 °C min^{-1}, and final time 1 min. Hu et al. (2010) provides details about the identification and quantification of the PCB congeners.

4.2.3 Statistical Analysis

Microsoft Excel Analysis Toolpak's ANOVA analysis and Students' t-test were used for statistical testing. The significance level of 0.05 was utilized to indicate whether the treatments were significantly different than the controls.

4.3 Results and Discussion

4.3.1 Measured versus Target Initial PCB Concentration

Although there was a targeted initial PCB concentration, the initial concentration after mixing and homogenization was measured to ascertain the starting concentration in both the mixture and single congener treatments. In each case, the measured concentration was different from the targeted concentration probably due to the heterogenity of the soil matrix despite efforts to obtain a homogenous mixture by repeatedly quartering the contaminated soil. The targeted initial concentration for each congener in the mixture (PCB 52, PCB 77 and PCB 153) was 500 ng g^{-1}. while the measured concentrations were 573± 29 ng g^{-1} (PCB 52); 512 ± 24 ng g^{-1}(PCB77) and 499 ± 25 ng g^{-1} (PCB153). For the single congener treatments, the targeted initial concentration was 1000 ng g^{-1} for each congener while the measured concentrations were 1120±58 ng g^{-1}, 1012 ± 3 ng g^{-1} and 1033 ±28 ng g^{-1} respectively for PCB 52, PCB 77 and PCB 153.

4.3.2 PCB 52 Dissipation in Switchgrass Planted Soil

The dissipation of PCB 52 in soil planted with switchgrass in a mixture containing the three congeners (PCB 52, 77 and 153) and a single congener treatment is depicted in Figure 4.1. The mixture recorded a 53% decrease in soil PCB 52 concentration while a 56 % reduction was observed for the single congener treatment. This represented a statistically significant decline when compared to the initial PCB 52 concentration in both the mixture and single congener treatments ($p<0.05$). While the mass reductions in the mixture and single congener treatment were different due to different initial concentration, percentagewise, the reductions were statistically similar ($P<0.05$).

For the unplanted systems there were 29% and 27% reductions in PCB 52 in the mixture and single congener treatments respectively. Both planted and unplanted systems had significant diminutions when compared to the initial PCB 52 concentration ($p<0.05$). However, the planted systems had significantly higher declines in soil PCB 52 concentration than the unplanted systems ($p<0.05$).

The mixtures of congeners in both planted and unplanted systems showed similar redutios in PCB52, although the planted system was clearly more effective at transforming PCB 52 than the unplanted system.

4.3.3 PCB 77 Dissipation in Switchgrass Planted Soil

With respect to PCB 77 dissipation, the switchgrass-planted system recorded a 44% diminution in soil PCB 77 concentration in the treatment containing the mixture while registering a 48% decline in the single congener treatment (Figure 4.2). In comparison, the unplanted systems showed significantly lower reductions with a 29%

decrease for the treatment containing the mixture and a 20% decline for the single congener treatment ($p<0.05$). Compared to the initial PCB 77 concentration, both the planted and unplanted systems had significant declines ($p<0.05$). Percentagewise, the reductions observed in the planted systems, were statistically similar for the mixture and single congener treatments.

4.3.4 PCB 153 Dissipation in Switchgrass Planted Soil

Figure 4.3 compares the concentration of PCB 153 in soil planted with switchgrass and unplanted soil after 32 weeks of incubation in the mixture and single congener systems. Similar to the other congeners in the mixture, the planted system showed significantly higher reduction of PCB 153 in the soil when compared to the unplanted system ($p<0.05$). The switchgrass planted system containing the mixture recorded a 46% reduction while the single congener treatment registered a 44% diminution (Figure 4.3). While both the planted and unplanted systems recorded significant decreases in PCB 153 concentration when compared to the initial PCB 153 concentration ($p <0.05$), the unplanted systems recorded significantly lower reductions of 30% for the treatment containing the mixture and 27% for the single congener treatment ($p<0.05$). Again, the percentagewise difference between the reductions experienced with the mixture and single congener treatments in the planted system was not significant ($p<0.05$).

4.3.5 PCB 52 Dissipation in Poplar Planted Soil

The PCB 52 concentration in soil planted with poplar and unplanted soil after 32 weeks of incubation is shown in Figure 4.4. Poplar was planted in soil containing a

mixture of PCB 52, PCB 77 and PCB 153 and also in soil contaminated only with PCB 52. A 54% decrease in PCB 52 concentration was obtained when the soil containing the mixture was planted with poplar, while the single congener treatment showed a 41% decline. On the other hand, the PCB 52 concentration in the unplanted systems diminished by 29% and 27% respectively, in the mixture and single congener treatments. Both the planted and unplanted systems recorded significant declines in the PCB 52 concentration in the soil ($p<0.05$), but the planted systems exhibited significantly higher reductions than the unplanted systems ($p<0.05$). In the planted systems, the reduction obtained with the mixture was significantly higher ($p<0.05$) than the reduction observed with the single congener treatment. This was contrary to what was expected, and was possibly due to synergistic effects in the mixture. Compared to the switchgrass planted soil, the reduction in soil PCB 52 concentration obtained with poplar was similar for the mixture ($p<0.05$), but significantly lower for the single congener treatment ($p<0.05$).

4.3.6 PCB 77 Dissipation in Poplar Planted Soil

The decrease in soil PCB 77 concentration in the mixture and when PCB 77 was the single contaminant, and planted with poplar, is depicted in Figure 4.5. There was a 46% diminution in soil PCB 77 concentration for the mixture whereas a significantly lower reduction of 39% was recorded for the single congener treatment ($p<0.05$). Again, this was unexpected and possibly due to synergistic effects in the mixture. In comparison to the planted systems, the unplanted systems recorded lower declines of 29% and 20% for the mixture and single congener treatments respectively. Similar to the situation with other planted systems, the reductions in soil PCB 77 concentration obtained with the poplar planted systems were significantly higher than the diminutions observed with the unplanted systems ($p<0.05$). With regards to PCB77 decrease in the mixture and plant

species, the results obtained with the poplar planted soil were statistically similar to those achieved with switchgrass planted soil ($p<0.05$). For the single congener treatment, a significantly lower reduction in soil PCB 77 concentration was obtained with poplar as the planted species when compared to switchgrass.

4.3.7 PCB 153 Dissipation in Poplar Planted Soil

Using poplar as the plant species, the PCB 153 concentration in the planted soil contaminated with the mixture was reduced by 48 %, and by 44% in the single congener treatment (Figure 4.6). In comparison, the unplanted systems showed a 30% decrease in the mixture and a 27% decline in the single congener treatment. While both the unplanted and planted systems had significant decreases in PCB 153 concentration ($p<0.05$), the diminutions in the planted systems were significantly higher than the declines in the unplanted systems ($p<0.05$). Similar to the other congeners in the mixture, the reduction in soil PCB 153 concentration achieved with the planted poplar system was statistically equivalent to that seen with the switchgrass planted system ($p<0.05$). However, unlike the other single congener treatments which registered significantly lower reduction in the poplar planted system when compared to the switchgrass planted system, the PCB 153 reduction in the poplar planted system was statistically analogous to that achieved in the switchgrass planted system.

4.3.8 PCB Biotransformation Products

In addition to dissipation of the parent compounds, several transformation products were also observed in the soil both in the planted and unplanted systems (Figure 4.7). The soil at the start of exposure was devoid of these transformation products (Figure

4.7). While similar transformation products were observed in both the planted and unplanted systems, there were significantly higher concentrations of some transformation products in the planted system relative to the unplanted system ($p<0.05$). The transformation products accounted for approximately all of the mass of parent compounds lost. The transformation products detected could be traced back to the parent compounds through dechlorination pathways, with PCBs 18, 35, 37, 99 and 101 being important intermediaries.

4.3.9 Distribution of PCB in Plant Material

Figure 4.8 shows the distribution of the parent compounds in plant material. It indicates that majority of the parent compounds detected resided in the roots most likely as a result of sorption. In terms of mass there was less than 0.1% of the parent compound in the above ground portion of the plant. This is consistent with the relatively high K_{ow} for these congeners, and it is highly unlikely that plant uptake will play a direct role in the dissipation and transformation of PCBs. Their major role seems limited to providing an enabling environment for rhizosphere degradation and transformation.

Both the unplanted and planted systems had significant reductions in PCB concentrations after 32 weeks of incubation. However, irrespective of the plant species, there was a significantly higher reduction in the planted systems when compared to the unplanted systems. This suggests that poplar and switchgrass were directly influencing the chemistry and biology of the soil directly under the influence of the roots (Table 4.1). There were significantly higher concentrations of the parent compounds in roots when compared to the shoots at the end of exposure (Figure 4.8).

The results are consistent with the fact that while several researchers have reported uptake of PCBs by plants, they have explicitly stated that the amounts taken up

were small (Weber and Mrozek, 1979, Chu et.al., 1999, Aslund et al., 2007,2008, Liu et al., 2009, Xu et al., 2010). For example, early work by Weber and Mrozek (1979) indicated while tall fescue grass were able to grow in 1,000,000 ng g^{-1} PCBs, without showing any effect on root growth they were only able to take up 0.17 % of a 20,000 ng g^{-1} concentration of applied Arochlor 1254 after 7 weeks of incubation. This was 10 times the amount taken up by soya bean in the same study.

The absence of any significant uptake of PCBs by poplar and switchgrass suggests that that significant reductions in parent PCB compound was driven by plant stimulated transformation in the rhizosphere. The similarity in biotransformation products in the unplanted and planted systems (Figure 4.7), suggests that similar microbial communities capable of reductive dechlorination existed in both planted and unplanted systems. Similar products implies similar processes with the common factor in both systems being the rhizospheric microbial population. The higher concentration of transformation compounds in the planted systems compared to the unplanted systems infers that poplar and switchgrass facilitated greater microbial numbers (activity) in the planted systems by influencing the chemical and biological processes associated with the reduction and transformation of the parent PCB compounds (Jordahl et al., 1997, Chekol et al.,2004). There was evidence of synergistic effects in the planted systems in that there was greater transformation in the mixtures when compared to the single congener treatments. This could indicate that having several congeners present induced a microbial community of PCB degraders that was more diverse and effective.

In the current study, there was a lag time of about 10 weeks before any significant reduction in parent PCB was observed (Figures 4.2 - 4.6). This lag time could have been due to an acclimation period for the competent rhizosphere microorganisms to adapt. This suggests that the declines observed were possibly due to cometabolic reduction. After the acclimation period there was a rapid decline parent PCB followed by a diminished rate of decline with the passage of time. The same trend was seen by Kuo et

al. (1999) for PCB 77 in which there was a rapid intial removal of a 40 µm concentration down 17 µm after which reduction ceased for 200 days. In another experiment they had to prime the reduction by adding PCB 77 when the concentration of PCB reached a certain level. After priming, the reduction accelerated. The retardation of the reaction was probably due to a styming of induction of the microbial enzyme responsible for degradation. The suppression of enzyme induction was probably propogated by the low concentration of the substrate. In this research, the reason for the diminishing of the reduction is not immediately apparent, because at the concentrations remaining, there should be sufficient substrate for continued rapid reduction. However it is possible that this retardation in PCB decline could have been due to the depletion of electron donor.

The strong influence of plants on the biology and chemistry in the rhizosphere is accomplished through the production of root exudates and secondary plant metabolites (Hernandez et al.,1997, Leigh et al., 2002, Ficko et al. 2011). These plant derived compounds typically cause rhizosphere dwelling microbes to significantly increase in number in comparison to their population in the bulk soil (Ionescu et al. 2009,de Carcer et al., 2007; Leigh et al. 2006, Slater et al., 2011), although they may also repress certain populations (Kamath et al., 2004, Rentz et al.,2004). Therefore, the rhizosphere normally supports and influences complex microbial communities through the production of nutrients released by root exudates, mucilage and decaying root material (de Carcer, 2007). Many plant exudates are analogues of xenobiotics, which may explain the ability of planted rhizospheres to significantly improve the reduction of xeniobiotics, possibly co-metabolically. In the case of PCBs, terpenes are structurally similar to biphenyls and as such may stimulate the cometabolic reduction of PCBs. Researchers have shown that plant exudates such as terpenoids are able to stimulate the same level of reduction as biphenyl (Leigh et al., 2002).

The ability of plant derived exudates to stimulate competent PCB degraders have resulted in experimentation with biostimulation using amendments of natural plant

material. (Dzantor et al. 2002, Singer et al.,2000a, 2000b, 2003b). However, while amendments do provide significanly better performances in unplanted treatments, there have been no additional benefits with planted systems, suggesting that there may be some threshold concentration of exudate which is necessary to induce enzyme activity or increase PCB bioavailabilty above which there is no substantial increase in benefit.

The switchgrass mediated microbial degradation observed in this study is consistent with the purported good performance of grasses in past investigations with PCB (Table 4.2). The reductions obtained with switchgrass, both with the mixture and single congener treatments ranged between 44% and 63%. Although they worked with a higher soil PCB concentration, the results obtained by Dzantor et.al (2000, 2001) and Chekol et al. (2004) working with grasses and legumes were similar to the results in the current study (Table 4.2). While some of the investigators did not attribute the observed decrease in PCB to microbial activity, it is highly probable that this was the major mechanism.

The observation that the results in terms of percentage reduction of soil concentration obtained with the current study straddled the range (30%- 77%) of other studies conducted with grasses despite differences in initial concentrations and length of exposure (Table 4.2), suggests that there may be some limiting effective range when using grass species over the time frames tested. The high end of the range represents a substantial reduction and augurs well for the potential use of grasses in phytoremediation. The occurrence of large reductions in the root zone is highly beneficial in comparison to uptake and translocation because it limits the transfer of the contaminants between reservoirs.

There were statistically similar percentagewise reduction of the parent compound for poplar and switchgrass in the mixture and statistically lower reduction in the poplar planted system for the PCB 52 and PCB 77 single congener treatments. In contrast to the the other single congener treatments PCB 153 showed similar reductions with poplar and

switchgrass. This suggests that switchgrass is just as effective as the more well known phyto remediatior poplar, in terms of rhizopheric PCB reduction potential. However, when the reductions are normalized to rootmass, poplar outperformed switchgrass with soil mass reductions ranging from 1.5 to 3 times more in the poplar planted systems than the switchgrass planted treatments.

4.4 Conclusions

The results have provided evidence of plant enhanced transformation of PCBs. Poplar and switchgrass planted rhizospheres consistently had significantly higher reduction of the tested PCB congeners and concomitant higher concentrations of transformation products when compared to unplanted systems. There was no significant plant uptake of the PCBs which indicate that the improvement in dissipation and transformation of the parent PCB compounds resulted from the plants providing an enabling environment for improved rhizosphere transformation of the PCBs. Microbial transformation is inferred by the similarity of transformation products in the planted and unplanted systems. The higher rates of transformation in the planted system suggest that plant exudates may provide electron donors which stimulate rhizospheric transformation. The transformation products obtained could be traced back by dechlorination pathways to the parent compounds and provided some insight into the metabolic pathways involved in the rhizospheric transformation of these PCB congeners.

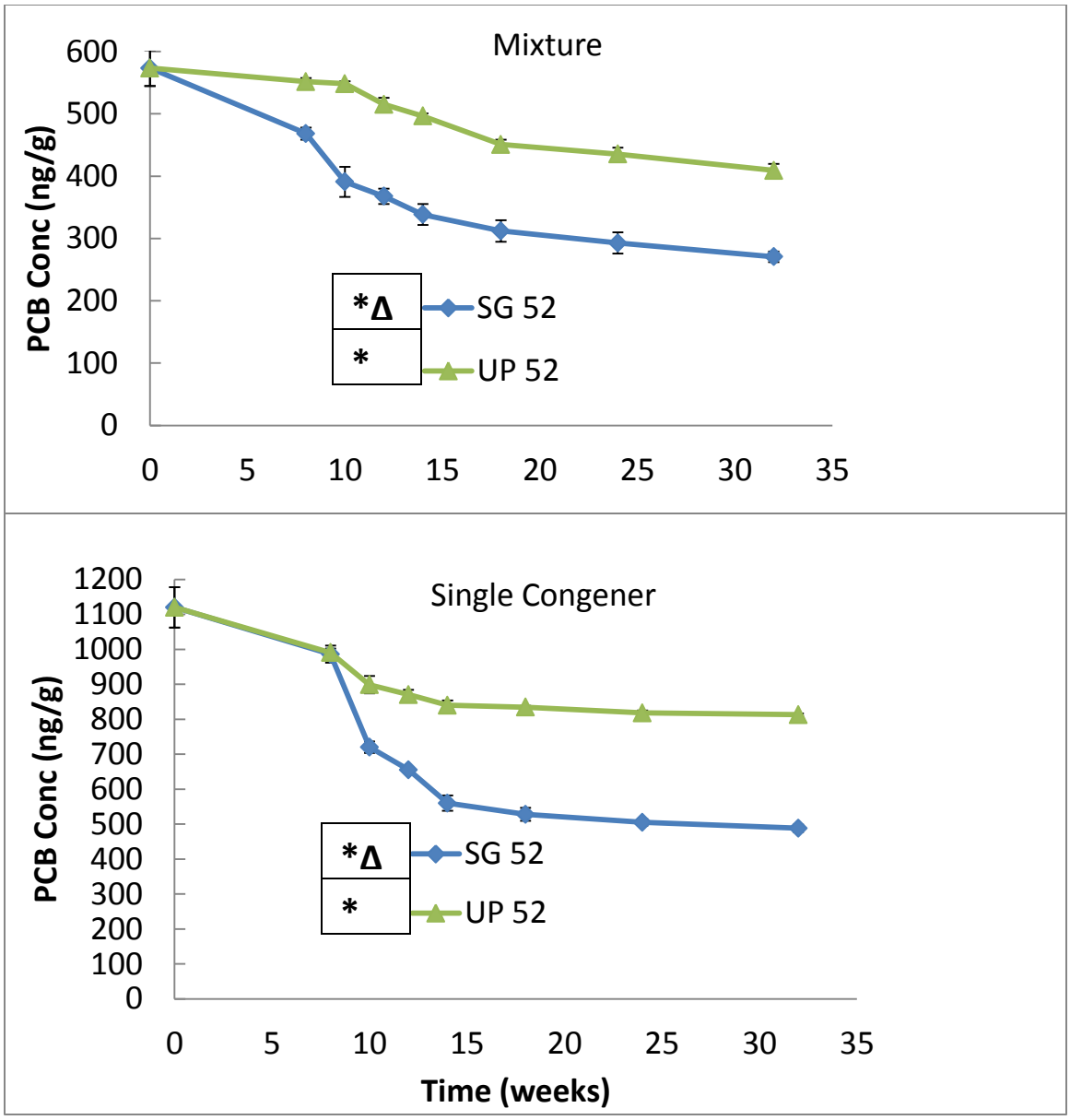

Figure 4.1: Dissipation of PCB 52 in switchgrass planted soil. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 52. SG = Switchgrass; UP = Unplanted system; PCB 52 was undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Δ = significant reduction when compared to unplanted system ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

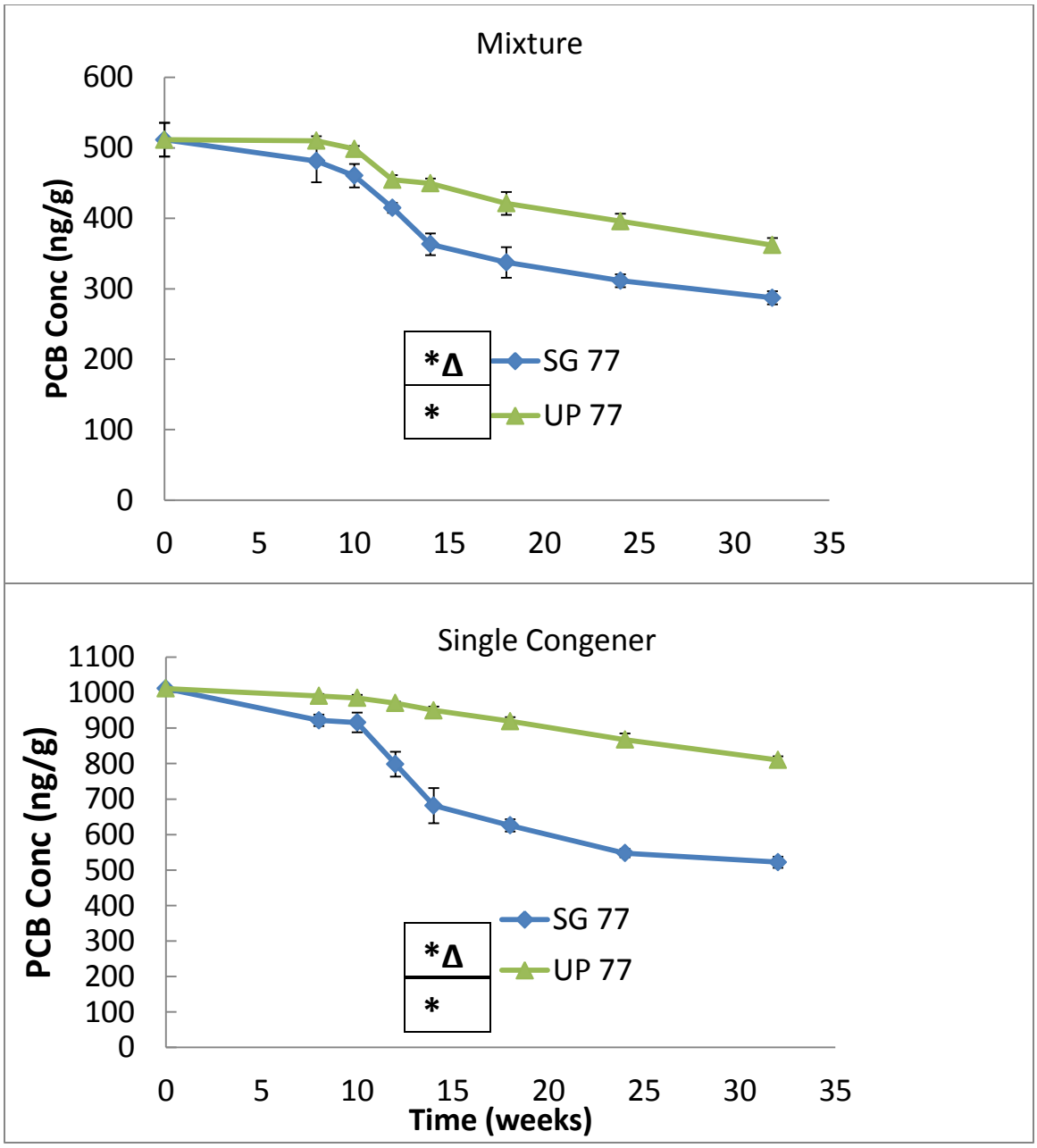

Figure 4.2: Dissipation of PCB 77 in switchgrass planted soil. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 77. SG = Switchgrass; UP = Unplanted system; PCB 77 was undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Δ = significant reduction when compared to unplanted system ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

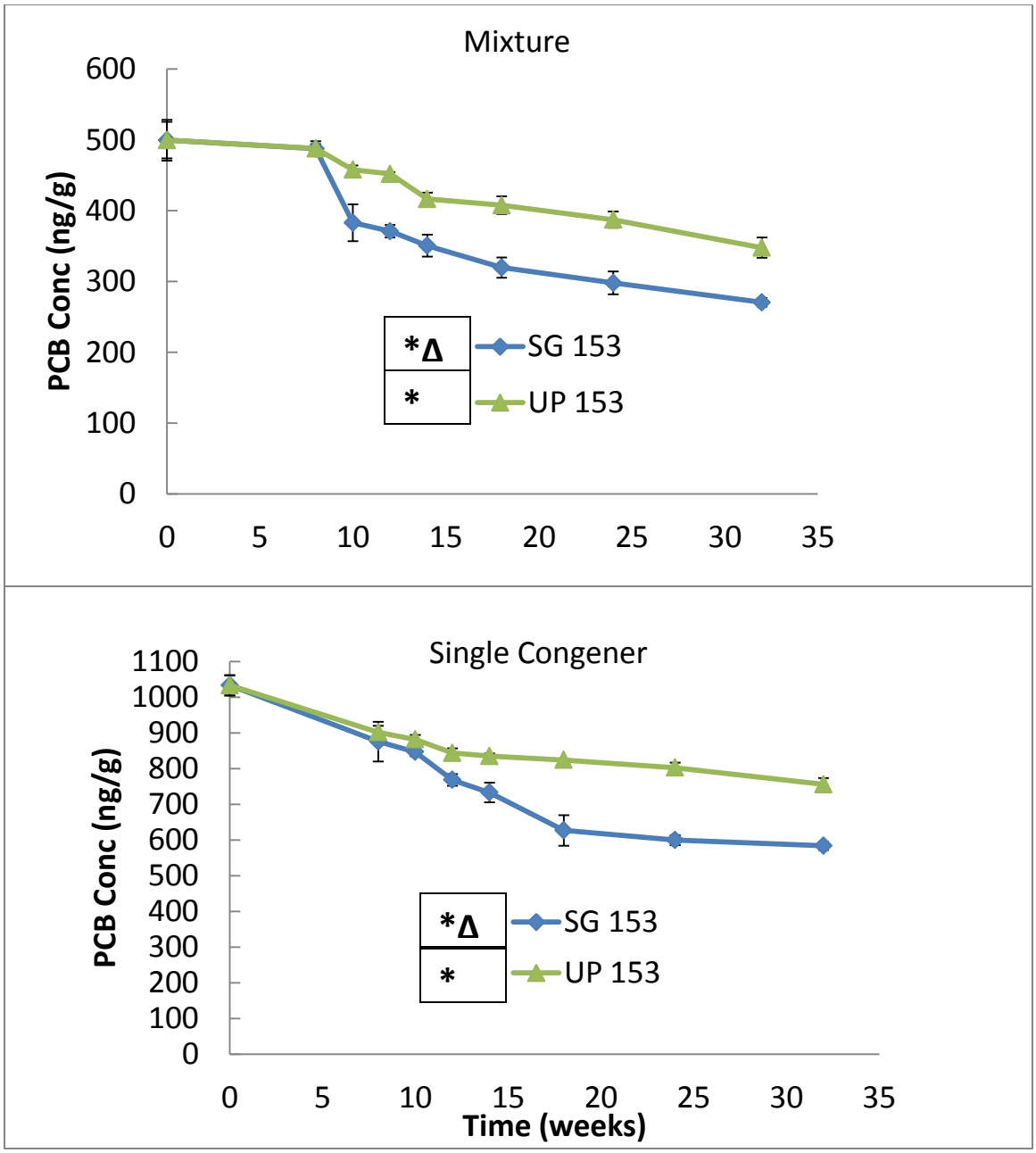

Figure 4.3: Dissipation of PCB 153 in switchgrass planted soil. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 153. SG = Switchgrass; UP = Unplanted system; PCB 153 was undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Δ = significant reduction when compared to unplanted system ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

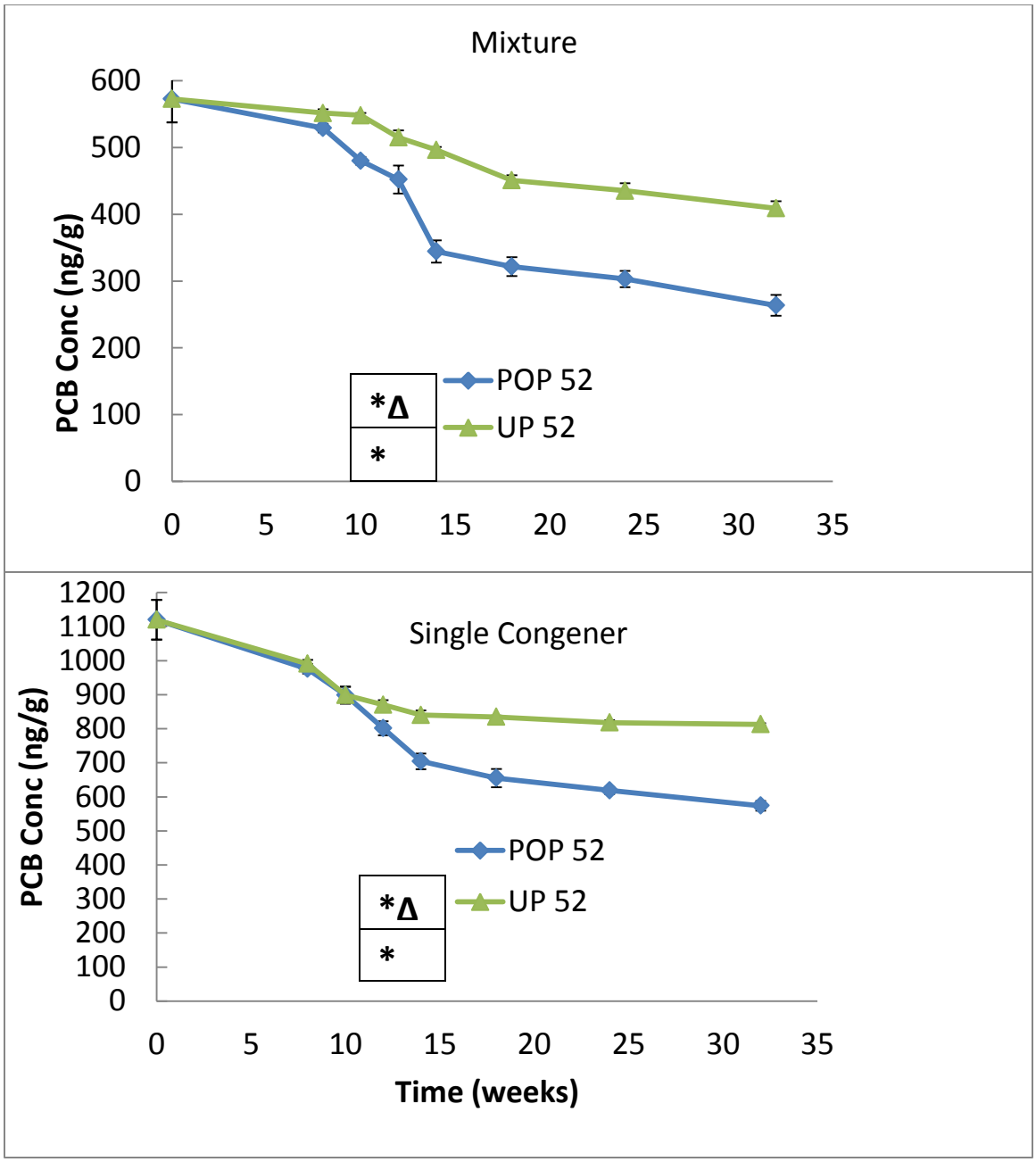

Figure 4.4: Dissipation of PCB 52 in poplar planted soil. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 52. POP = Poplar; UP = Unplanted system; PCB 52 was undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Δ = significant reduction when compared to unplanted system ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

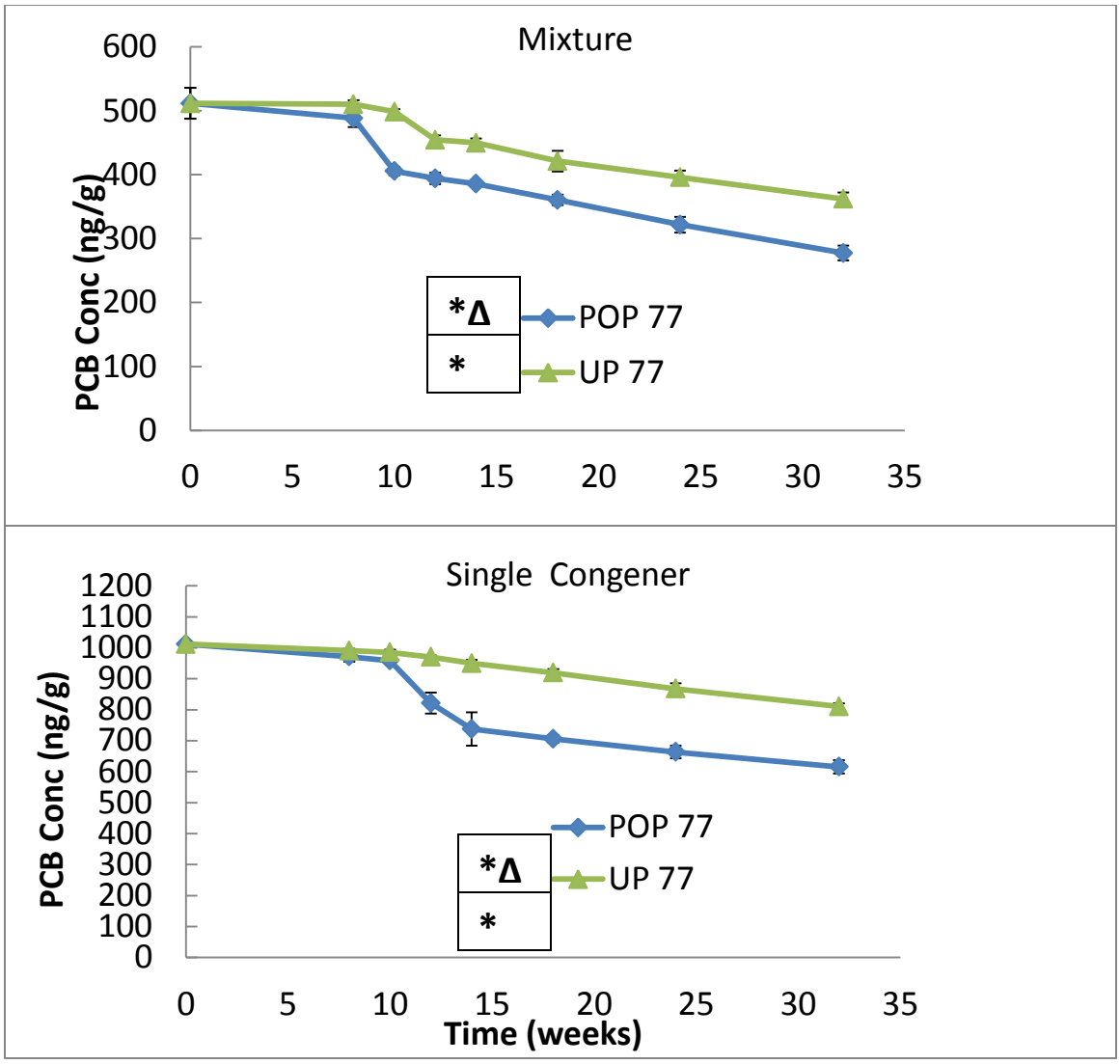

Figure 4.5: Dissipation of PCB 77 in poplar planted soil. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 77. POP = Poplar; UP = Unplanted system; PCB 77 was undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Δ = significant reduction when compared to unplanted system ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

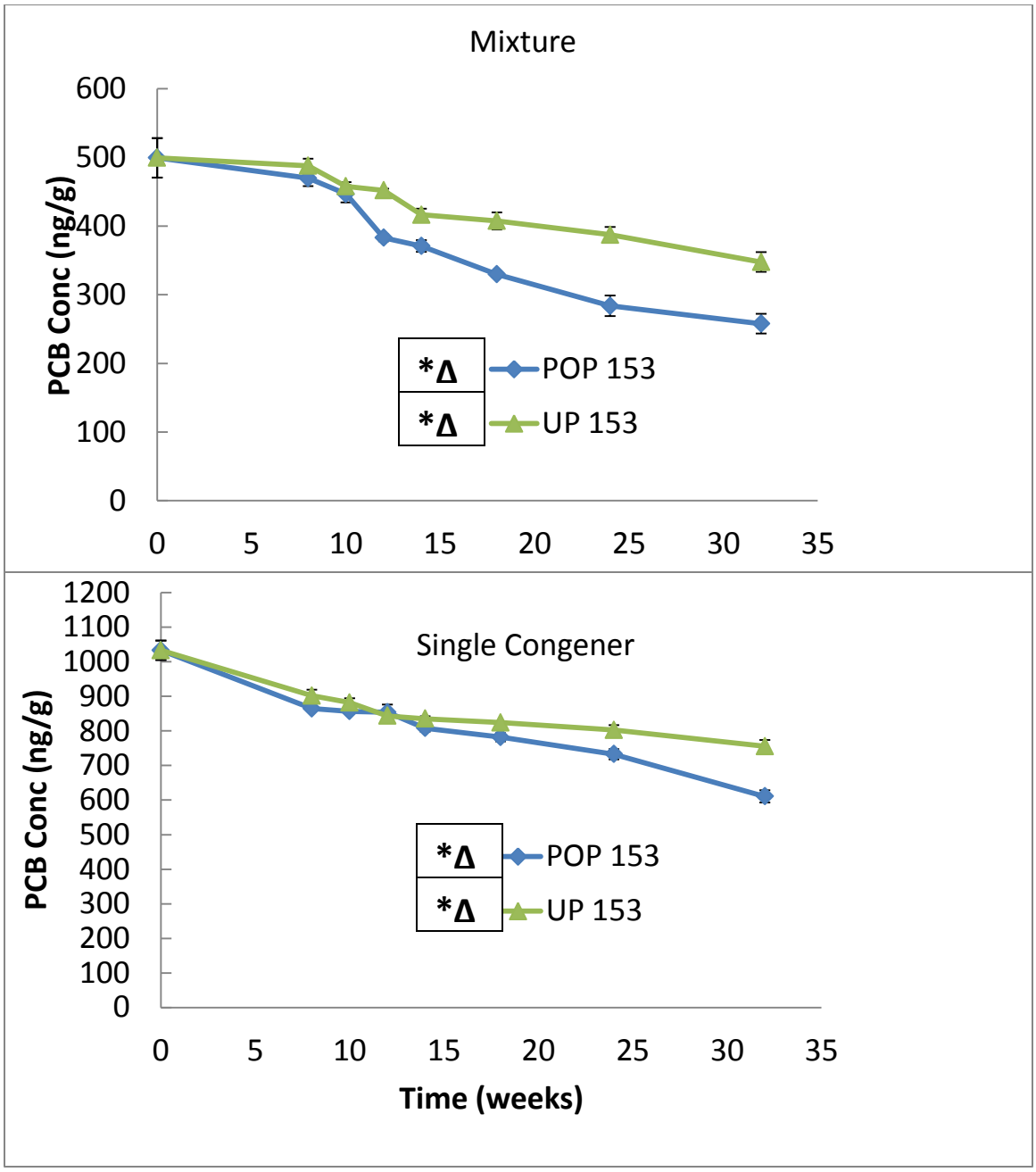

Figure 4.6: Dissipation of PCB 153 in poplar planted soil. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB153. POP = Poplar; UP = Unplanted system; PCB 153 was undetectable in the controls which were not spiked. *= significant reduction when compared to initial concentration ($p<0.05$). Δ = significant reduction when compared to unplanted system ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

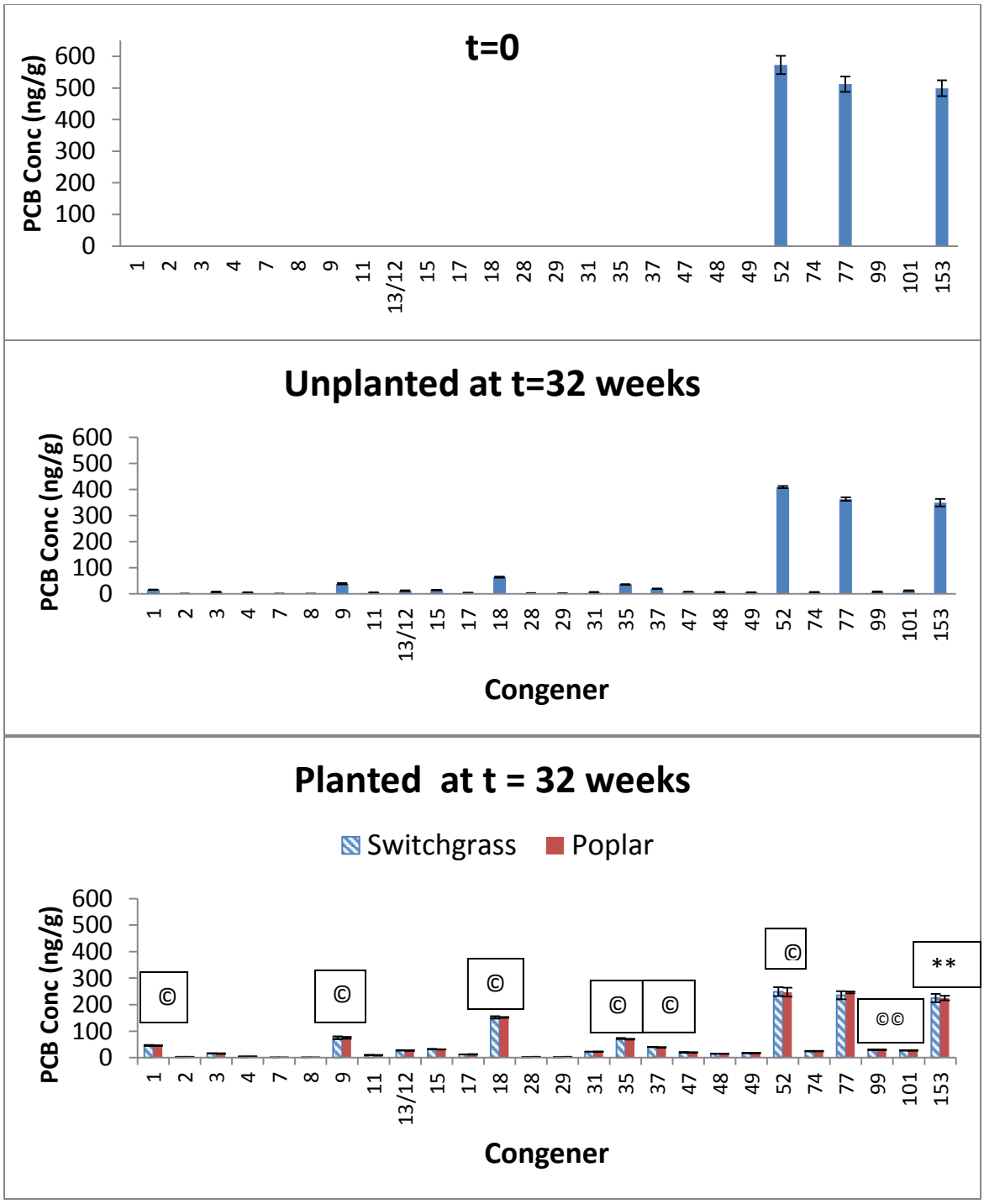

Figure 4.7: PCB congener profile for soil contaminated with a mixture of PCB 52, PCB77, and PCB 153 after 32 weeks of exposure. Profile at t=0 (top panel); profile for unplanted system (middle panel); profile for planted system (bottom panel). © = significant increase when compared to unplanted system ($p<0.05$). *= significant reduction in comparison to initial concentration ($p<0.05$).

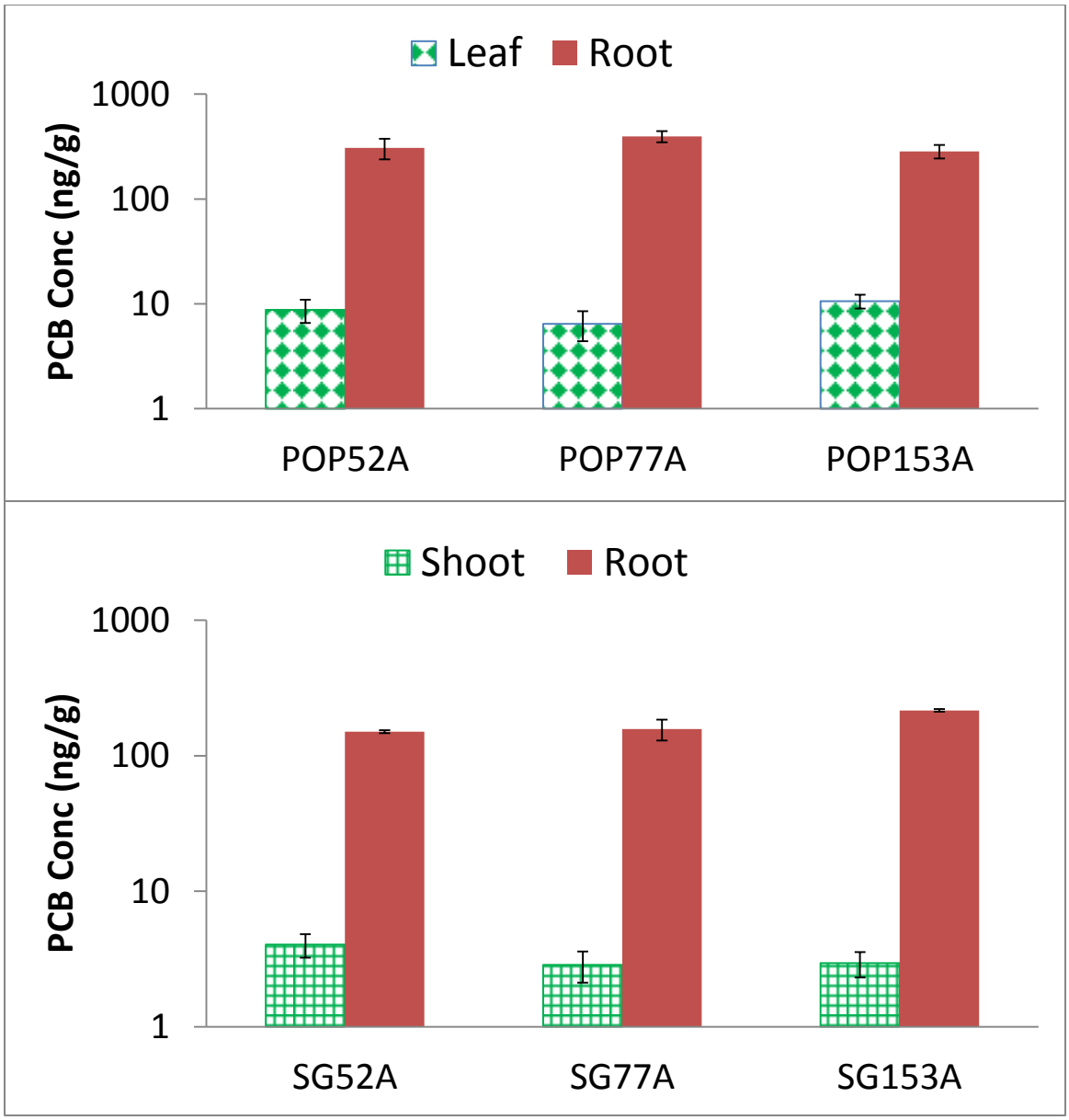

Figure 4.8: Distribution of parent compounds in plant material after 32 weeks of planting in PCB contaminated soil. Top chart is poplar; bottom chart is switchgrass. Error bars are 1 standard deviation and are based on triplicate measurements.

Table 4.1: Mass Compartmentalization of PCB in Poplar and Switchgrass Planted Systems

	Parent Compound In Soil (%)	**Transformation Products in Soil (%)**	**Roots (%)**	**Shoots (%)**
PCB52 Poplar	55.9	40.3	3.8	<0.1
PCB52 Switchgrass	48.3	48.8	2.9	<0.1
PCB77 Poplar	65.0	32.1	2.9	<0.1
PCB77 Switchgrass	56.4	38.3	5.3	<0.1
PCB153 Poplar	65.0	30.8	4.2	<0.1
PCB153 Switchgrass	61.8	35.0	3.2	<0.1

Table 4.2: Comparison of grass mediated dissipation of PCB in selected studies

STUDY	**Initial PCB**	**Time**	**Plant Species**	**Reduction**

	Concentration **(ng g^{-1})**	**frame**		**(%)**
Current Study	499±25 - 1120±58	224 days	Switchgrass	44-56
Dzantor et al. 2000	100,000	180 days	Switchgrass	52
Dzantor et al. 2000	100,000	180 days	Reedcanary grass	59
Dzantor et al. 2000	100,000	180 days	Tall fescue grass	32
Dzantor et al. 2000	100,000	180 days	Deertongue grass	30
Dzantor et al. 2001	50,000	100 days	Reedcanary grass	41
Chekol et al. 2004	100,000	120 days	Switchgrass	61-72
Chekol et al. 2004	100,000	120 days	Reedcanary grass	70-77
Chekol et al. 2004	100,000	120 days	Tall fescue grass	67
Chekol et al. 2004	100,000	120 days	Deertongue grass	72

CHAPTER 5 RHIZOSPHERE BIOTRANSFORMATION PRODUCTS OF PCB 52,77AND 153 AND DECHLORINATION PATHWAYS IN SOIL MICROCOSMS

5.1 Introduction

Since production began in the 1930's an estimated 1.5 million tons of polychlorinated biphenyls (PCB) have been manufactured (Abraham et al., 2002). PCBs are one the most persistent organic pollutants in the environment today (Bedard 2008, Borja et al., 2005, Kacalkova and Tlustos, 2011). They are consistently included in the top 10 most toxic compounds on the United States Agency for Toxic Substances and Disease Registry (ATSDR) list of priority pollutants (ATSDR,2011). Lower chlorinated congeners (1-4 chlorines) usually are more amenable to degradation under aerobic conditions, while highly chlorinated congeners are more readily degraded under anaerobic conditions by reductive dechlorination (Abraham 2002, Borja et al., 2005, Fagervold et al., 2005, Pieper, 2005). Research has exploited the use of sequential aerobic and anaerobic treatment to optimize reduction of PCB mixtures (Fathepure and Vogel, 1991, Master et al., 2002,), and there have been reports of plant assisted microbial degradation of PCBs both in the lab and under field conditions (Leigh et al. 2006, Mackova et al., 2007, Slater et al., 2011). However, these and other studies have mostly focused on dissipation of parent compounds from mixtures or on phytoextraction (Aslund Whitfield et al., 2007,2008, Chekol et al., 2004, Dzantor et al., 2000, 2002, Ficko et al., 2011, Javorska et al., 2011, Singer et al., 2003, White et al., 2006, Zeeb et al., 2006). To date, no study has attained a mass balance or elucidated the transformation products and pathways in the rhizosphere.

Rhizoremediation is an attractive alternative to the current treatment options for PCB remediation, but the intermediates and pathways of transformation must be known.

This study seeks to enhance rhizosphere biotransformation of selected PCB congeners by combining the benefits of plant assisted transformation with the manipulation of redox conditions in the rhizosphere to enhance dechlorination. Here, the rhizosphere is defined as the soil in the direct vicinity and influence of roots. Three representative congeners were used in single congener studies, and also combined in a mixture to determine if there are synergistic or antagonistic interactions and to better elucidate single congener pathways.

The transformation of PCB 52 is thought to be particularly difficult by the most common route of PCB degradation for lower chlorinated PCBs, i.e. 2,3 (5,6) dioxygenase attack and aerobic oxidation. The rationale behind this hypothesis is that in PCB 52, the normal sites of attack are occupied by chlorine substituents. Ortho substituents are particularly difficult to remove due to steric hindrances which mitigate against an enzymatic approach to those sites. However, it is possible that PCB 52 can be degraded by a 3, 4 (4,5) dioxygenase attack or even a monoxygenase mechanism (Bedard et al., 1987, Komancova et al.,2003). Therefore, PCB 52 (2, 2’5, 5’ tetrachlorobiphenyl) was selected because it is difficult to degrade, due to the chlorine substituents in the ortho positions on both rings.

Due to their toxicity, studies involving coplanar PCBs are particularly important from a public health perspective (Ah-receptors). One microcosm study investigated the ability of microorganisms obtained from PCB contaminated sediments to degrade five coplanar congeners (Zanaroli et al., 2006). One congener, PCB 77 (3, 3'4, 4' tetrachlorobiphenyl), showed 90% conversion, to products such as 3, 4 dichlorobiphenyl (PCB 12), 3, 4’dichlorobiphenyl (PCB 13) and 3, 3' dichlorobiphenyl (PCB11). Therefore, PCB 77 was selected because it is one of the most toxic PCB congeners (with coplanar properties exhibiting dioxin-like properties), and it should be readily degraded because it is devoid of chlorine substituents in the ortho position.

The other congener selected was PCB 153 (2, 2', 4, 4', 5, 5'hexachlorobiphenyl), which has six chlorine moieties and a tendency to bioaccumulate in humans. In addition, it has ortho substituents on both rings, making it not readily amenable to microbial degradation. However, microbial degradation of PCB 153 has been observed previously with *Burkholderia* LB 400 and *Alcaligenes eutrophus (now Ralstonia eutropha)* H850 (Bedard et al., 1986, Leigh et al. 2006). The plant species chosen for this study were switchgrass (*Panicum virgatum)* and hybrid poplar (*Populus deltoids x nigra* DN34)**.** Switchgrass is perennial, deep rooted, hardy, widespread, and possesses a C4 mechanism for photosynthesis. Poplar is deep rooted, a model plant genetically and widely used in phytoremediation applications, and it has been shown to effectively stimulate the biotransformation of several xenobiotic compounds (Kacalkova and Tlustos, 2011,Liu et al., 2009, Zhai et al., 2010).

The objective of this study is to identify rhizospheric biotransformation products of the tested PCB congeners and determine transformation pathways based on the identified products. Furthermore the transformation of PCBs is investigated by subjecting the rhizosphere to intermittent cycles of flooding. It is hypothesize that anoxic or anaerobic conditions induced by flooding will facilitate PCB dechlorination. Intermittent flooding is necessary to produce the redox conditions required for reductive dechlorination, without compromising the health of the plants.

This study is the first to report transformation products and corresponding pathways in the rhizosphere of planted microcosms cycled at low redox conditions to enhance reductive dechlorination. It demonstrates dechlorination of PCB congeners under marginally aerobic conditions and provides closure or near-closure of the mass balance based on these dechlorination products alone.

5.2 Materials and Methods

5.2.1 Experimental Set up

Silty loam soil from Amana colonies IA, was passed through a 60 mesh sieve and artificially contaminated with tetra chlorinated PCB 52, PCB77 and hexa chlorinated PCB 153(99% pure) separately and as a mixture of the three congeners. Prior to spiking the soil, the PCB congeners were dissolved in five liters of hexane and then added to the soil for a target concentration of 1000 ngg^{-1} (3.42 $nmolg^{-1}$ for PCB 52 and PCB 77 and 2.77 $nmolg^{-1}$ for PCB 153) in the single congener treatments. The target concentration for each congener in the mixture was 500 ngg^{-1}(1.71 $nmolg^{-1}$ for both PCB 52 and PCB 77 and 1.39 $nmolg^{-1}$ for PCB 153). The soil was homogenized using the quartering technique. It consisted of dividing the soil into four quadrants on a quartering canvas, and making several diagonal trajectories to mix the soil components together. Twenty passes (diagonal trajectories) were made to homogenize the soil thoroughly. To account for heterogeneity, 20 subsets of soil were collected from different locations, homogenized and analyzed in triplicate. Triplicate concentration measurements were performed to establish the initial concentration, and it was compared to the target concentration for the purpose of quality assurance. The contaminated soil was aged for two months at 25^{o}C in sealed tubs to reduce bioavailability and to ensure conditions more representative of field sites (Alexander et al., 2000, Thompson et al., 1998, White et al., 2006). The aged, contaminated soils were then planted separately with switchgrass (*Panicum virgatum)* seeds from Adams-Briscoe Seed Co. (Jackson, GA) and with 22.9 cm poplar (*Populus deltoids x nigra* DN34) cuttings from Segal Ranch Hybrid Poplars Nursery (Grand View, WA).

Treatments consisting of switchgrass and poplar planted in uncontaminated soil was used as blanks and functioned as volatilization controls. Plants were grown under a 16 hour light/8 hour dark photo period with a light intensity of 200 µmol $m^{-2}s^{-1}$ and at a temperature of 25^{o}C. Rubbermaid^{TM}, plastic shoe boxes (33.8 cm x 21.6 cm x 11.9 cm (LxWxD) with 1.9 cm holes drilled in the covers, lined with aluminum foil and containing 2500 g of soil were used as reactors. Holes (1.9 cm) were bored in covers of the reactors to facilitate planting of the poplar cuttings, growth of the mature grass and measurement of redox potential and dissolved oxygen concentration during periods of flooding (low redox conditions). The use of covered reactors with holes drilled in the covers also reduced the potential for loss of PCBs by volatilization. The plants were allowed to establish themselves for four (4) weeks. After 4 weeks, the treatments were subjected to alternate cycles of seven (7) days flooding (to induce anaerobic conditions) and seven (7) days of no flooding (aerobic conditions). To achieve flooding, three liters of deionized water was initially added to the reactors and supplemented as dictated by transpiration rates. Transpiration typically functioned as an efficient mechanism for water withdrawal, so there was no need to withdraw excess water at the end of the flooding cycle. The experiment was conducted for thirty two (32) weeks.

Twelve (12), five (5) g samples of soil were collected randomly from each treatment using a Lock and Hold^{TM} syringe (Ben Meadows Company WI, USA) and homogenized prior to analysis. At the end of exposure (32 weeks) soil and plant materials were sampled and analyzed for PCB content using GC/MS/MS triple quadropole mass spectrometry (Agilent Technologies 6890N GC with an Agilent 7683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA)). PCBs 14, d65, and 166 were used as surrogate standards and PCB 204 was the internal standard. The experimental set up and analyses were carried out in triplicate. A photograph and a schematic representation of the reactors used in the experimental setup can be seen in the Appendix (Figure A10).

5.2.2 PCB Extraction

Denaturation and extraction of PCB in soil and plant material was conducted by adding three (3) milliliters per gram of a 1:1 hexane/acetone mixture to 5 grams of grounded homogenized soil and sonicating for 1 hour. Prior to sonification, the samples were spiked with 50 ng of PCB14 (3,5-dichlorobiphenyl), deuterated PCB65 (2,3,5,6-tetrachlorobiphenyl) and PCB166 (2,3,4,4′,5,6-hexachlorobiphenyl) (Cambridge Isotope Laboratories, Inc.), which were used as surrogate standards. Surrogate recoveries ranged from 87.24±3.09% to 94.41±4.37% for PCB 14, 95.17±4.44% to 105.07±4.23% for PCB 65 and 93.19±2.49% to 106.37±2.46% for PCB 166.

The sonicated material was centrifuged at 3000 rpm for 5 minutes, after which the supernatant was transferred to a fresh vial. A second extraction was performed and the supernatants combined. The combined supernatant was evaporated to dryness using rotary evaporation and the solvent changed to hexane. Any loss by rotary evaporation was accounted for using the surrogate standard recoveries. Removal of lipids and other polar substances was achieved by double extraction with concentrated sulfuric acid (H_2SO_4) and hexane. This hexane extract was concentrated to approximately 0.5 ml under a gentle stream of nitrogen. The concentrate was eluted with 10 ml of hexane through a filter consisting of 0.1g of silica (70−230 mesh, Fisher Scientific, Inc.), 0.1g of anhydrous sodium sulfate (Na_2SO_4) and 0.9 g silica gel acidified with H_2SO_4 (2parts silica:1part H_2SO_4). The eluent was concentrated and PCB204 (2,2′,3,4,4′,5,6,6′ octachlorobiphenyl) was added as an internal standard to facilitate quantification before analysis by GC/MS/MS triple quadropole mass spectrometry (Agilent Technologies 6890N GC with an Agilent 7683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA)). The gas chromatogram (GC) was fitted with a Supelco SBP-Octyl capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness) with

helium as carrier gas at a constant flow rate of 0.8 ml min^{-1}. The GC operating conditions were as follows: injector temperature 270 °C, interface temperature 290 °C, initial temperature 75 °C, initial time 2 min. The GC temperature program was 75 to 150 °C at 15 °C min^{-1}, 150 to 290 °C at 2.5 °C min^{-1}, and final time 1 min. Identification and quantification of PCB congeners in the samples was performed using a calibration standard consisting of all 209 congeners and is described elsewhere (Hu et al. 2010).

5.2.3 Measurement of Redox Conditions

Dissolved oxygen (DO) concentrations were measured in the saturated soilwater with a Hach Sension 156 meter and probe, while redox measurements were obtained using a Hanna Instruments Redox/pH combination meter. The DO probe was calibrated in water saturated air according to the manufacturer's instructions. The accuracy was also checked by measuring and comparing the DO concentration in water at a specific temperature with a known saturation concentration. In addition, the probe was chemically zeroed to compensate for positive error readings of 0.02-0.05 mgl^{-1} which is possible when taking measurements below 1 mgl^{-1}. The accuracy of the oxidation–reduction potential (ORP) meter was checked with 475 mv and 229 mv redox buffers (Aqua Solutions).

5.2.4 Statistical Analysis

Microsoft Excel Analysis Toolpak's ANOVA analysis and Students' t-test were used for statistical testing. The significance level of 0.05 was utilized to indicate whether the treatments were significantly different than the controls.

5.3 Results

5.3.1 Dissolved Oxygen and Redox Measurement

Dissolved oxygen measurements indicated that the water column in the planted systems was hypoxic during flooding, but oxygen was not sufficiently depleted to produce anaerobic conditions (Appendix, Figure A11). The dissolved oxygen measurements in the planted systems ranged from 0.45±0.17 mgl^{-1} to 0.52±0.15 mgl^{-1}. The unplanted systems, which ranged from 0.15±0.11 mgl^{-1} to 0.21±0.17 mgl^{-1}, had lower dissolved oxygen concentration than the planted systems and bordered on being anoxic. Soil redox measurements confirmed the absence of anaerobic or even anoxic conditions in the planted systems (Appendix, Figures A12-A14). On the other hand, based on the soil redox measurements, anoxic conditions, specifically sulfidogenic conditions (0 to -200mv) were clearly present in the unplanted systems (Appendix, Figures A11-A14). The absence of anoxic conditions in the planted systems was probably due to plant transfer of oxygen to the rhizosphere during photosynthesis. This hypothesis is supported by the fact that the unplanted systems showed greater propensity towards sulfidogenic conditions. Although it would have been desirable to have at least a methanogenic environment to facilitate reductive dechlorination, there have been reports of reductive dechlorination also occurring under sulfidogenic conditions (Fava et al., 2003, Kuo et al., 1999).

5.3.2 PCB Dissipation: Exposure to a Mixture of PCB 52, PCB77 and PCB 153

Table 5.1 shows the results for a mixture of PCB 52, PCB 77, and PCB 153 spiked and aged into soil and planted with switchgrass and poplar after 32 weeks of incubation. It also shows the results when the soil was spiked with the individual congeners. In the mixture, the targeted initial PCB 52 concentration was 500 ng g^{-1} (1.71 $nmolg^{-1}$) while the measured concentration was 573± 29 ng g^{-1} (1.96± 0.10 $nmolg^{-1}$); the difference likely due to the heterogeneity of the soil matrix despite efforts to obtain a homogenous mixture by repeatedly quartering the contaminated soil. A 57% decrease in PCB 52 was observed for both the switchgrass and poplar planted systems after 32 weeks of incubation (Table 5.1). This represented a statistically significant decline when compared to the initial PCB 52 concentration in the mixture ($p<0.05$).

For PCB 77, while the targeted initial concentration was 500 ng g^{-1} (1.71 $nmolg^{-1}$), the heterogeneity of the soil matrix resulted in a measured intial concentration of 512 ± 24 ng g^{-1} (1.75± 0.08 $nmolg^{-1}$). With respect to PCB 77 dissipation, the switchgrass-planted system containing the mixture recorded a 54% decline while the poplar-planted system was quite similar with a 53% diminution (Table 5.1) ($p<0.05$). Compared to the initial PCB 77 concentration, both the planted and unplanted systems had significant declines ($p<0.05$).

The targeted PCB 153 concentration in the mixture was 500 ng g^{-1} (1.39 $nmolg^{-1}$) while the measured initial concentration was 499 ± 25 ng g^{-1} (1.38± 0.07 $nmolg^{-1}$). There were simlar decreases in PCB 153 concentration in both the switchgrass and poplar planted systems. Both planted systems showed a dimunition of 55% in soil PCB 153 after 32 weeks exposure (Table 5.1).

Figure 5.1 shows the congener profile for both the planted and unplanted systems in the soil contaminated with the mixture after 32 weeks of incubation. The congener profile at the start of the experiment is also depicted. It illustrates that compared to the soil profile at the start, several transformation products from the degradation of PCB 52, PCB 77 and PCB 153 were obtained in both the planted and unplanted systems. For the unplanted systems, the yield of any single degradation product did not surpass 10% of the initial total PCB concentration. In contrast, significantly higher yields ($p<0.05$) of similar transformation products were observed within the planted systems. PCBs 18 (2,2',5 trichlorobiphenyl) and 9 (2,5 dichlorobiphenyl) which are potential degradation products of PCB 52 and 153 showed prominent increases in concentration. For PCB 77, the trichlorinated PCB 37 (3,4,4' trichlorobiphenyl) and PCB 35(3,3',4 trichlorobiphenyl) were the major products. Aerial deposition, from laboratory air to the soils, as a route for the appearance of transformation products was ruled-out because of the relatively high concentrations detected, and because the congener profile for the unspiked treatment was quite different from that observed in the spiked treatments (Appendix Figure A15).

5.3.3 PCB Dissipation: Single Congener Exposure to Plants with PCB 52

The result for the decrease in PCB 52 concentration in the single congener exposure is shown in Table 5.1. The targeted initial concentration for PCB 52 in the single congener exposure experiment was 1000 ng g^{-1} (3.42 $nmolg^{-1}$), however the measured initial concentration was 1120 ±58 ng g^{-1} (3.84± 0.20 $nmolg^{-1}$). The difference was likely due to the difficulty of accurately sampling the heterogeneous soil matrix. After 32 weeks of exposure, there was a 63% decline in the soil PCB 52 concentration for the switchgrass planted system , and a 54 % decrease for the poplar planted system.

Overall, this represented a significant decrease in PCB 52 concentration in both the switchgrass and poplar planted systems, when compared to the initial concentration ($p<0.05$).

Figure 5.2 shows the congener profile in the soil contaminated with PCB 52 as the single congener for both the planted and unplanted sytems after 32 weeks of incubation. It also depicts the soil congener profile at the start of the experiment (t=0). The only congener that was detected at the start of the experiment was PCB 52. However, there were significant increases in the concentration of various transformation products in both the switchgrass and poplar planted systems at the end of exposure. For both planted systems the yields of comparable transformation products in the single congener (PCB 52) treatment were significantly higher than what was obtained in the mixture ($p<0.05$). As with the experiment containing a mixture of congeners, the yields of PCB 52 transformation products in the single congener treatment were higher in the planted sytems than the unplanted system ($p<0.05$). Both the switchgrass and poplar planted systems mirrorred each other in terms of the mass conversion to major transformation products.

For example, in the switchgrass planted system, PCB 18 (2,2',5 trichlorobiphenyl) was the major degradation product accounting for approximately 34% of the remaining PCB concentration in the soil with PCB 9 (2, 5 dichlorobiphenyl) and PCB-1 (2 chlorobiphenyl) accounting for 18% and 13%, respectively. Analagous to the switchgrass planted soil, PCB 18 was also the major degradation product in the poplar planted soil accounting for approximately 30 % of the remaining PCBs with PCB 9, and PCB 1 accounting for 14% and 11%, respectively. Based on the transformation products observed in the single congener exposure, a degradation pathway for the rhizospheric transformation of PCB 52 is proposed in Figure 5.3.

5.3.4 PCB Dissipation: Single Congener Exposure with PCB 77

The target initial concentration for PCB 77 as the sole contaminant was 1000 ng g^{-1} (3.42 $nmolg^{-1}$) and the measured concentration was 1012 ± 3 ng g^{-1} (3.47±0.01 $nmolg^{-1}$). The soil PCB 77 concentration in the switchgrass planted system diminished by 54 % while the poplar planted system recorded a 47% decline (Table 5.1). Despite the significant difference between the decrease observed in the poplar and switchgrass planted systems ($p< 0.05$), both showed a signifcant loss when compared to the the initial PCB concentration ($p<0.05$).

Compared to the congener profile at the beginning, there were several transformation products of PCB 77 in the soil rhizosphere after 32 weeks of exposure (Figure 5.4). The yield of the major degradation products was significantly higher in the single congener treatment than the mixture for both the planted and unplanted systems ($p<0.05$). Similar to the trend observed in other exposures discussed thus far, the yields obtained with the planted systems were much higher than with the unplanted system, at least 10 times as much. The major products of dechlorination were PCB 35 (3,3',4 trichlorobiphenyl) and PCB 37 (3,3,4' trichlorobiphenyl). PCB 35 accounted for 19 % of the final PCB concentration in the switchgrass planted soil and 17% in the poplar planted system. PCB 37 was responsible for 11% of the final soil PCB concentration in both the switchgrass and poplar planted systems. Figure 5.5 shows potential degradation pathways through PCB 35 and PCB 37 based on the transformation products observed.

5.3.5 PCB Dissipation: Single Congener Exposure with PCB 153

The targeted initial concentration for PCB 153 in the single congener exposure experiment was 1000 ng g^{-1} (2.77 $nmolg^{-1}$), and the measured initial concentration was nearly identical at 1033 ±28 ng g^{-1} (2.86.±0.01 $nmolg^{-1}$). The soil PCB 153 concentration diminished by 51% reduction in the switchgrass planted treatment and declined by 50% in the poplar planted treatment (Table 5.1). The dimunitions in both switchgrass and poplar planted systems were statistically significant compared to the the initial PCB concentration ($p<0.05$).

The congener profiles for the soil contaminated with PCB 153 as the sole contaminant and planted with switchgrass and poplar after 32 weeks of incubation are shown in Figure 5.6. Also shown is the congener profile at the start of the exposure, together with the congener profile for the unplanted system (Figure 5.6). Compared to the congener profile at the start of exposure, a wide range of degradation products were obtained spanning from pentachlorinated compounds to dichlorinated congeners in both the planted and unplanted systems (Figure 5.6). The yields were small, individually, and did not exceed 10% for any one congener. However, the summation of all the transformation products accounted for 99% of the total PCB 153 converted. The yields of pentachlorinated compounds PCB 101 (2,2',4,5,5'pentachlorobiphenyl) and PCB 99 (2,2',4,4',5pentachlorobiphenyl) and tetrachlorinated PCB 74 were higher in the planted systems than in the unplanted system. Based on the the degradation products observed, two potential pathways for PCB 153 transformation are depicted in Figures 5.7 and 5.8. The first (Figure 5.7) shows the route through PCB 101 and the second (Figure 5.8) shows the transformation via the intermediary PCB 99.

5.4 Discussion

Significantly higher reductions in parent PCB congeners were obtained with the planted systems when compared to unplanted systems. This is demonstrated by data in Figures 5.1,5.2, 5.4 and 5.6. It is likely the result of rhizosphere stimulation by plant exudates (Jordahl et al.,1997). The most important role for the plants in PCB phytoremediation is in creating an enabling environment for increased microbial activity through the production of food and biostimulants which serve as electron donors for the microbes. Plant exudates such as flavonoids and terpenes have been shown to stimulate growth of competent microrganisms and have increased PCB degradation by organisms in the lab and the field (Donnelly et al.,1994, Hernandez et al.,1997 Ionescu et al., 2009, Leigh et al., 2002,2006, Slater et al., 2011). It is possible that the plant exudates derive their abilty to stimulate microbial metabolism of PCBs due to structural similarities with biphenyl, a well known inducer of microbial PCB degradation. It is likely that these exudates faciltate cometabolic transformation of PCBs or act as a source of carbon for growth.

These reductions were largely a consequence of enhanced rhizosphere transformation, rather than a direct function of plant uptake and biotransformation. The reason for this conclusion is two fold. Based on the log K_{ow} and water solubility of these congeners, they will not be taken-up by plants and enter the transpiration stream (Liu et al., 2008, 2009, Schnoor et al.,1995). Second, on a mass basis, all the parent PCBs detected in the plant material resided in the roots (Table 5.2) and were not translocated to shoots, and at least 95% of the PCBs detected at the end of exposure was resident in the soil (Table 5.2).

Transformation products seen in the planted systems are similar to those in the unplanted systems (Figures 5.1,5.2,5.4&5.6). This suggests that the microbial reductive

community is similar in both systems but the activity is greater in the planted systems. Exposure to individual PCB congeners and an Aroclor mixture resulted in a greater level of potential PCB degrading microorganisms and a greater abundance of BPH genes in an artificial soil (Correa et al.,2010).

The transformation pathways are consistent with reductive dechlorination seen in other studies. Two different cultures of *Dehalococcoides* were able to degrade PCB 153 to PCB 47 and PCB 52 in Aroclor 1260 mixture going through PCB 99 and 101 respectively (Adrian et al., 2009,Bedard et al., 2007). Regarding PCB transformation in the literature, dechlorination of PCB 77 to biphenyl was observed in one study (Rhee et al., 2003). In terms of degradation products, Kuo and colleagues reported that there was active dechlorination of PCB 77 in sediments, but only saw dechlorination to the dichlorinated PCB 11 and nothing further (Kuo et al., 1999). Looking at the congener profiles for PCB 77 in this study, both in the mixture and as a single congener, reduction to monochlorinated PCB 3 was evident, which extends the pathway further than reported by Kuo and collaborators. Kuo et al. (1999) did not report the precursors of PCB 11, whereas in the current study, the progression from tri- to di- and to monochlorinated PCBs was clearly evident in PCB 77 degradation.

For PCB 77 degradation products, Zanaroli and coworkers reported the presence of PCB 11, 12, and 13, but did not report any trichlorinated precursors in the reductive dechlorination process (Zanaroli et al., 2006). However, these trichlorinated intermediates may have been rapidly degraded to less chlorinated products observed in the study. PCBs 11, 12 and 13 were also detected in this study as transformation products of PCB 77, including detection of some direct precursors. However, PCBs 12 and 13 were co-eluted in this study; therefore it is difficult to ascribe quantifiable proportions to each. The high conversion rate (90%) of the parent compound by Zanaroli and colleagues was much higher than observed in this study, but it is quite possibly that these high rates

were a consequence of the methanogenic conditions under which they were working in estuarine sediments from a PCB-polluted environment.

While identification of the soil microbial community was not an objective of this study, the importance of understanding the role of the microbial community in the rhizoheric transformation of the congeners tested is fully appreciated and is the subject of another ongoing study.

The ability to detect aerobic products was limited by use of GC/MS/MS, and as such, polar metabolites (hydroxyl-PCBs, ring cleavage products) or mineralization to carbon dioxide and HCl could not be measured. But the fact that the reductive dechlorination transformation products summed to approximately the total mole fraction (lost) suggests that these aerobic processes (oxidation, mineralization) were not large. In spite of the soil remaining marginally aerobic during flooding, it is possible that highly reduced microzones within soil particles could still exist and provide the opportunity for reductive dechlorination to take place. Typically, ring cleavage and mineralization would require a different consortium of aerobic organisms with dioxygenase enzymes, to be predominant. Nonetheless, dechlorination seems to have predominated in these experiments, and dechlorination is a vital and necessary first step in the achievement of ring cleavage and mineralization.

Reductive dechlorination reactions were consistent with the products observed even though strict anaerobic conditions did not prevail. Microzones of low redox conditions in the rhizosphere are likely responsible for the dechlorination which occurred. As it stands, the results from this experiment show clearly that reductive dechlorination can occur even where soils are marginally aerobic in the root zones of plants. There were few differences in rhizosphere transformation rates or pathways between the two plant species examined here (*Populus* spp. and *Panicum virgatum*). But there was a clear and statistically significant tendency for planted microcosms to achieve more substantial biotransformation than unplanted controls, although the pathways were similar in both

systems. In addition, the transformation seen in the mixture was explained by the individual congener transformation giving credence to the proposed pathways.

Table 5.1: Summary Table for Transformation of Parent PCB in Switchgrass and Poplar-Planted Soil after 32 weeks

EXPOSURE **Initial Conc. (ng g^{-1})** [nmolg^{-1}]	**SYSTEM**	**DECREASE** PCB 52 (%)	**DECREASE** PCB 77 (%)	**DECREASE** PCB153 (%)
MIXTURE (PCB52,77,153) **PCB 52 – 573 ±29** [1.96± 0.10] **PCB 77 – 512 ±24** [1.96± 0.10] **PCB 153 – 499 ±26** [1.38± 0.07]	Unplanted	29.8 ±1.8	33.5 ±1.5	35.1 ±1.9
	Switchgrass	57.2 ±0.6	54.3 ±0.7	55.2 ±0.2
	Poplar	57.5 ±3.2	52.6 ±0.8	55.4 ±0.8
SINGLE CONGENER PCB 52 – 1120 ±58 [3.84± 0.20]	Unplanted	29.1 ±3.9	-	-
	Switchgrass	63.1 ±0.5	-	-
	Poplar	54.4 ±2.2	-	-
SINGLE CONGENER PCB 77 – 1012 ±3 [3.47±0.01]	Unplanted		20.1 ±2.0	
	Switchgrass	-	54.2 ±1.1	-
	Poplar	-	47.3 ±0.8	-
SINGLE CONGENER PCB 153 – 1033 ±28 [2.86.±0.01]	Unplanted	-	-	30.1 ±1.2
	Switchgrass	-	-	51.5 ±0.4
	Poplar	-	-	50.4 ±0.4

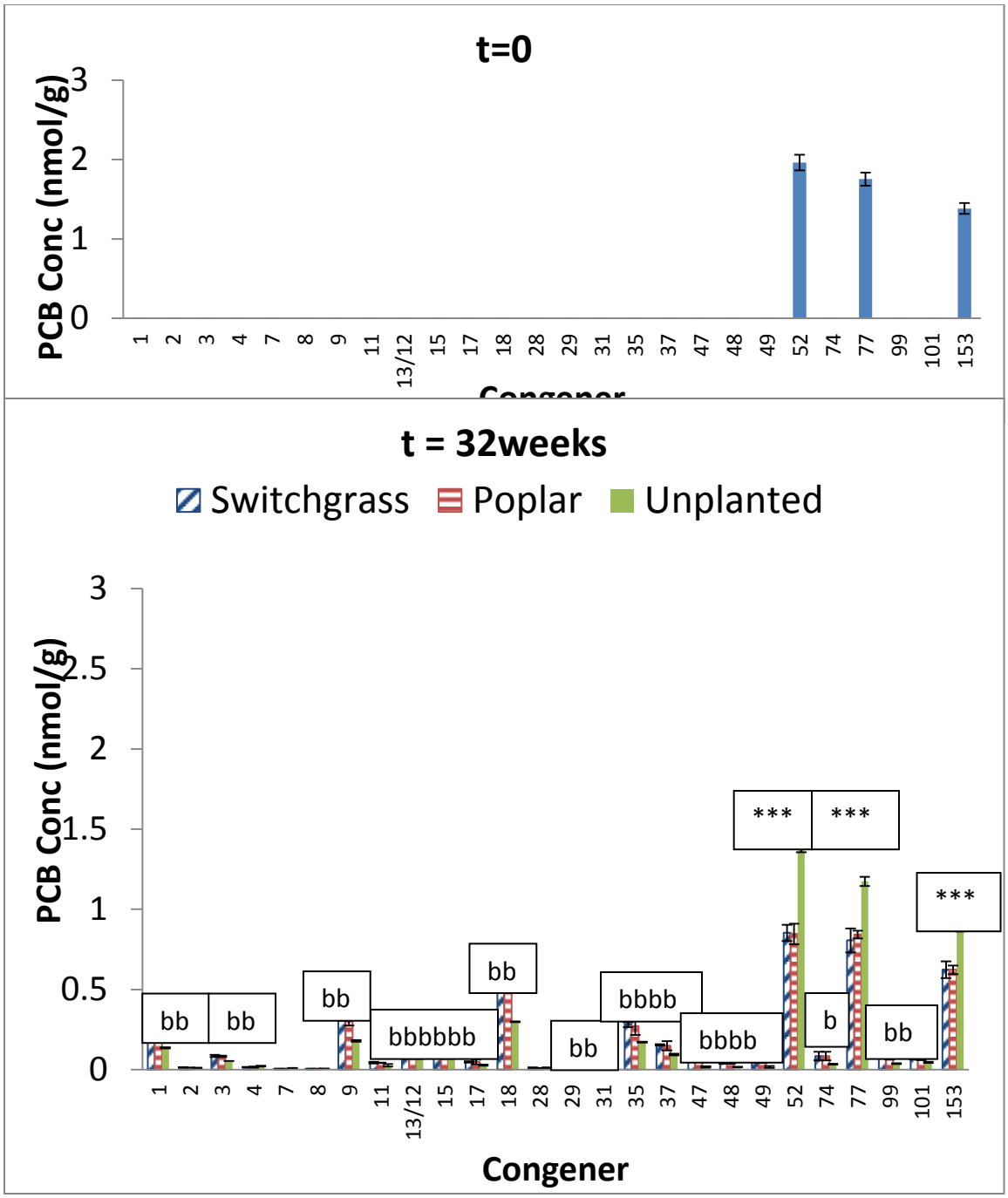

Figure 5.1: PCB congener profile for soil contaminated with mixture of PCB 52, PCB77, and PCB 153, and undergoing alternate cycles of flooding and no flooding after 32 weeks of exposure. b = significant increase in concentration for the planted system when compared to unplanted system ($p<0.05$). *= significant decrease in parent PCB concentration in comparison to initial concentration ($p<0.05$). Profile at t=0 (top panel); profile for planted and unplanted systems (bottom panel). Error bars are 1 standard deviation and are based on triplicate measurements.

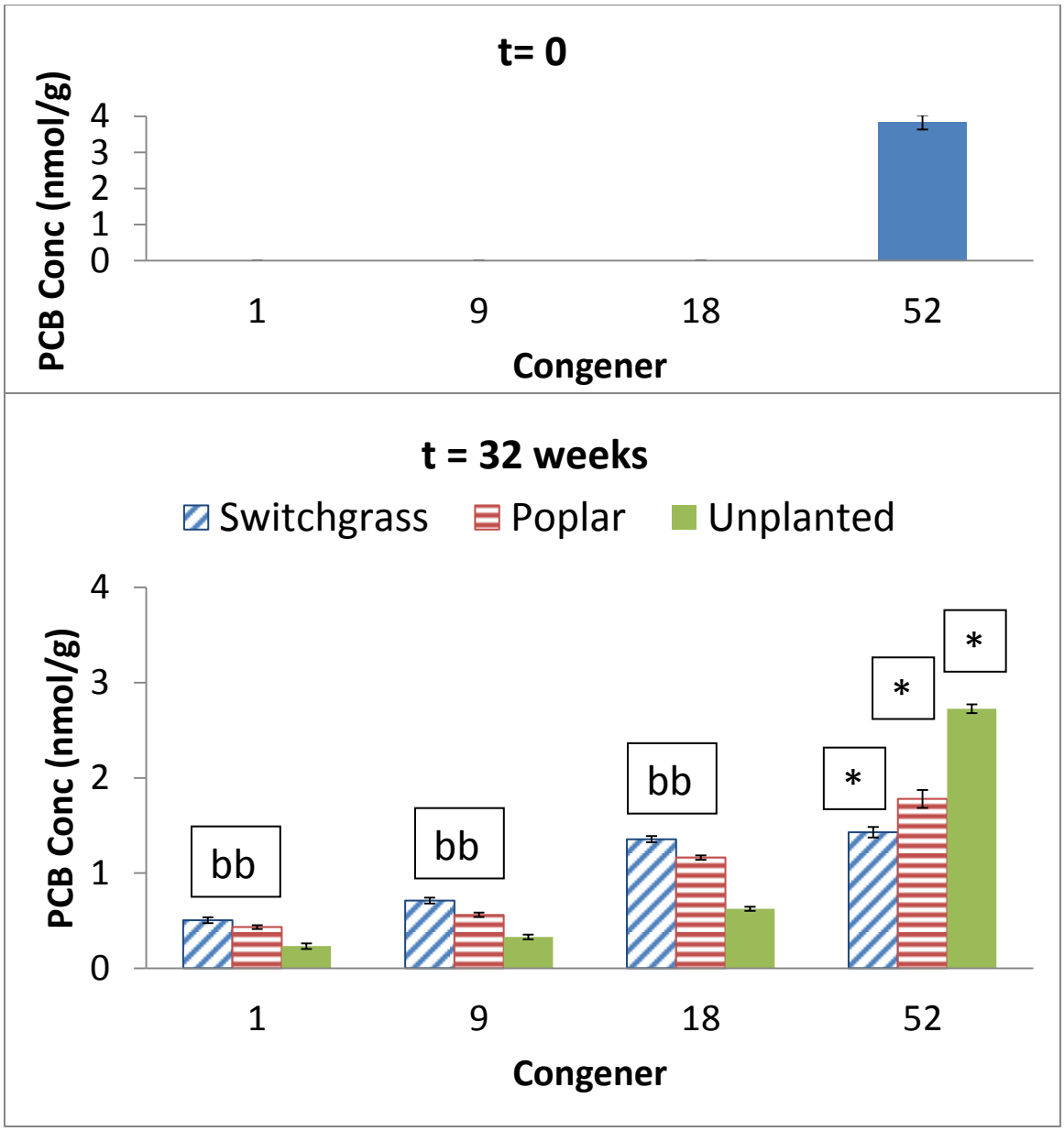

Figure 5.2: PCB congener profile for soil contaminated with PCB 52 and undergoing alternate cycles of flooding and no flooding after 32 weeks of exposure. b = significant increase in concentration for the planted system when compared to unplanted system ($p<0.05$). *= significant decrease in parent PCB concentration in comparison to initial concentration ($p<0.05$). Profile at t=0 (top panel); profile for planted and unplanted systems (bottom panel). Error bars are 1 standard deviation and are based on triplicate measurements

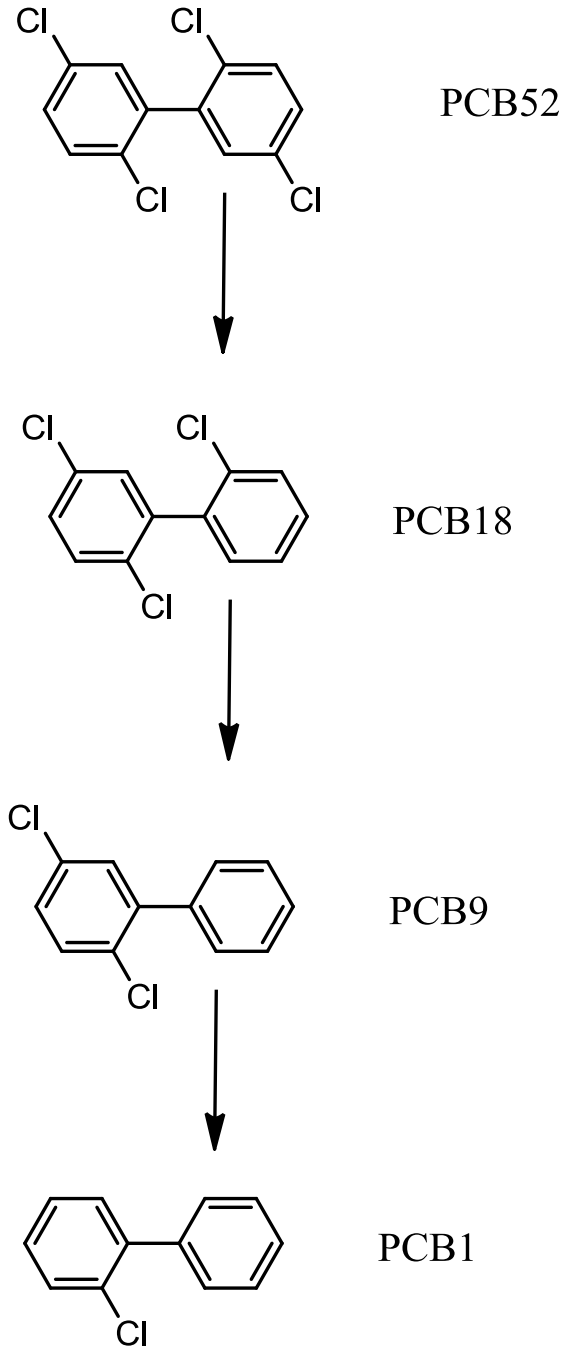

Figure 5.3: Potential pathway for PCB 52 degradation based on transformation products observed.

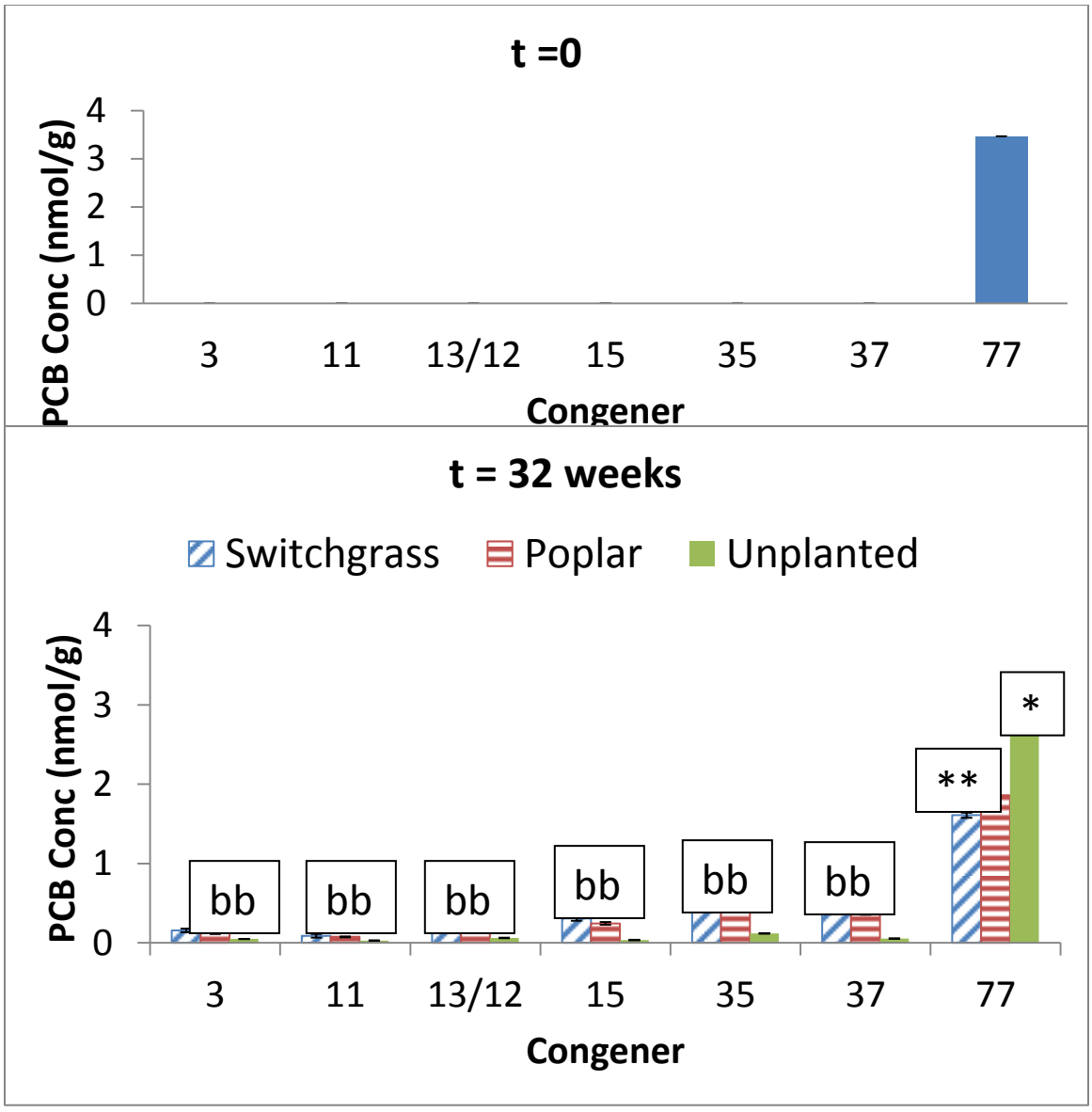

Figure 5.4: PCB congener profile for soil contaminated with PCB 77 and undergoing alternate cycles of flooding and no flooding after 32 weeks of exposure. b = significant increase in concentration for the planted system when compared to unplanted system ($p<0.05$). *= significant decrease in parent PCB concentration in comparison to initial concentration ($p<0.05$). Profile at t=0 (top panel); profile for planted and unplanted systems (bottom panel). Error bars are 1 standard deviation and are based on triplicate measurements.

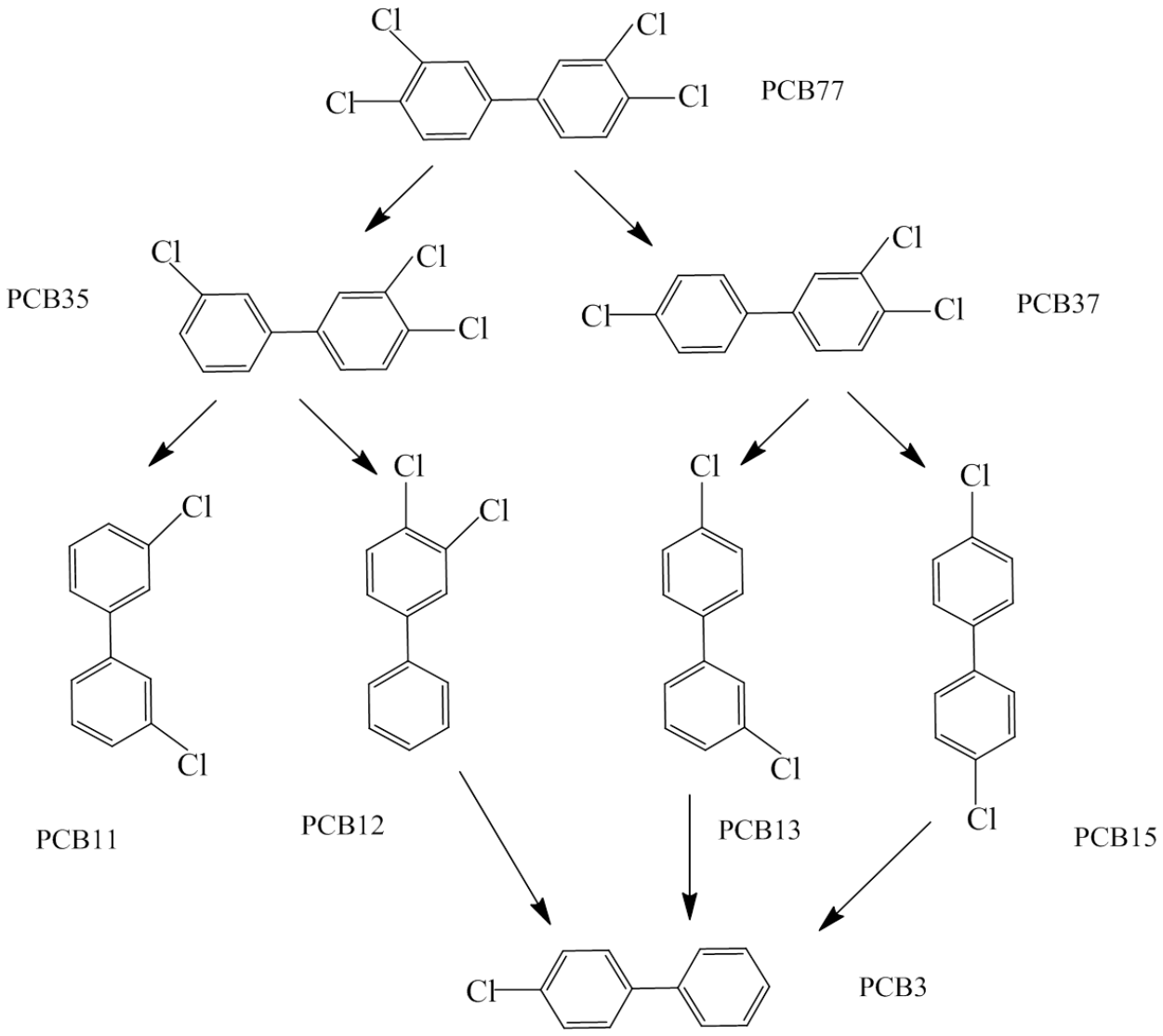

Figure 5.5: Potential pathway for PCB 77 degradation based on transformation products observed.

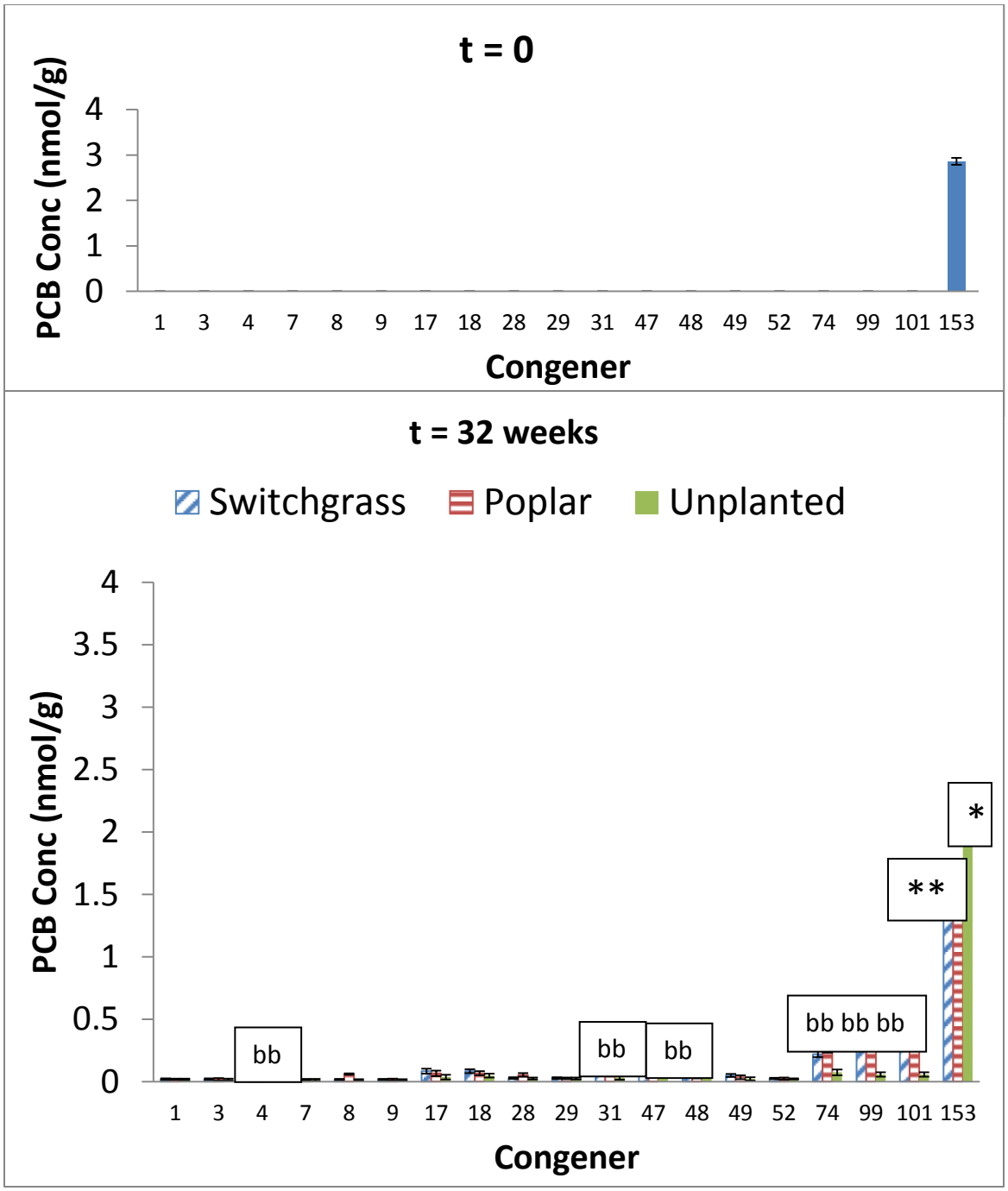

Figure 5.6: PCB congener profile for soil contaminated with PCB 153 and undergoing alternate cycles of flooding and no flooding after 32 weeks of exposure. b = significant increase in concentration for the planted system when compared to unplanted system ($p<0.05$). *= significant decrease in parent PCB concentration in comparison to initial concentration ($p<0.05$). Profile at t=0 (top panel); profile for planted and unplanted systems (bottom panel). Error bars are 1 standard deviation and are based on triplicate measurements.

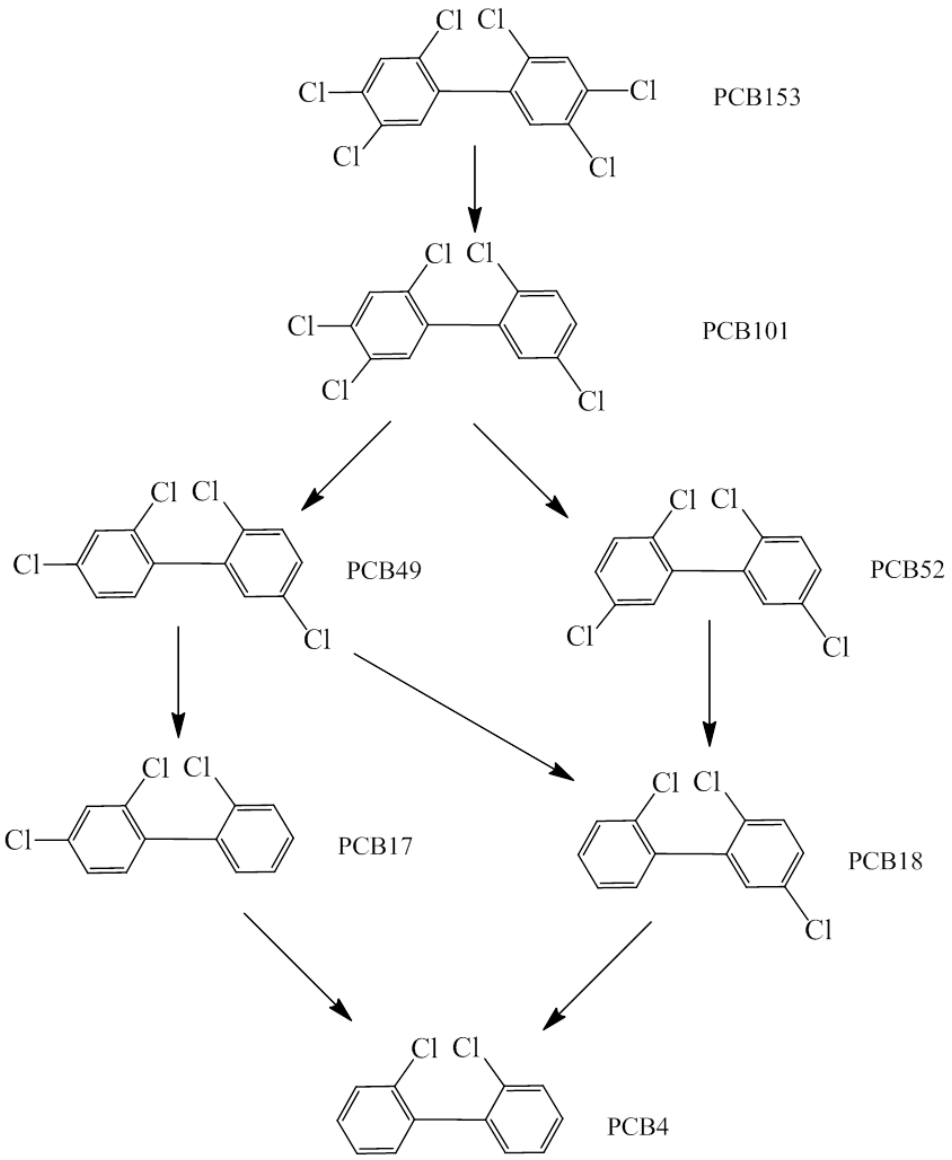

Figure 5.7: Some products observed with PCB 153 transformation and potential degradation pathway through the PCB 101 intermediary.

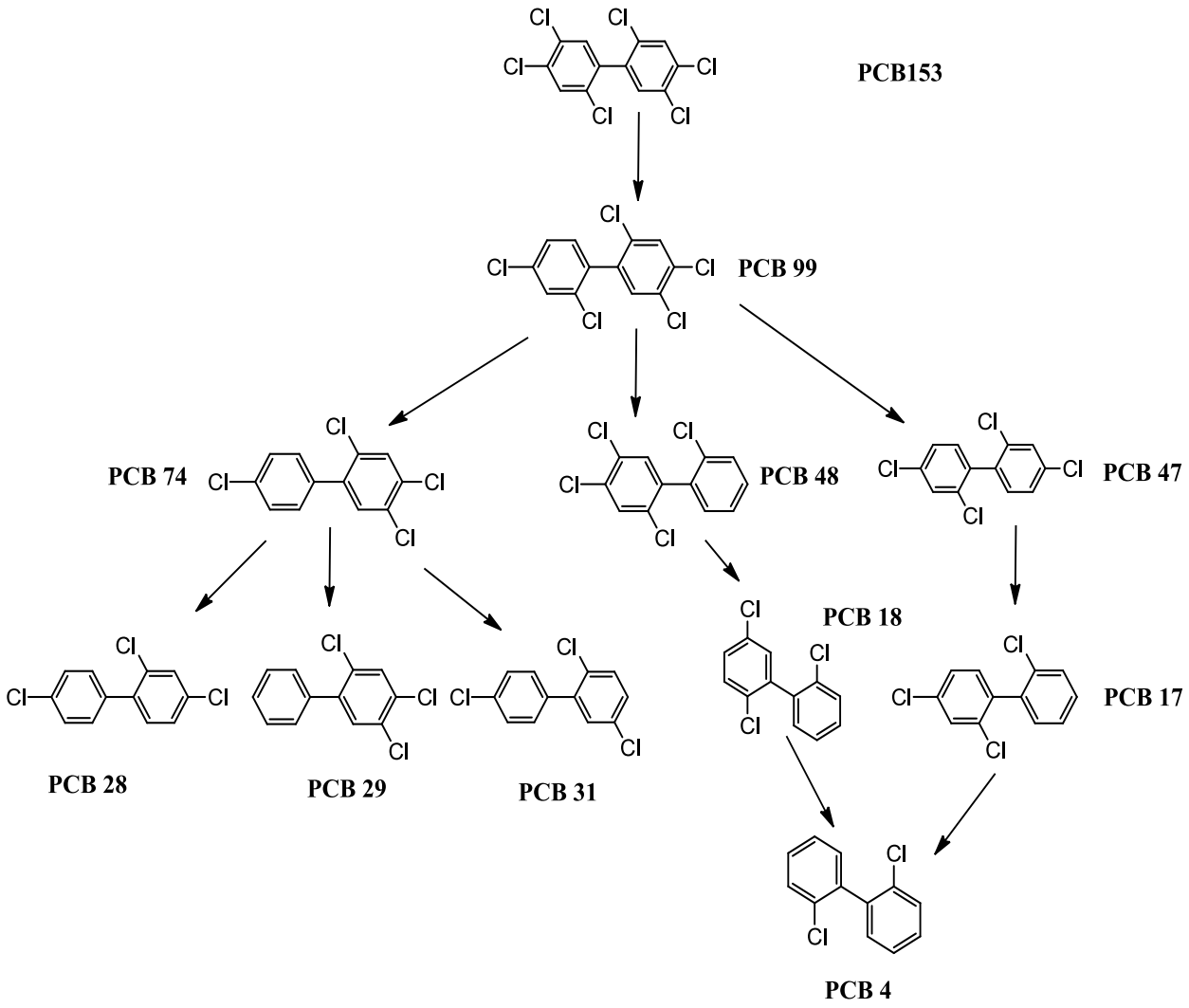

Figure 5.8: Some products observed with PCB 153 transformation and potential degradation pathway through the PCB 99 intermediary.

Table 5.2: Molar Mass Balance in Poplar and Switchgrass Planted Soil (Average of n=3)

	Parent Compound In Soil (mole %)	**Transformation Products in Soil (mole %)**	**Roots (mole%)**	**Shoots (%)**	**Total Recovered (mole%)**
PCB52 Poplar	45.9	56.2	3.4	<0.1	105.6
PCB52 Switchgrass	37.2	63.0	3.5	<0.1	103.7
PCB77 Poplar	53.5	47.4	2.6	<0.1	103.5
PCB77 Switchgrass	46.4	55.5	4.5	<0.1	106.4
PCB153 Poplar	51.0	47.7	4.3	<0.1	102.4
PCB153 Switchgrass	49.0	46.9	2.2	<0.1	98.1

CHAPTER 6 CONCLUSIONS, ENGINEERING SIGNIFICANCE, AND RECOMMENDATIONS FOR FUTURE WORK

6.1 Summary

Sequential redox cycles of dechlorination followed by aerobic bio-oxidation are desirable to achieve complete degradation of a mixture of higher and lower chlorinated PCBs. In this research, soil was artificially contaminated with polychlorinated biphenyls (PCBs) in mixture and as single congeners, aged, and planted with two different plant species. The PCB congeners were PCB 52, PCB 77 and PCB 153. Alternating redox cycles were created in the root zone of plants by flooding and draining the soil. Over 32 weeks, switchgrass (*Panicum virgatum)* and poplar (*Populus deltoids x nigra* DN34) planted systems that were exposed to alternate cycles of flooding performed significantly better in reducing parent PCBs than planted systems that were not cycled. There was difficulty in developing strictly anaerobic conditions in the planted systems during flooding, possibly due to plant transfer of oxygen to the roots. However, during flooding, the development of highly reduced microzones in the soil particles was possible. This coupled with diurnal redox fluctuations, provided opportunity for reduction of the parent compounds. Multiple cycles were necessary to achieve significant differences between the cycled and uncycled treatments.

Poplar (*Populus deltoids x nigra* DN34) and switchgrass (*Panicum virgatum*) planted rhizospheres demonstrated significant reduction of PCB 52,77 and 153 in aged soil microcosms relative to unplanted soil when the congeners were either combined as a mixture or used in single conger exposures over a period of 32 weeks. The planted rhizospheres also consistently had significantly higher dissipation and transformation of the tested congeners when compared to unplanted spiked systems. Even though the

decrease obtained with the planted system were higher than the unplanted system, the decline was not the the result of plant uptake because most of the parent compounds and transformation products resided in the soil. This suggests that secretion of plant secondary compounds and exudates which stimulated rhizosphere transformation accounted for the enhanced degradation observed in the planted microcosms. This enhanced degradation in the planted rhizosphere could have resulted from the presence of more organisms, a different microbial community or more active degraders in the planted system relative to the unplanted treatments.

There was evidence of reductive dechlorination in the poplar (*Populus deltoids x nigra* DN34) and switchgrass (*Panicum virgatum*) planted systems and also in the unplanted systems. Higher concentrations of transformation products were detected in the planted systems than the unplanted. This suggests that the greater reduction and transformation in the planted systems resulted from specific interactions between the microbial community in the root zone and the conditions provided by the plant roots in the rhizopshere. Measured transformation products, which excluded polar metabolites and possible mineralization products accounted for approximately all of the mass of parent compounds lost. The transformation products could be tracked back to the parent compounds via dechlorination pathways and provided insight into the metabolic processes involved in the rhizospheric biotransformation of these PCB congeners. While lower reductions were obtained when poplar was the planted species, the difference in reduction was not statistically significant in comparison to switchgrass planted soil.

Commercial garden soil had trace contamination (37.9 ng g^{-1} in total) of lower chlorinated PCBs congeners. Planting the contaminated soil with Poplar (*Populus deltoids x nigra* DN34), resulted in the complete removal of 26 of the 29 congeners after 96 days. The unplanted soil only had complete removal of 2 of the 29 congeners after 96 days. In addition to removal of the trace contaminants, the planted treament performed better in diminishing the concentration of a PCB 77 spike than the unplanted treatment.

Uptake of the depleted PCBs by plants was minimal, and most of the remaining PCBs resided in the soil. This suggests that the plants influenced the chemistry and biology in the root zone resulting in better performance in the planted treatments relative to the unplanted. The results also suggest that phytoremediation can be an effective tool in cleaning up commercially available garden soil that is lightly contaminated with PCBs.

In summary;

- PCB congeners decrease at significantly faster rates in soils planted with switchgrass and poplar compared to unplanted soils. Switchgrass planted soil recorded somewhat higher but not statistically significant declines than poplar planted soil.
- In the plant, most of the PCB resided in the root (probably sorption) without significant translocation.
- Select PCB congeners (52,77 and 153) decline in soil reactors in conjunction with the appearance of less chlorinated congeners in significant concentrations which account for approximately all of the mass lost. Thus, there is evidence for reductive dechlorination.
- A pathway can be proposed for rhizospheric biodegradation of PCB 52 to PCB 18,PCB 9 and PCB1.
- Another pathway can be proposed for the rhizospheric biodegradation of PCB 77 through the intermediaries PCB 35 and PCB 37.
- A pathway for the rhizospheric biotransformation of PCB 153 through PCB 101 and PCB 99 can also be discerned.
- There was difficulty in lowering the redox potential to sulfidogenic conditions in the reactors designed here. This suggests plant oxygen transfer downwards to the roots and soils. Planted reactors became redox "poised" at about +20 mv.
- Poplar planted rhizosphere enhanced the degradation of lower chlorinated PCB contaminants in a commercial garden soil relative to unplanted treatment after 96

days. Therefore lightly contaminated garden soil obtained commercially can be "cleaned" by poplars.

6.2 Significance of Research

This research was able to demonstrate that systems planted with switchgrass and poplar were able to significantly improve the decline and subsequent transformation of PCBs 52, 77 and 153 relative to the unplanted treatments. Phyto- stimulated rhizosphere transformation of PCBs as shown in this research, provides an addition to the current limited options available for PCB remediation, which are the expensive and energy intensive option of excavation and incineration ,and the expensive option of excavation and storage .However the benefits associated with phytoremediation of PCB contaminated soils or sediments are likely to be site specific because of the importance of the microbial community which appears to be the major driver of the transformations seen in this research. While most of the PCBs remained in the soil, the transformation resulted in the accumulation of less chlorinated congeners and the toxicity of PCB is related to their total chlorine content. PCB 77 in particular, is one the most toxic PCB congers and its transformation is a positive outcome. While bio-oxidation and subsequent mineralization of would have been a more desirable outcome, and there was no indication that there was bio-oxidation of the PCBs, reductive dechlorination to produce lower chlorinated PCBs is an important first step in achieving bio-oxidation

Cycling of moisture content through the creation of alternate cycles of flooding and no flooding, while having a statistically significant impact on PCB decline and transformation after multiple cycles, is not as pronounced as the

differences seen between the planted and unplanted treatments. However, an interesting outcome of this research is that there was reductive dechlorination in systems that were not deeply anaerobic in the bulk environment as evidenced by redox potential measurements. This suggests that the reduction and transformations seen may be cometabolic in nature and as such results will be site specific.

6.3 Recommendations for Potential Future Work

While there were clear differences in the decline and transformation of PCB 52, 77 and 153 between the planted and unplanted systems, nothing is known about the microbial communities in these systems. The fact that declines and transformations were seen in the unplanted systems suggests that microbes were the main drivers of the declines and transformation. Therefore, future work could focus on the role of microbes in the decline and transformation of the tested PCB congeners. Research could be focused on identification of microbial community members and also examine changes, if any, in the microbial community structure over time in both the planted and unplanted systems. In addition, there could be research on the level of activity in the planted systems compared to the unplanted systems.

In addition, it was observed that there appeared to be a stalling of the declines in the planted systems after about 24 weeks. This is commonly encountered in remediation of other organic pollutants. The reason for this stalling is not readily apparent and could be due to any number of reasons including reduction in the production of electron donors or reduction in microbial enzymatic activity. Tracking microbial enzymatic activity over time could provide some clues if that is reason for the apparent stalling. Similarly tracking the production of electron donors over time in the planted system through the

measurement of dissolved organic carbon (DOC) and total organic carbon TOC could provide some answers. In addition, the effect of adding electron donors (like molasses) to the system after that 24 week period could be investigated.

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APPENDIX

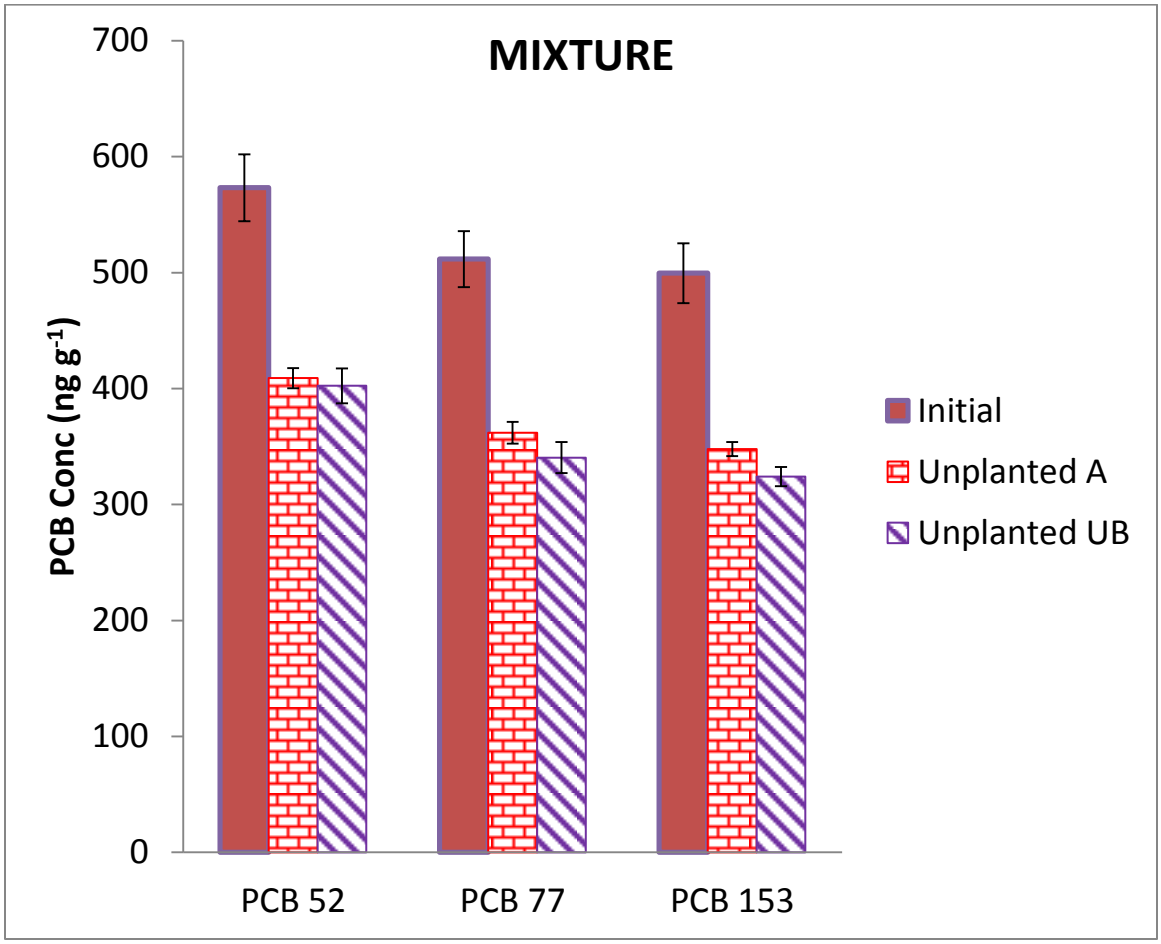

Figure A1: Dissipation of PCB in unplanted soil contaminated with mixture of PCB 52, PCB 77 and PCB 153 A = uncycled treatment i.e. treatment that was not flooded during exposure; UB = cycled treatment i.e. treatment that underwent sequential cycles of flooding and no flooding during exposure. Unlike the switchgrass and poplar planted systems, moisture content cycling did not increase the removal of parent compounds in the unplanted system. The spike congeners were undetectable in the controls which were not spiked. Error bars are 1 standard deviation and are based on triplicate measurements.

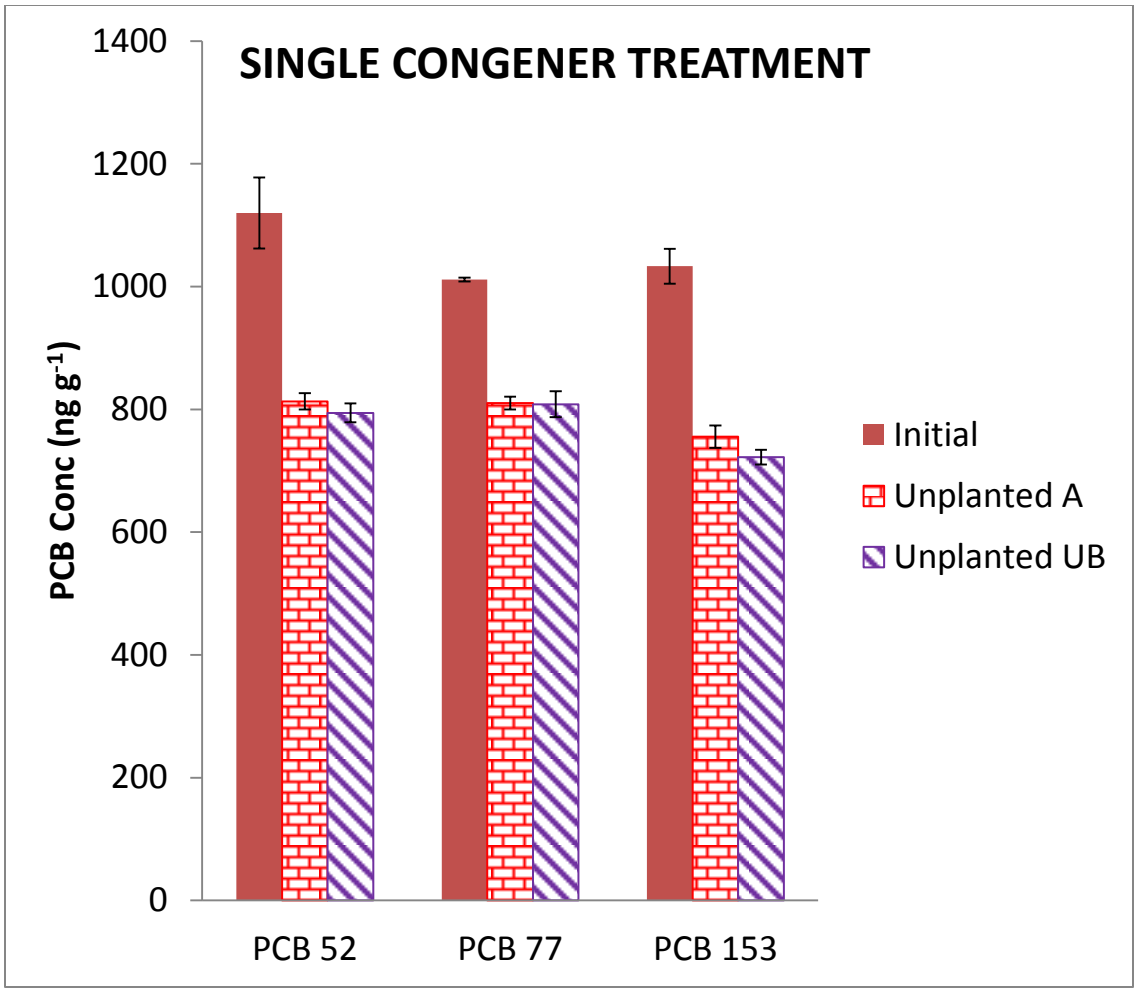

Figure A2: Dissipation of PCB in unplanted soil contaminated with PCB 52, PCB 77 and PCB 153 as the sole contaminants A= uncycled treatment i.e. treatment that was not flooded during exposure; UB = cycled treatment i.e. treatment that underwent sequential cycles of flooding and no flooding during exposure. Unlike the switchgrass and poplar planted systems, moisture content cycling did not increase the removal of parent compounds in the unplanted system. The spiked congeners were undetectable in the controls which were not spiked. Error bars are 1 standard deviation and are based on triplicate measurements.

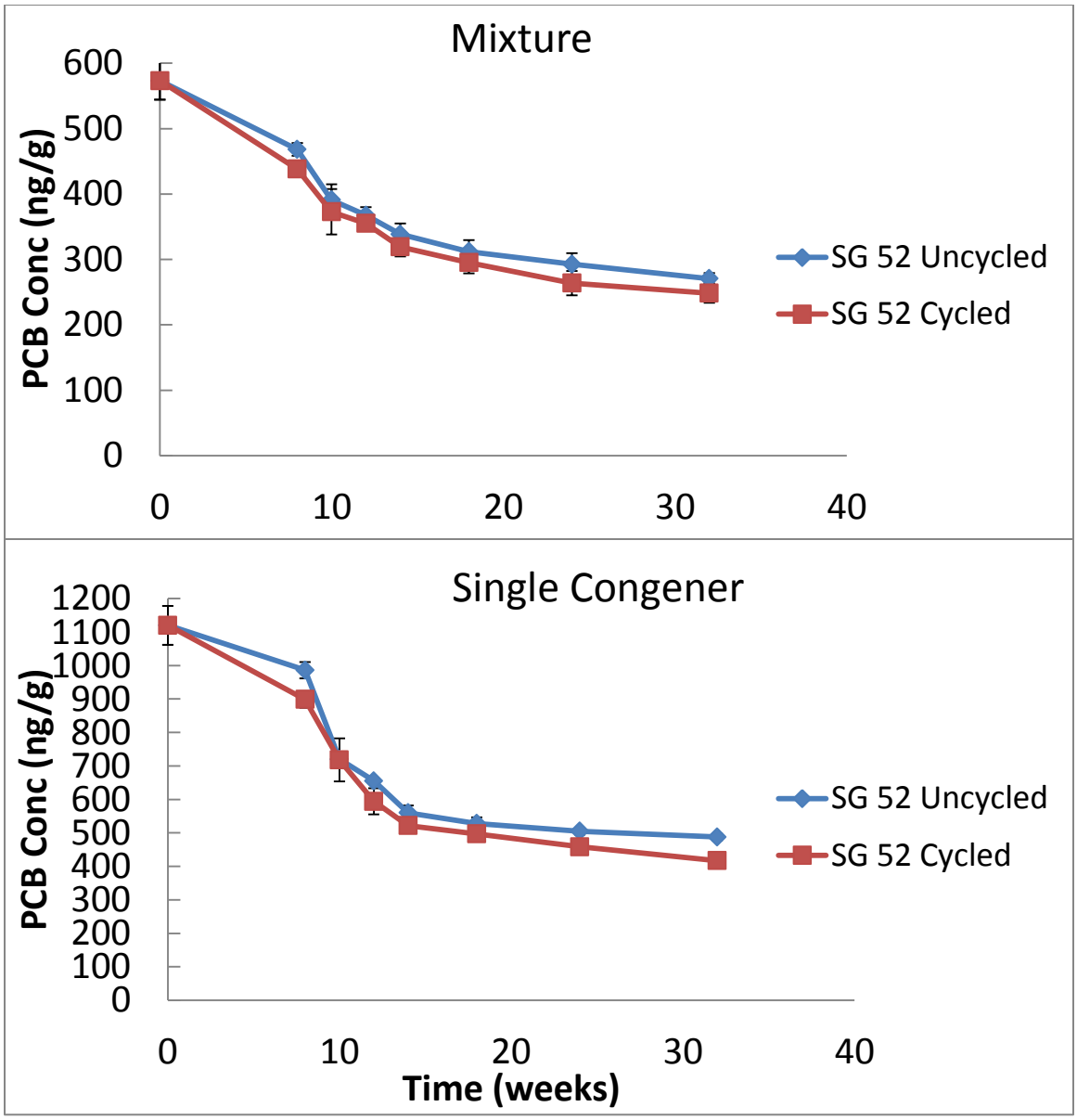

Figure A3: Comparison of PCB 52 dissipation in switchgrass planted soil that had sequential 7 days cycles of flooding and no flooding with planted soil that did not have this cycling. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 52. SG = Switchgrass. Multiple cycles were required to obtain a significant difference in decrease of PCB 52 concentration between the cycled and uncycled treatments in the single congener exposure ($p<0.05$), while there was not a significant difference between the cycled and uncycled treatment in the mixture ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

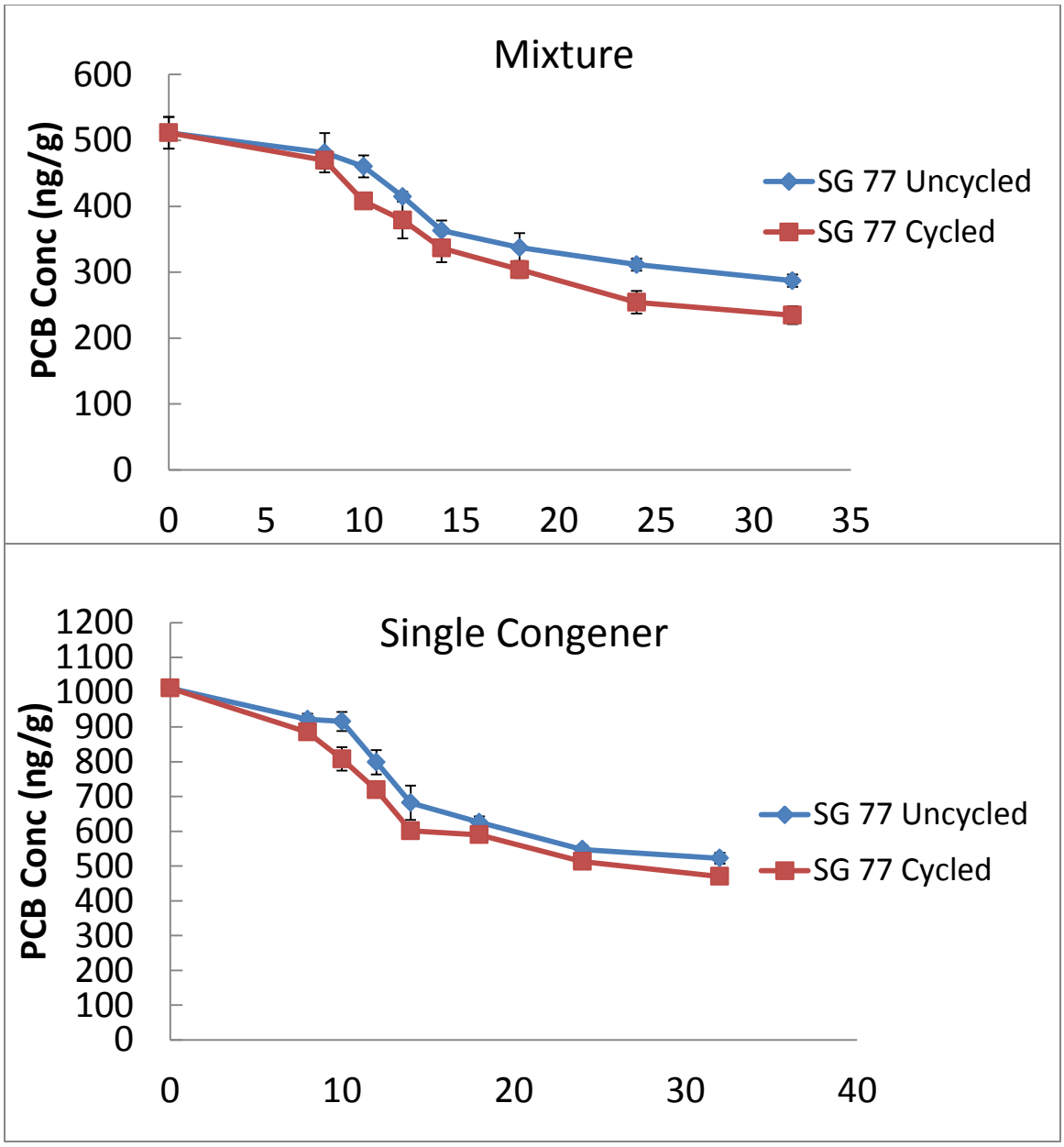

Figure A4: Comparison of PCB 77 dissipation in switchgrass planted soil that had sequential 7 days cycles of flooding and no flooding with planted soil that did not have this cycling. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 77. SG = Switchgrass. Multiple cycles were required to obtain a significant difference in decrease of PCB 77 concentration between the cycled and uncycled treatments in both the mixture and single congener treatments ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

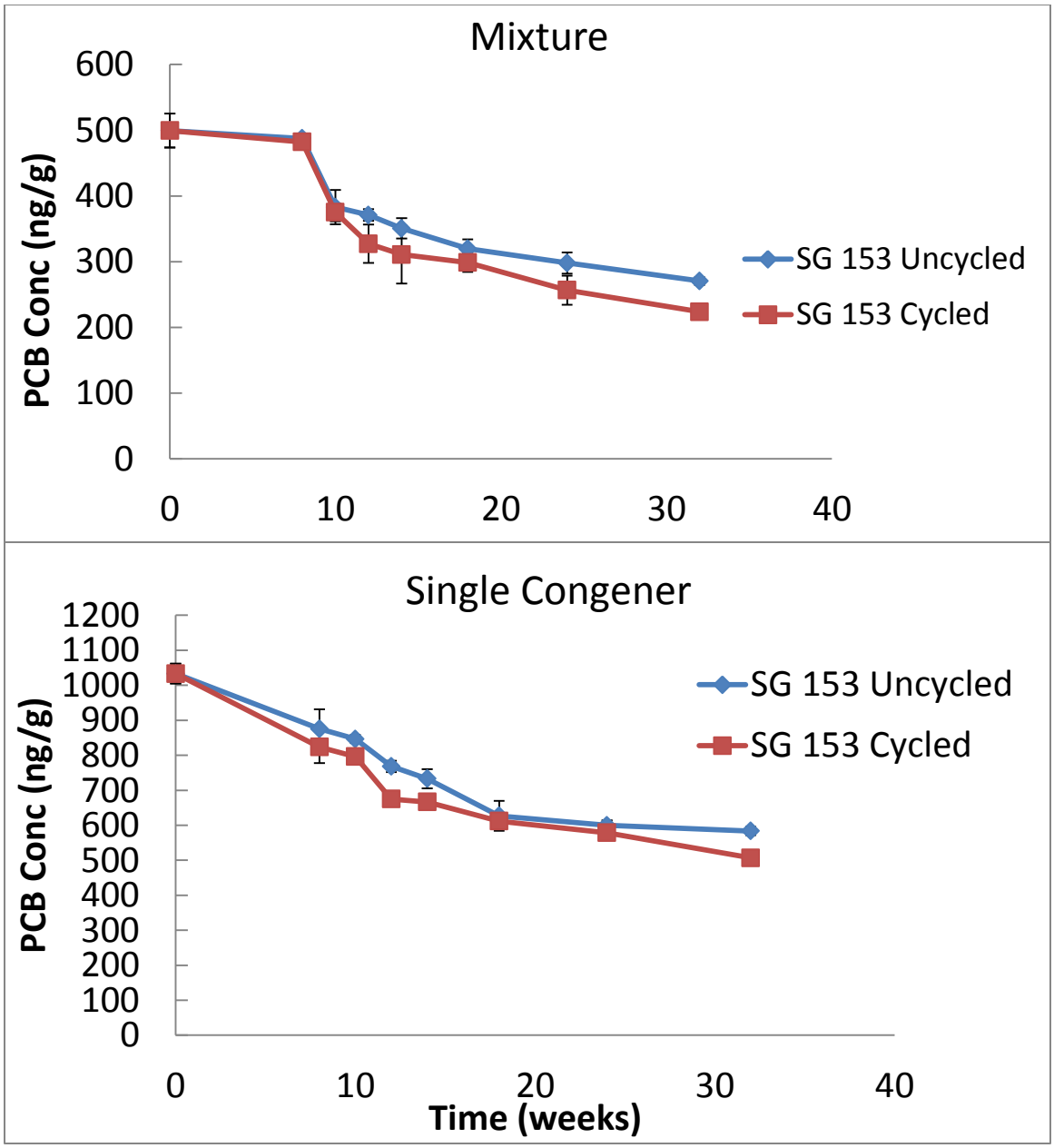

Figure A5: Comparison of PCB 153 dissipation in switchgrass planted soil that had sequential 7 days cycles of flooding and no flooding with planted soil that did not have this cycling. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB153. SG = Switchgrass. Multiple cycles were required to obtain a significant difference in decrease of PCB 153 concentration between the cycled and uncycled treatments. in both the mixture and single congener treatments ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

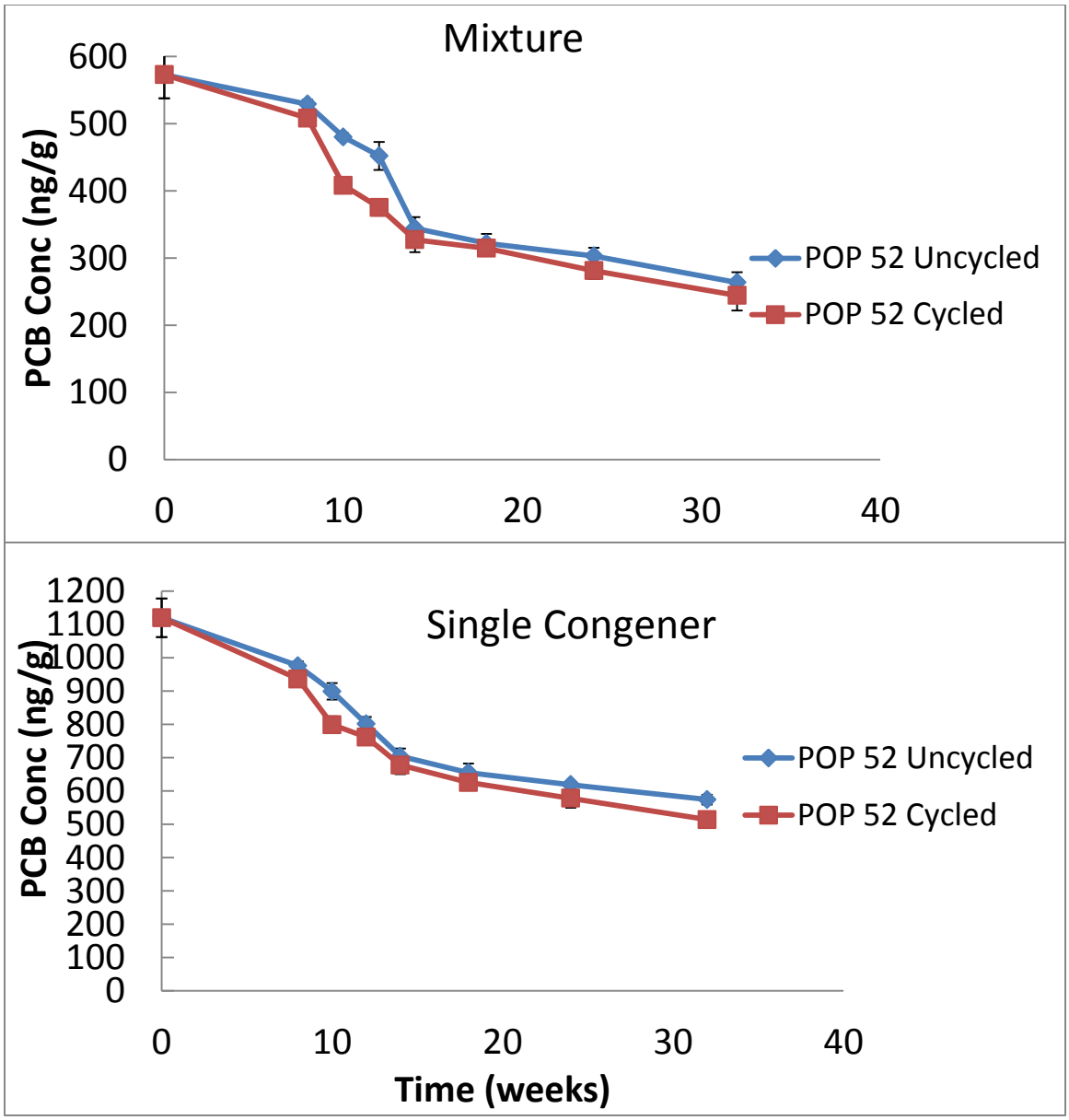

Figure A6: Comparison of PCB 52 dissipation in poplar planted soil that had sequential 7 days cycles of flooding and no flooding with planted soil that did not have this cycling. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 52. POP = Poplar. Multiple cycles were required to obtain a significant difference in decrease of PCB 52 concentration between the cycled and uncycled treatments in the single congener exposure ($p<0.05$), while there was not a significant difference between the cycled and uncycled treatment in the mixture ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

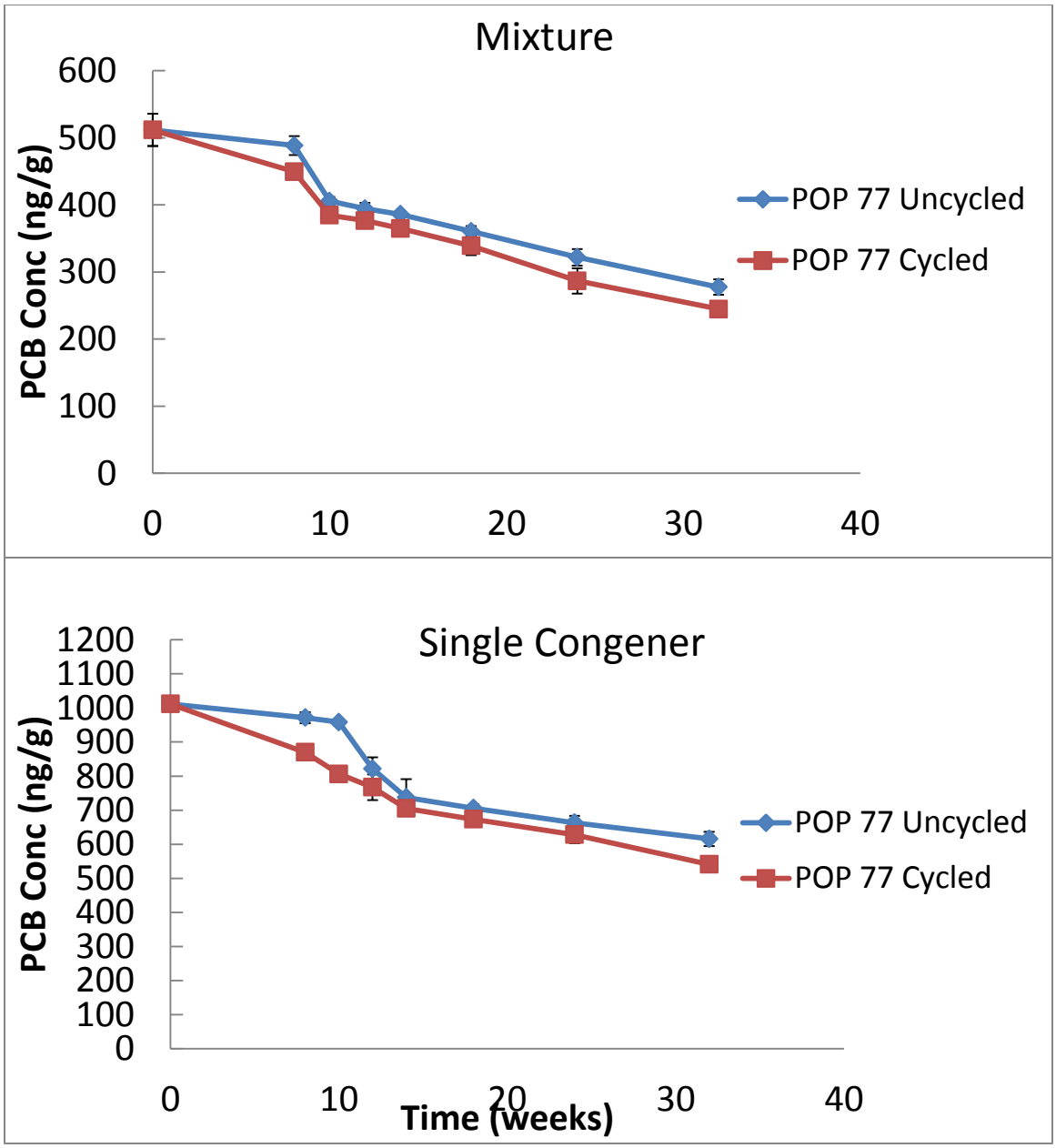

Figure A7: Comparison of PCB 77 dissipation in poplar planted soil that had sequential 7 days cycles of flooding and no flooding with planted soil that did not have this cycling. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 77. POP = Poplar. Multiple cycles were required to obtain a significant difference in decrease of PCB 77 concentration between the cycled and uncycled treatments in both the mixture and single congener treatments ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

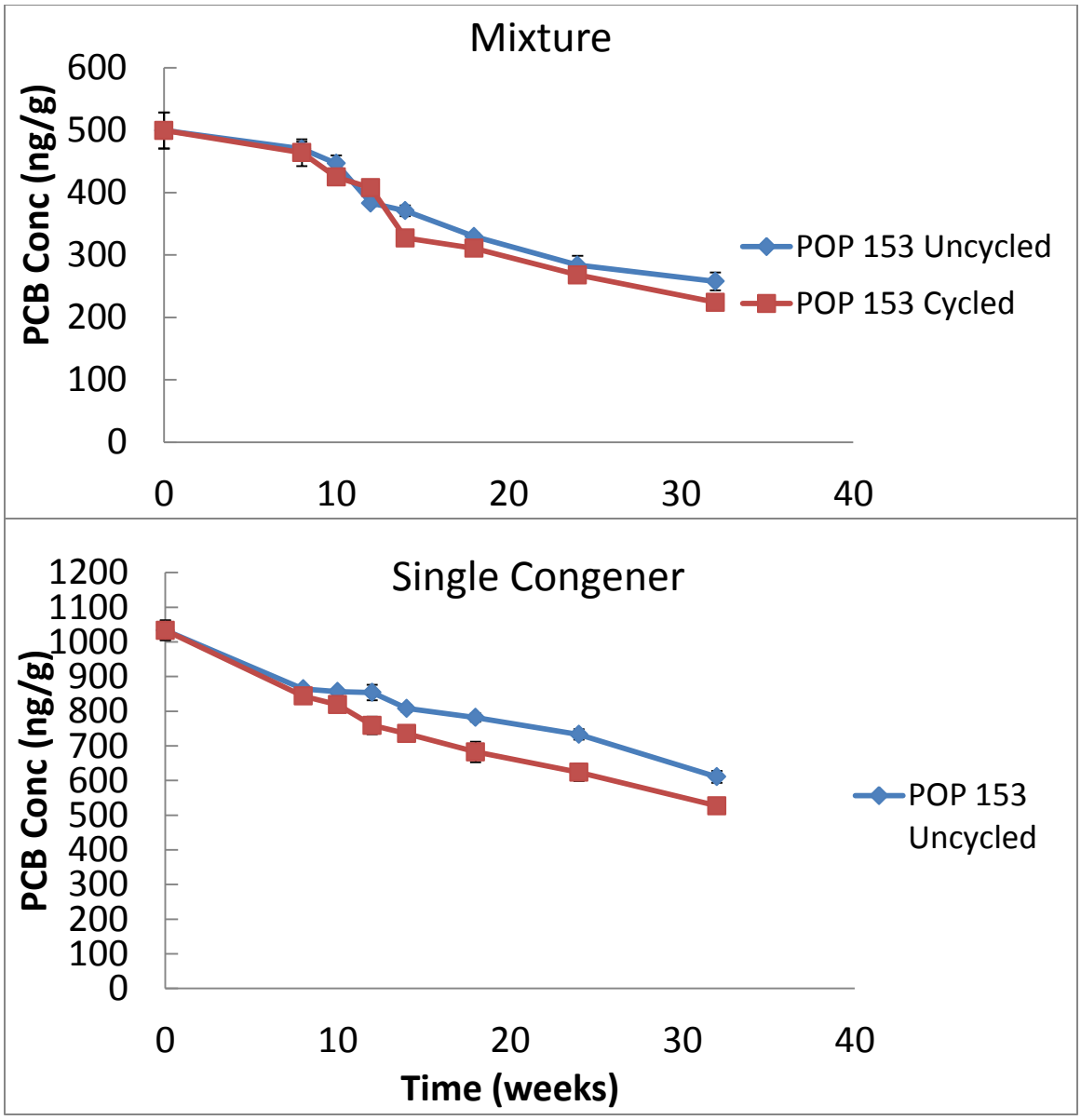

Figure A8: Comparison of PCB 153 dissipation in poplar planted soil that had sequential 7 days cycles of flooding and no flooding with planted soil that did not have this cycling. Top panel shows the decrease in soil containing a mixture of PCB 52, 77 and 153. Bottom panel shows reduction in soil containing only PCB 153. POP = Poplar. Multiple cycles were required to obtain a significant difference in decrease of PCB 153 concentration between the cycled and uncycled treatments in both the mixture and single congener treatments ($p<0.05$). Error bars are 1 standard deviation and are based on triplicate measurements.

Figure A9: Photograph of reactor used in experiment with soil from Amana colonies and that had cycling of moisture content and redox potential. The switchgrass planted system is used as an example.

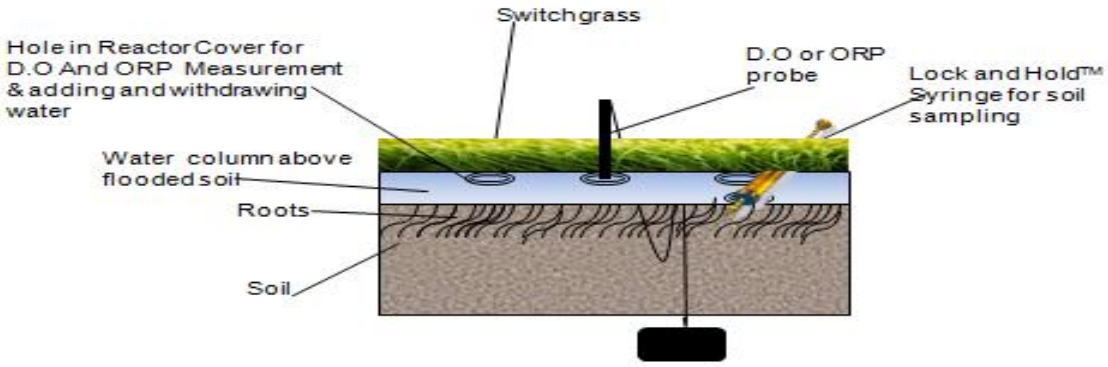

Figure A10: Schematic of reactor used in experiment with soil from Amana colonies and that had cycling of moisture content and redox potential. Samples were collected using a Lock and Hold syringe to make a vertical core in the soil column D.O and ORP.measurements were taken by inserting the probe through a 1.9 cm hole drilled in the cover of the reactor. The switchgrass planted system is used as an example.

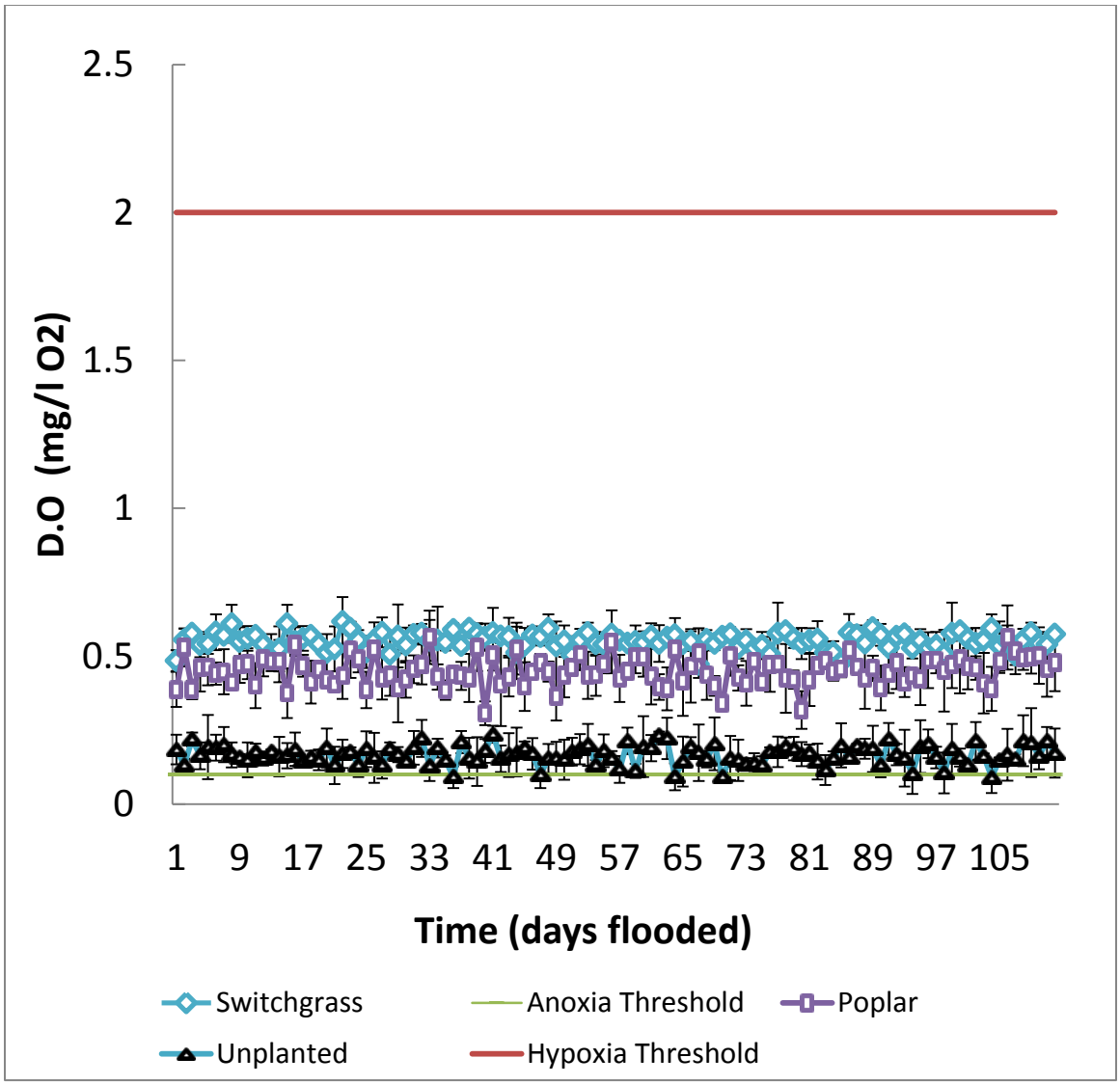

Figure A11: Dissolved oxygen (DO) concentration in water column above flooded soil contaminated with a mixture of PCB 52, PCB 77 and PCB 153. During flooding the water column was hypoxic in the switchgrass and poplar planted systems. The unplanted system was also hypoxic during flooding, but had lower DO readings than the planted systems and bordered on being anoxic. The treatments with the mixture are used as an example, but similar results were obtained with the treatments that were spiked with single congeners (PCB 52, PCB 77and PCB 153).

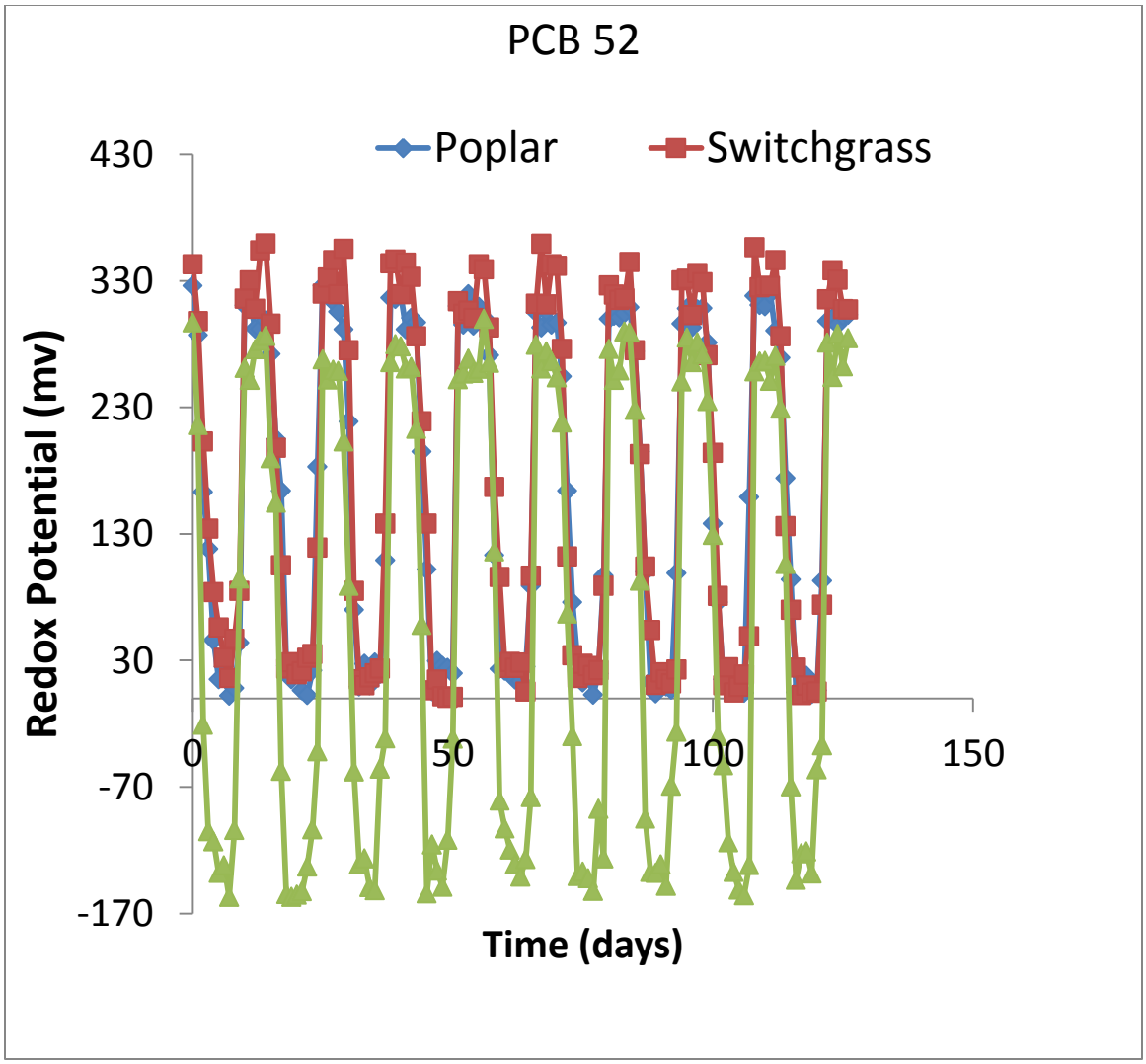

Figure A12: Redox potential in soil artificially contaminated with PCB 52 and planted with either switchgrass or poplar and undergoing alternate 7 days cycles of flooding and no flooding. There were variations in redox potential in the planted systems that mirrored the cycles of flooding and no flooding with higher redox potential when there was no flooding and lower redox potential when there was flooding. Also shown is the redox potential in the unplanted system, which showed the same trend in redox variation as the planted system.

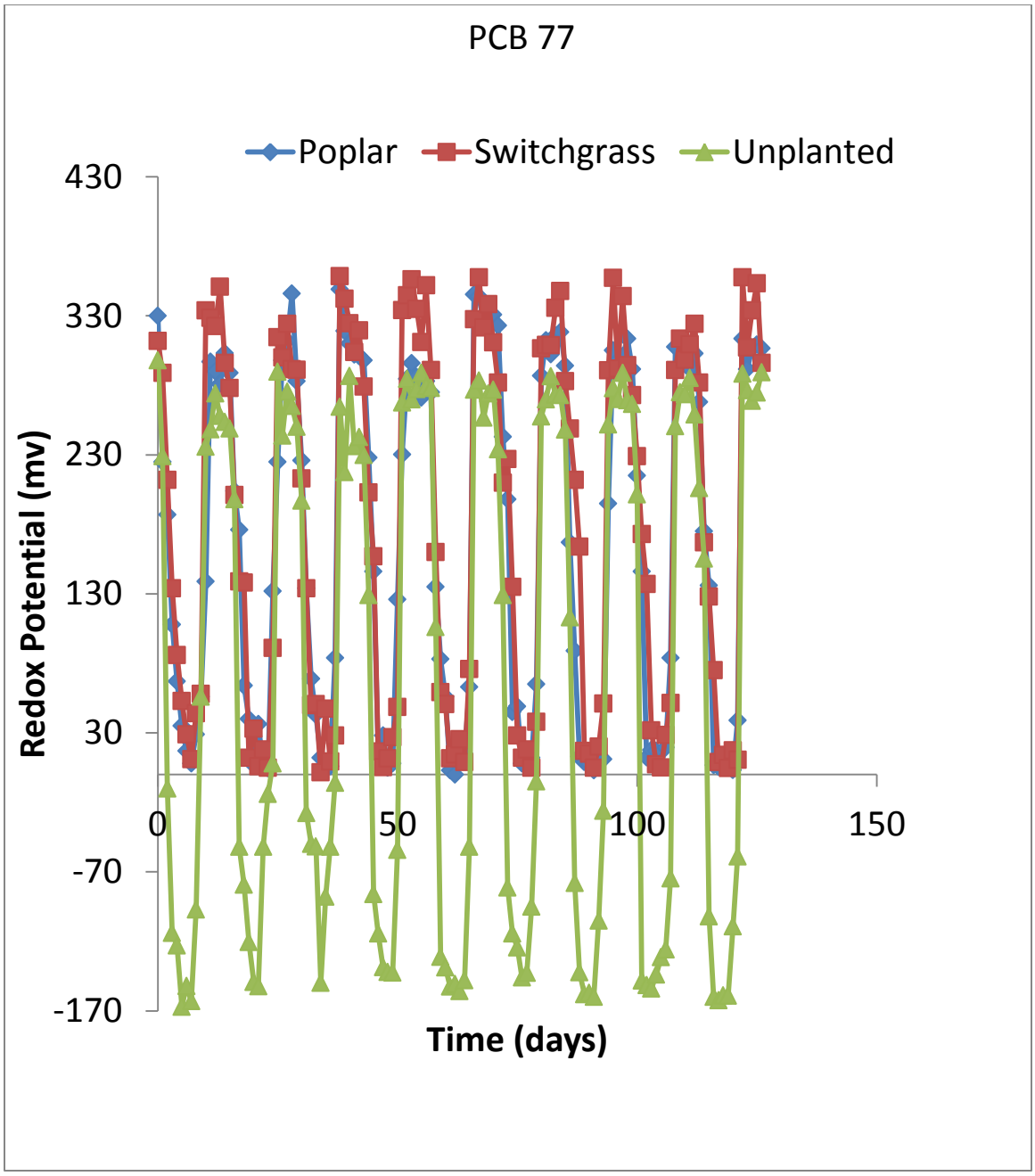

Figure A13: Redox potential in soil artificially contaminated with PCB77 and planted with either switchgrass or poplar and undergoing alternate 7 days cycles of flooding and no flooding. There were variations in redox potential in the planted systems that mirrored the cycles of flooding and no flooding with higher redox potential when there was no flooding and lower redox potential when there was flooding Also shown is the redox potential in the unplanted system, which showed the same trend in redox variation as the planted system.

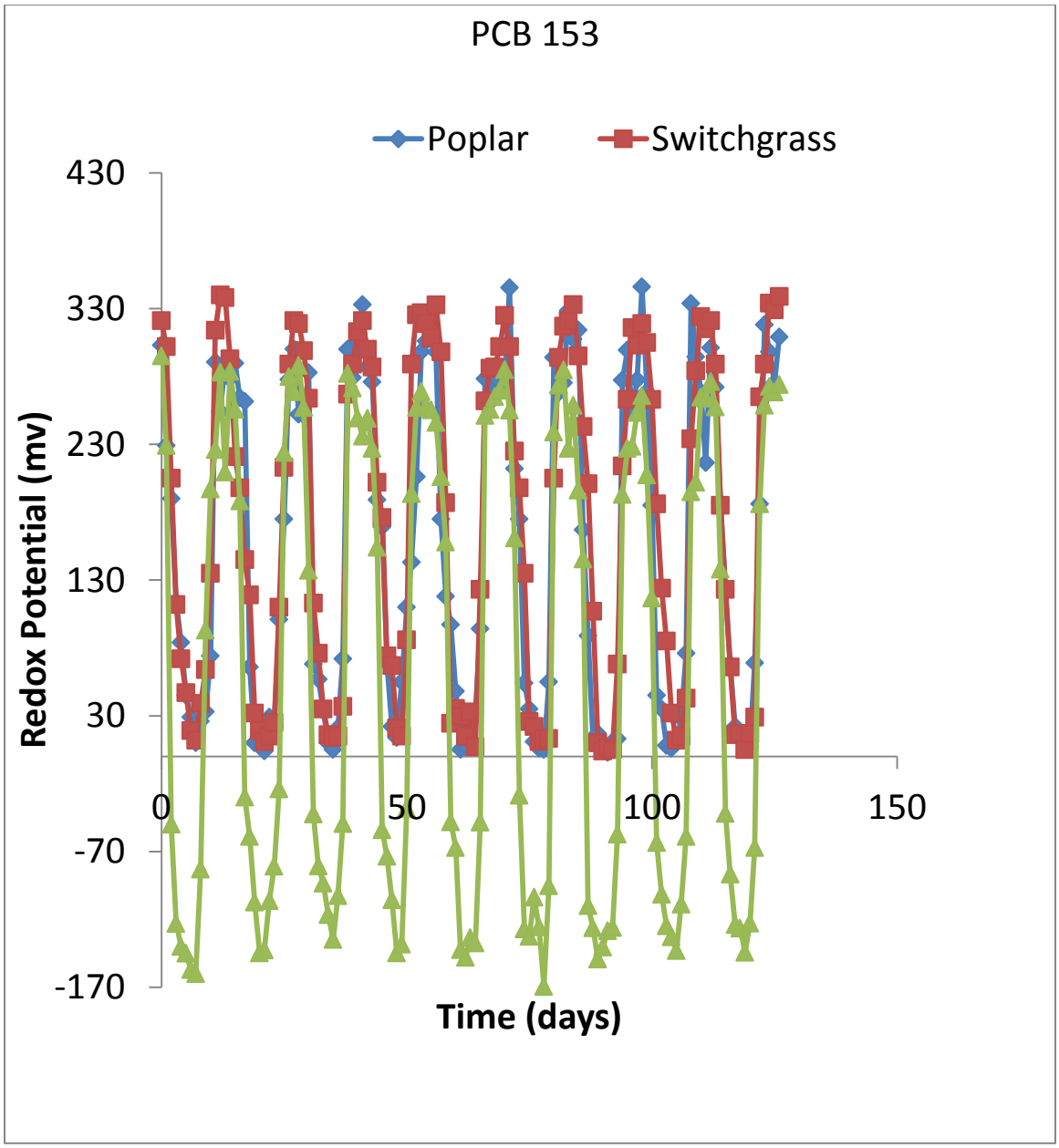

Figure A14: Redox potential in soil artificially contaminated with PCB153 and planted with either switchgrass or poplar and undergoing alternate 7 days cycles of flooding and no flooding. There were variations in redox potential in the planted systems that mirrored the cycles of flooding and no flooding with higher redox potential when there was no flooding and lower redox potential when there was flooding Also shown is the redox potential in the unplanted system, which showed the same trend in redox variation as the planted system.

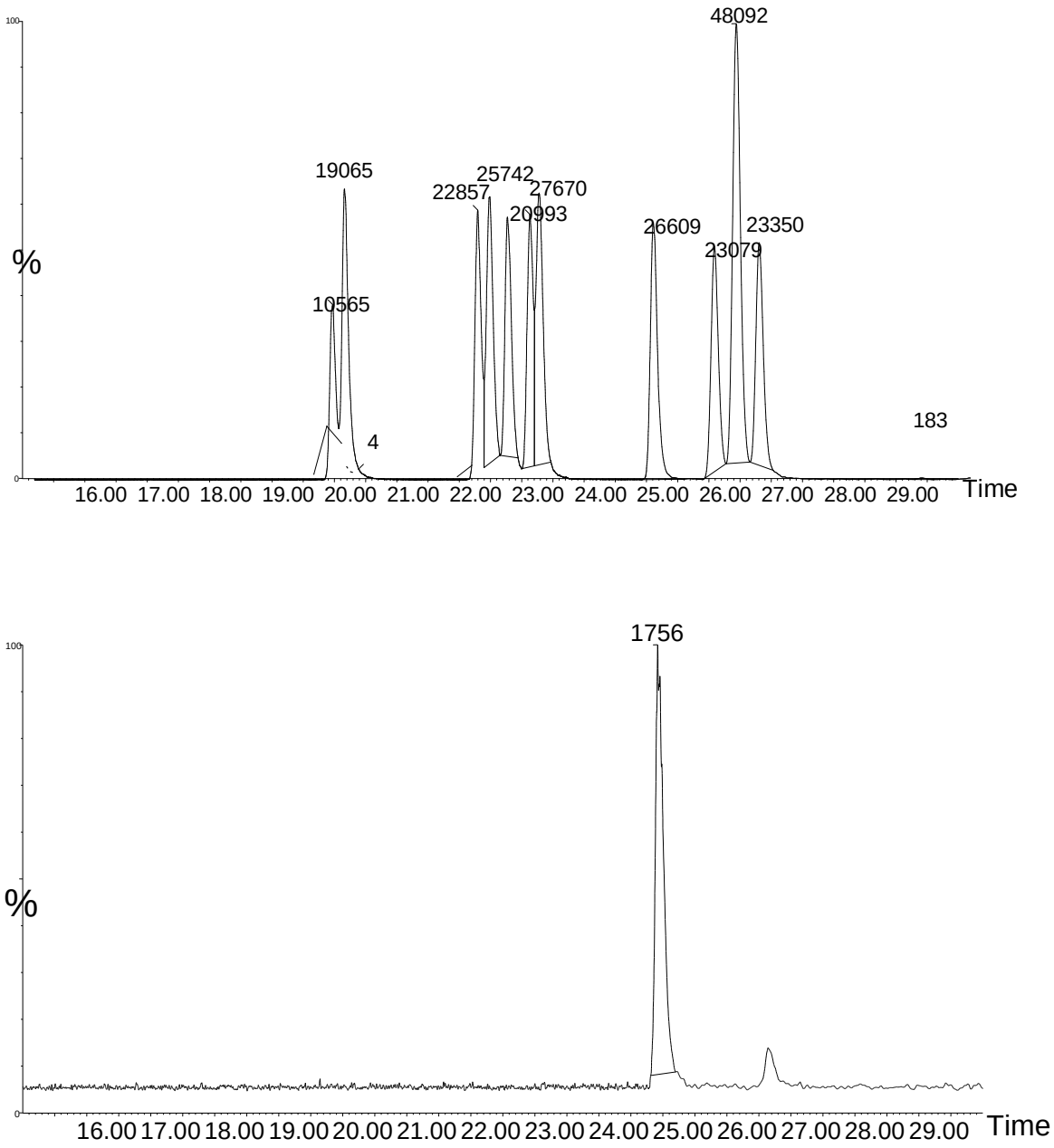

Figure A15: Representative GC/MS/MS chromatograms for trichlorinated congeners in soil for spiked sample (top panel), blank (bottom panel).at the end of 32 weeks in redox and moisture content cycling experiment. This shows that the spiked and unspiked treatments had different chromatogram profiles indicating that there was no deposition of PCBs from the air in the blank (unspiked) treatment suggesting that aerial deposition was not responsible for the PCB congeners seen in the spiked treatment.

Table A1: Molar Mass Balance in Unplanted Reactors in Cycling Experiment

	Parent Compound In Soil (mole %)	**Transformation Products in Soil (mole %)**	**Total Recovered (mole%)**
PCB52 Uncycled	72.6	26.6	99.2
PCB5 Cycled	70.9	30.9	101.8
PCB77 Uncycled	80.1	15.1	95.2
PCB77 Cycled	79.9	19.7	99.6
PCB153 Uncycled	73.1	30.3	103.4
PCB153 Cycled	69.9	36.4	106.3

Table A2: Characterization of Silty Loam Amana Soil conducted by A&L Heartland Laboratory, Iowa

TEST	**RESULT**
pH	6.5
Phosphorus(P)	59 ppm
Potassium (K)	141 ppm
Calcium (Ca)	2316 ppm
Magnesium (Mg)	414 ppm
Organic Matter	3.7%
% Potassium (K)	2.3%
% Calcium (Ca)	75.2%
% Magnesium (Mg)	22.4%
Cation Exchange Capacity	15.4 meq/100g

Table A3: Characterization of Scotts Lawn Soil conducted by A&L Heartland Laboratory, Iowa

TEST	RESULT
pH	6.7
Phosphorus(P)	37 ppm
Potassium (K)	89 ppm
Calcium (Ca)	2397 ppm
Magnesium (Mg)	622 ppm
Organic Matter	5.4%
% Potassium (K)	1.3%
% Calcium (Ca)	68.9%
% Magnesium (Mg)	29.8%
Cation Exchange Capacity	17.4 meq/100g

Table A4: Example Calculation for Molar Mass Balance using PCB 153 Single Congener Cycled Treatment as the example.

	Molecular Wt of homolog (MW)	Conc (ng/g)	Mass (ng)	Mass(ng)/MW*10^9 (nmol)	Mole Fraction (%)
Soil Mono	188.6541	7.10	1.77E+04	9.40E-08	1.3%
Soil Di	223.0993	10.64	2.66E+04	1.19E-07	1.7%
Soil Tri	257.5446	78.06	1.95E+05	7.58E-07	10.6%
Soil Tetra	291.9898	56.77	1.42E+05	4.86E-07	6.8%
Soil Penta	326.4351	246.31	6.16E+05	1.89E-06	26.4%
Soil Hexa	360.8803	505.83	1.26E+06	3.50E-06	49.0%
Root Mono	188.6541	14.95	1.30E+03	6.90E-09	0.1%
Root Di	223.0993	7.47	6.50E+02	2.91E-09	0.0%
Root Tri	257.5446	3.84	3.34E+02	1.30E-09	0.0%
Root Tetra	291.9898	3.14	2.74E+02	9.37E-10	0.0%
Root Penta	326.4351	2.84	2.47E+02	7.58E-10	0.0%
Root Hexa	360.8803	600.76	5.23E+04	1.45E-07	2.0%
Shoot Mono	188.6541	3.08	5.09E+02	2.70E-09	0.04%
Shoot Di	223.0993	2.28	3.77E+02	1.69E-09	0.02%
Shoot Tri	257.5446	1.12	1.86E+02	7.21E-10	0.01%
Shoot Tetra	291.9898	1.09	1.79E+02	6.15E-10	0.01%
Shoot Penta	326.4351	1.16	1.91E+02	5.85E-10	0.01%
Shoot Hexa	360.8803	1.07	1.76E+02	4.87E-10	0.01%
Initial	360.8803	1032.92	2.58E+06	7.16E-06	

Note: Soil mass =2500g;Shoot Mass =165g;Root Mass=87g

Table A5: Example Calculation for Molar Mass Balance using PCB 153 Single Congener Uncycled Treatment as the example.

	Molecular Wt of homolog (MW)	Conc (ng/g)	Mass (ng)	Mass(ng)/MW*10^9 (nmol)	Mole Fraction (%)
Soil Mono	188.6541	6.78	1.70E+04	8.99E-08	1.3%
Soil Di	223.0993	10.17	2.54E+04	1.14E-07	1.6%
Soil Tri	257.5446	64.42	1.61E+05	6.25E-07	8.7%
Soil Tetra	291.9898	54.25	1.36E+05	4.65E-07	6.5%
Soil Penta	326.4351	205.85	5.15E+05	1.58E-06	22.0%
Soil Hexa	360.8803	579.48	1.45E+06	4.01E-06	56.1%
Root Mono	188.6541	15.63	3.27E+03	1.73E-08	0.2%
Root Di	223.0993	10.86	2.27E+03	1.02E-08	0.1%
Root Tri	257.5446	4.75	9.93E+02	3.86E-09	0.1%
Root Tetra	291.9898	2.41	5.04E+02	1.73E-09	0.0%
Root Penta	326.4351	2.84	5.93E+02	1.82E-09	0.0%
Root Hexa	360.8803	216.87	4.53E+04	1.26E-07	1.8%
Shoot Mono	188.6541	2.39	3.15E+02	1.67E-09	0.02%
Shoot Di	223.0993	3.17	4.18E+02	1.87E-09	0.03%
Shoot Tri	257.5446	1.05	1.39E+02	5.40E-10	0.01%
Shoot Tetra	291.9898	1.55	2.05E+02	7.02E-10	0.01%
Shoot Penta	326.4351	1.93	2.54E+02	7.79E-10	0.01%
Shoot Hexa	360.8803	1.41	1.86E+02	5.15E-10	0.01%
Initial	360.8803	1032.92	2.58E+06	7.16E-06	

Note: Soil mass =2500g;Shoot Mass =132g;Root Mass=209g