THE EFFECT OF PCBs AND VASCULAR PLANTS ON THE MICROBIAL ASSEMBLAGE OF LOWER HUDSON RIVER SEDIMENT

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ABSTRACT

Microbial degradation of polychlorinated biphenyls (PCBs) occurs under anaerobic and aerobic conditions. A common wetland plant, *Typha angustifolia*, exchanges methane in the sediment with atmospheric oxygen potentially creating a unique habitat facilitating microbial PCB degradation. The goals of this study were to: 1) measure changes in the abundance of total bacteria and domain-level microorganisms (Bacteria and Archaea) associated with *Typha* treated with PCBs; specifically Aroclor 1242 and 2) determine if PCB degradation occurred. Sediment from plants (n=70) collected from North Tivoli Bay at the Hudson River National Estuarine Research Reserve, Annandale, NY on 26 June 1998 was removed from the rhizome, treated with either Aroclor 1242 in acetone (35 g:300 ml) or acetone only (control), potted, and maintained in a greenhouse at the Louis Calder Center, Armonk, NY through October 1998. Gas exchange to the rhizome was inactivated for one-half of the plants by cutting the stem from the root. Bacteria and Archaea were quantified in sediments closely and distally associated with the rhizomes at 0, 30, 60, and 90 d using 16S rDNA whole-cell hybridization; total bacterial number was determined by DAPI. Total bacterial abundance per mass organic matter (number x 10^9 g $AFDM^{-1}$) in the Aroclor 1242 treatment was significantly greater ($P < 0.001$) at 60 d (8.6) and 90 d (13.3) than in controls (60d: 5.7; 90d: 8.4). Whereas greater Bacteria abundance occurred at 60 d (Aroclor: 6.1; control: 3.7), Archaea abundance was nearly twice as high in the treatment (2.2) than in the control (1.2) at 90 d. No difference was found in these measurements between the active and inactive roots. Aroclor dechlorination was detected using a gas chromatograph with electron capture detector (GC-ECD)in which long-retention time peaks decreased and slow-retention time peaks increased. Increases in bacterial abundance and change in the Aroclor 1242 GC-ECD profile suggests that portions of the microbial assemblage associated with the rhizomes utilize the compound for growth.

TABLE OF CONTENTS

ABSTRACT....II-2

LIST OF TABLES AND FIGURES....II-4

INTRODUCTION....II-5

MATERIALS AND METHODS....II-7

RESULTS....II-11

DISCUSSION....II-18

ACKNOWLEDGEMENTS....II-21

REFERENCES....II-22

LIST OF TABLES AND FIGURES

Table 1. Comparison of the total number of bacteria, Bacteria, and Archaea in the rhizosphere and nonrhizosphere subsamples of the active cattail root treatments.

Table 2. Gas chromatography-electron capture detector (GC-ECD) analysis of Aroclor 1242 in control and PCB-treated active root samples showing the relative peak percentages over the 90 d experiment time period.

Figure 1. Comparison of the total number of bacteria g $AFDM^{-1}$ (A), Bacteria g $AFDM^{-1}$ (B), and Archaea g $AFDM^{-1}$ (C) in the rhizosphere sediments of an active cattail in PCB-treated and control samples.

Figure 2. Comparison of the total number of bacteria g $AFDM^{-1}$ (A), Bacteria g $AFDM^{-1}$ (B), and Archaea g $AFDM^{-1}$ (C) in the nonrhizosphere sediments of an active cattail in PCB-treated and control samples.

INTRODUCTION

Bioremediation of highly recalcitrant contaminants can occur under aerobic and anaerobic conditions or may require both for complete degradation as is the case with polychlorinated biphenyls (PCBs) (Fish and Principe 1994). There are 209 individual PCB compounds (i.e., congeners) that differ in the arrangement of up to 10 chlorine atoms on a biphenyl. Mixtures of these congeners were sold in the United States under the trade name Aroclor, the most abundant being Aroclor 1242 (Brown et al. 1984). The bioaccumulative , persistent, and carcinogenic (Safe 1989) nature of these compounds presents environmental health concerns.

PCBs undergo reductive dechlorination by microorganisms in anaerobic habitats whereby the chlorine atoms from highly substituted PCB congeners are replaced by hydrogen atoms (Bedard et al. 1996, Brown et al. 1987,Fish and Principe 1994, Nies and Vogel 1990, Quensen et al. 1990, Rhee et al. 1993, Williams 1994, Wu et al. 1997, Ye et al. 1995). Although species have not been identified, the reductive dechlorination of PCBs has been linked to methanogenic microorganisms in the domain Archaea (Ye et al. 1992, 1995). Bacteria, such as the sulfate reducers, and an unidentified group of bacteria called dechlorinators, have also been shown to reductively dechlorinate PCBs (Kim and Rhee 1997). Fewer chlorines on the congener reduces the toxicity and enables the resulting PCB to be degraded by aerobic bacteria (Bedard et al. 1996, Focht 1993, Quensen et al. 1988, 1990) such as certain *Proteobacteria*. (Pellizari et al. 1996) and *Alcaligenes* sp. (Mondello et al. 1997). Aerobically PCBs are broken down oxidatively by dioxygenase enzymes that insert oxygen into the aromatic rings, breaking apart the biphenyl (Mondello et al. 1997).

The rhizosphere, which is the zone of soil immediately adjacent to plant roots, has different kinds of microorganisms, numbers, and/or activities that differ from the bulk soil not associated with the rhizosphere (i.e. nonrhizosphere) (Sylvia et al. 1998). The

leaves and stems of cattails growing in wetlands or estuaries help to vent methane from and transport atmospheric oxygen to the rhizosphere submerged in the anaerobic sediment (Schutz et al. 1989). Thus, the rhizome permeates the anaerobic zone with a relatively large surface area of aerobic plant tissue. In this way, the rhizome, rhizosphere, and adjacent anaerobic sediments may create an aerobic-anaerobic habitat of unique and intense microbial activity.

Methane, a metabolic end product produced by obligate anaerobic methanogenic microorganisms (i.e., Archaea), is used as a substrate for growth by the aerobic methane oxidizing bacteria (Gilbert and Frenzel 1995) called methanotrophs (i.e., Bacteria), which are found in the rhizosphere. In turn, the methanotrophs generate CO_2 (Schutz et al. 1989) which is needed by the methanogens. The removal of methane from the soil and increased availability of CO_2 may enhance the growth of PCB degrading methanogens and increase PCB dechlorination.

The objectives of this study were to examine the effects of PCB contamination on the microbial assemblage found in the sediments associated with a common estuarine macrophyte, *Typha angustifolia*. On a broad level of assessment, changes in the assemblage were measured by quantifying the total number of bacteria, the abundance of the domain Bacteria, and of the domain Archaea in sediment around *Typha* rhizomes treated with and without PCBs. As is the current convention (Singleton 1997) "bacteria" refers to all prokaryotes and "Bacteria" to the group formerly referred to as the eubacteria. Changes in these measurements were associated with dechlorination and degradation of PCBs as monitored by gas chromatography. In this way, changes in the microbial abundance could be related to PCB exposure over a 90 day period and the influence of the rhizosphere habitat as well as the anaerobic sediments (i.e., nonrhizosphere) could be assessed.

MATERIALS AND METHODS

Experimental Design. T. angustifolia (n=70) were removed from North Tivioli Bay at the Hudson River National Estuarine Research Reserve, Annandale, NY on 26 June 1998. About 14 cm of sediment was dug around the plant to a depth of 15.0 cm, the excess soil cut away, and the plants placed into plastic pots (12.5 cm x 12.5 cm x 15.0 cm). Potted plants and sediment were transported to the Louis Calder Biological Field Station greenhouse in Armonk, NY where they were maintained for the duration of the experiment.

At the greenhouse, the soil was washed from the roots of the plants using Hudson River water. The dislodged sediment was allowed to settle overnight and the standing water was removed the following day. To chemically treat the mixtures, equal amounts of sediment was distributed by volume among four containers and mixed with water (2 soil:1 water; v:v) making a slurry. Enough slurry (198 liters) was produced to re-pot two groups of 35 plants with sediment.

Controls consisted of sediment slurry mixed with acetone only ($5.1 \mu l\ g^{-1}$) dry soil weight). Acetone serves as a vehicle to solubilize hydrophobic PCBs in hydrophilic mixtures such as sediment slurry. Given considerable mixing, the hydrophobic PCBs will coat the surface of soil particles. Therefore, the PCB treatment consisted of 35 g of Aroclor 1242 (Accustandard Inc., New Haven, CT) dissolved in 300 ml of acetone to produce a final sediment concentration of $250\ \mu g\ g^{-1}$ dry soil weight. For both sediment slurries, 12 ml of either acetone only or acetone + PCB were added at five min intervals for one hour with constant stirring. To help homogenization of the mixture and adherence of PCBs to particles, the slurries were stirred for an additional 1.5 h. The slurries were allowed to settle for eight hours so that standing water could be removed.

Plant mesocosms were constructed for the control and PCB-treated sediments by re-potting *Typha* in the plastic pots with the treated sediments and placing them in one of two plastic pools (100 cm dia) . The mesocosms were filled weekly with either Hudson

River water from Tivoli Bay mixed with groundwater or with just groundwater. Plants in each mesocosm were divided randomly into either those with an active root (i.e., plants and root was not altered) and those in which the root was inactivated by severing the stem from the rhizome (n=15 for PCB and control each). Because all plants were intact at the time the mesocosms were established, no inactive plants could be sampled at 0 d.

Sample collection and processing. Sampling from each of the four treatments (active root-control, active root-PCB, inactive root-control, inactive root-PCB) was done at 0, 30, 60, and 90 days (n=5). After removing the sediment and rhizome from the plastic pot, sediment was collected from areas closely associated with the rhizome (i.e., rhizosphere) and from areas distally related to the rhizosphere (i.e., nonrhizosphere) and placed in sterile Whirl-pak bags (Nasco, Ft. Atkinson,WI). Rhizosphere sediment was sampled by scraping sediment from the root with a sterile spatula, then using a scalpel to collect the soil closely associated with the root and root hairs. Subsamples were taken for microbial analysis and for dry weight and organic matter determination and the remaining sample was frozen until extraction for GC-ECD analysis could be completed.

For microbial analysis, microorganisms were dislodged from the 2.5 g of wet sediment subsample which had been placed into a sterile 50 ml Falcon tube (Becton Dickson, Franklin Lakes, NJ). The sediment was mixed with 8.4 ml of sodium pyrophosphate and 25 ml of preservative (0.5xPBS and 4% paraformaldehyde) (1X PBS = [7.6 g NaCl, 1.9 g $Na_2HPO_4 \cdot 7H_2O$, 0.7 g Na $H_2PO_4 \cdot 2H_2O$][liter 0.2 μm-filtered $dH_2O]^{-1}$) then sonicated at 5W for 2 min using a probe sonicator (Fisher Scientific, Springfield, NJ). Sonciation conditions to dislodge the greatest number of microorganisms had been empirically determined prior to sample collection. The mixture was allowed to settle for one day after which 20 ml of the supernatant was transferred to a second Falcon tube and stored at 4 °C until analysis. The preserved samples were diluted 400x for microbial quantitation assays.

The percent moisture of a 2.5 g subsample was determined by the difference in sample weight before and after drying overnight at 105 °C in a preashed aluminum weigh pan. The percent organic matter, ash free dry mass (AFDM), was determined by the difference between ashed (550 °C for 24 h) and dried sample weights.

Microbial analysis. The total number of microorganisms was enumerated using 4'6-diamindino-2-phenylindole (DAPI) following a modified method of Porter and Fieg (1980). Briefly, 1 ml of diluted sample was added to 1 ml of sterile filtered dH_2O placed into a 25 mm Millipore filter stand (Millipore Corp, Bedford, MA). The cells were stained with DAPI (Sigma Chemical CO., St. Louis, MO) (0.1 g ml^{-1} final concentration) for 3 min and collected onto a 0.22 μm-pore black polycarbonate filter (Poretics, Livermore, CA) backed with a 0.45 μm-pore cellulose filter (Micron Separations Inc.[MSI], Westborough, MA).

The domains Bacteria and Archaea were enumerated by wholecell hybridization following the quantitative method of Lemke et al. (1997b). A sample volume of 1 ml was filtered onto a 0.2 μm-pore Anodisc (Whatman International LTD, Maidenstone, Eng.) supported on a 0.22 μm-pore acetate (MSI, Westborough, MA) and hybridized with either S-D-Bact-0388-a-A-18 (i.e., EUB338; Amann et al. 1990a) or S-D-Arch-0915-a-A-20 (i.e., ARC915; Amann et al. 1990b) probes, respectively. Probes (40 μl), labeled with Texas red (Genosys, The Woodlands, TX), were applied to the filter surface in a ratio of one part probe (50 ng μl^{-1}) to nine parts hybridization buffer (6X SSC, 0.02 M Tris [pH 7.0], 0.1% sodium dodecyl sulfate [SDS], 0.01% PolyA) and placed into a hybridization chamber (i.e., Petri Dish) to prevent desiccation. Hybridizations with the Bacterial probe were at 48 °C for 4 h and washed twice (0.9 M NaCl, 0.02 Tris [pH 7.2], 0.1% SDS) at the same temperature for 10 min. Hybridization specificity was verified by the inclusion of positive (i.e., *Burkholderia cepacia* [ATCC#25416] (American Type Culture Collection, Manassas, VA) and *Acinetobacter calcoaceticus [ATCC#23055]*) and negative controls (i.e., *Methanosarcina thermophila* [acquired from K. Sowers, Center of

Marine Biotechnology, Baltimore, MD] and *Methanobacterium wolfei* [ATCC #43096]). Controls were fixed and preserved in the same manner as the samples. Hybridizations with the Archaea probe were for 4 hours at 45.5 °C and washed twice at 48 °C for 20 minutes using the same hybridization and wash buffers as listed above. Positive controls were *M. thermophila* and *M wolfei* and negative controls were *B. cepacia* and *A. calcoaceticus*. The *M. wolfei* cultures were grown in a specific media (Winter et al. 1985) under an atmosphere of 80:20, H_2 :CO_2 at 2 atm using gassing cannulas as described by Sowers and Noll (1995).

Chemical Analysis. Equal weights of replicate soil samples were combined to form a composite sample (about 2 g dry weight). Extraction of the PCBs was done using the ultrasonic method as described in EPA standard method 3550B (EPA 1985) for samples with high concentrations. Following extraction, the samples were concentrated to 1 ml using a Turbovap 500 concentrator (Zymark, Hopkinton, MA). The concentrated samples were brought to a 10 ml final volume with hexane and diluted 100 fold in hexane in order to bring the concentration of PCBs in the sample within the range of the calibration curve for the Gas Chromatograph. Diluted samples were injected onto a HP 6890 Gas Chromatograph (Hewlett Packard, San Fernando, CA) with a DB-1701 front column and a DD-17 (30m x 0.32 mm) (JW Scientific, Folsom, CA) rear column and analyzed following EPA method 8081 (EPA 1985). Injection port temperature was 225 °C and the detector temperature was 325 °C. Oven temperature was elevated from 100 °C to 160 °C at 10 °C min^{-1}, then from 160 °C to 200 °C at 2 °C min^{-1} and from 200 °C to 250 °C at 5 °C min^{-1}. Concentration of Aroclor 1242 in the samples was calculated from a 5 point calibration curve ranging from 50 μg $liter^{-1}$ to 2000 μg $liter^{-1}$. Aroclor 1242 was identified based on the retention times of six signature peaks.

Statistical analysis. Data was analyzed using the SigmaStat (ver. 2.0, Jandel Scientific Software, San Rafael, CA). A three-way ANOVA (P = 0.05) was run using the general linear model: y = treatment + time + rhizome and was followed by Tukey's

multiple comparisons test or T-test depending on number of groups per factor. The number of groups in each factor were defined as: treatment = 2 (PCB or control); time = 4 (0 d, 30 d, 60 d, and 90 d), and rhizome = 4 (active rhizome, active nonrhizome, inactive rhizome, inactive nonrhizome). The model was run for each measured variable (y), which was: number of bacteria g $AFDM^{-1}$; number of hybridized Bacteria g $AFDM^{-1}$, and number of hybridized Archaea g $AFDM^{-1}$. A two-way ANOVA ($P<0.05$) was run using the model: y = treatment + zone followed by T-tests for active root samples only. The number of groups in treatment were the same as above and zone = 2 (rhizosphere and nonrhizosphere). To evaluate differences over time, a one-way ANOVA model ($P<0.05$) was run using the model y = time.

RESULTS

Data for the three measurements of the microbial assemblage (i.e., total bacterial number and two Domain-level hybridizations were analyzed using a three-way ANOVA for each variable. Factors for the ANOVA were rhizome activity, chemical treatment, and time. Of the three factors, no significant differences existed in rhizome activity (i.e., amongst active rhizome, active nonrhizome, inactive rhizome, and inactive nonrhizome) for total number of bacteria (P=0.738), Bacteria (domain) (P=1.0) and Archaea (domain) (P=0.497). The remaining analysis (two-way ANOVA) focused on the results obtained from the sample zone (rhizome vs nonrhizome) and treatment (control vs PCB) at each sampling date for data obtained from active root plants only.

The effect of rhizosphere habitat on microbial abundance. Overall, few differences were detected in the microbial analysis of subsamples taken in the rhizosphere and nonrhizosphere zones of the mesocosms (Table 1). Even though the average number of total bacteria ranged from 5.5 $x10^9$ to 13.3 $x10^9$ g $AFDM^{-1}$, no significant difference in abundance existed in sediment collected from the rhizosphere and the nonrhizosphere samples. In contrast, the number of Bacteria was significantly lower in the rhizosphere of control samples and higher in the nonrhizosphere samples at 30 d. Nonrhizosphere

samples had more Archaea cells than the rhizosphere at 0 d in the PCB-treated samples (P = 0.002) and at 90 d in the control samples (P = 0.002).

Changes in microbial numbers in PCB-treated samples. Differences were seen in all three measurements of the microbial assemblage in sediment subsamples collected from *Typha* rhizospheres (Figure 1) and nonrhizospheres (Figure 2) treated with Aroclor 1242 (i.e., PCBs). In both subsample zones, more bacteria were detected in PCB-treated samples at 60 d and 90 d (P<0.001 for both; 2-way ANOVA) (Figure 1A and 2A). Even though no difference was seen between treatments in the nonrhizsophere at 30 d, lower numbers were found in the PCB treatment in rhizosphere subsamples (P=0.042, t-test).

A trend emerged in the change in total number of bacteria over time in the rhizosphere data where numbers in the PCB and control treatment declined and then increased, the PCB treatment increased at 60 d while the control increased at 90 d (Figure 1A). Numbers in the control were similar at 0 d and 30 d, however 60 d numbers were less than 0 d, and 90 d was greater than 60 d (P=0.007, 1-way ANOVA; P<0.05, using the Tukey's Test). In the PCB treatments, total bacterial number in the organic portion of the soil showed an incremental, significant increase from 30 d to 60 d to 90 d (P<0.001, 1-way ANOVA); 0 d numbers were not different from 60 d numbers. There was no significant difference between the bacteria number over time in the non-rhizosphere PCB-treated samples and only 60 d numbers were higher than 0 d in the control samples (P=0.006) (Figure 2A).

More Bacteria were found in the PCB-treated samples in both zones at 60 d (P=0.014, 2-way ANOVA) (Figure 1B and 2B). Samples from the 30 d nonrhizosphere sediment showed an opposite affect with greater Bacteria number in the control samples (P<0.001, t- test). Although an overall treatment difference was found in the 0 d data (P=0.01, 2-way ANOVA), no differences were detected upon t-test analysis. Bacteria number g $AFDM^{-1}$ ranged from 1.5×10^9 to 6.3×10^9 in these samples (Table 1).

Table 1. Comparison of the total number of bacteria, Bacteria, and Archaea in the rhizosphere and nonrhizosphere subsamples of the active cattail root treatments (Numbers = avg x 10^9 g $AFDM^{-1}$; SEM in parentheses; n = 5 unless indicated; *P = 0.05 - 0.01; **P = 0.01 - 0.001; ***P<0.001; ND = no data).

	Active Root: Control		Active Root: PCB	
	Rhizosphere	Nonrhizosphere	Rhizosphere	Nonrhizosphere
Total Number of bacteria				
0 d	8.6 (0.6)	9.5 (1.2)	9.7 (0.9)	10.4 (1.9)
30 d	7.8 (0.6)	8.2 (0.9)	5.5 (0.7)	8.7 (1.7)
60 d	6.4 (0.4)	7.9 (0.1)	12.9 (0.9)	8.1 (0.7)
90 d	8.9 (0.4)	7.9 (0.1)	12.9 (0.9)	13.3 (0.4)[1]
Bacteria				
0 d	3.1 (2.4)	2.6 (0.1)	2.4 (0.2)	2.3 (0.1)
30 d	2.1 (0.2)**	5.7 (0.3)**	2.4 (0.1)***	1.5 (0.1)[1]***
60 d	6.3 (0.1)	5.1 (0.1)	5.2 (1.7)	3.8 (0.3)
90 d	3.7 (0.1)	4.8 (0.1)	4.3 (0.2)	3.6 (0.4)
Archaea				
0 d	1.0 (<0.1)	1.1 (<0.1)	0.9 (<0.1)*	1.3 (<0.1)*
30 d	0.7 (<0.1)	0.6 (<0.1)	1.2 (0.2)	1.3 (<0.1)
60 d	ND	0.8 (0.2)[2]	1.1 (<0.1)[2]	1.0 (<0.1)[2]
90 d	1.0 (<0.1)*	1.4 (<0.1)*	2.3 (0.1)	2.2 (<0.1)

[1] n = 4
[2] n = 2

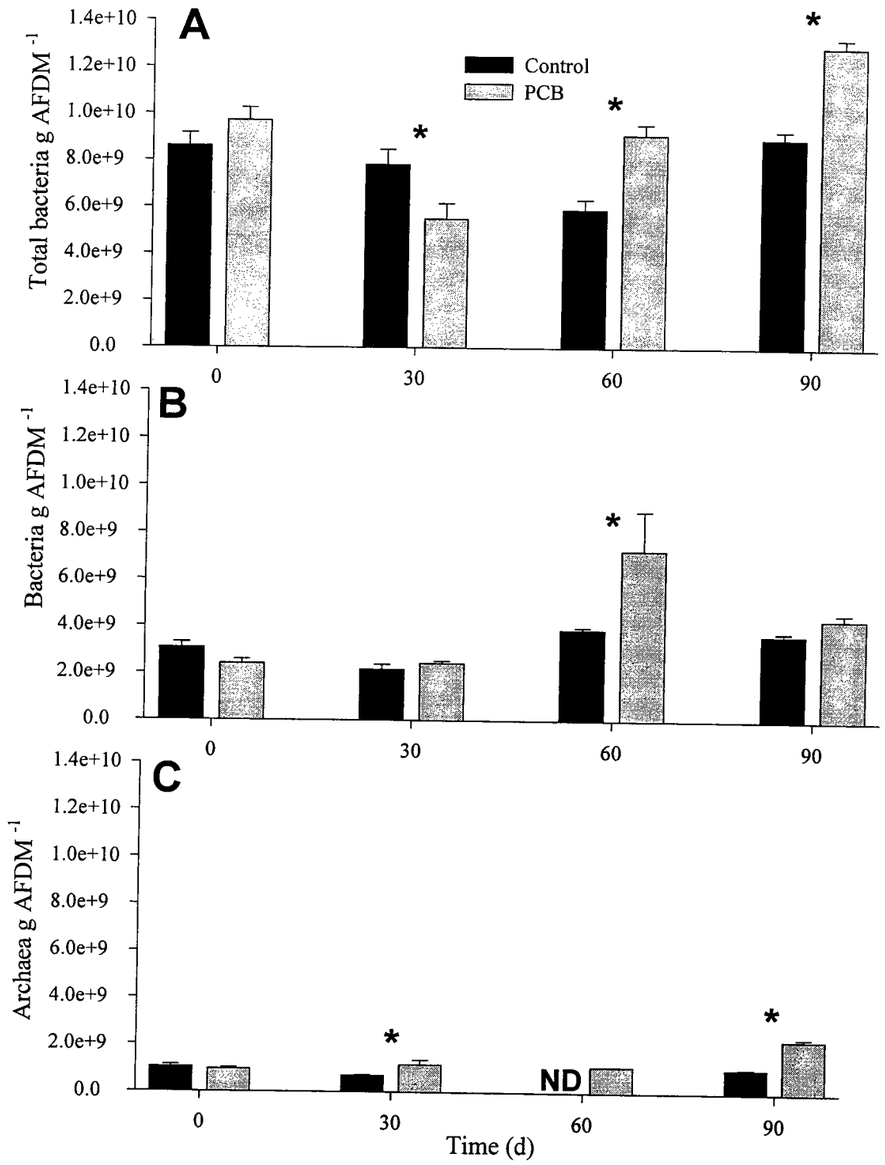

Figure 1. Comparison of the total number of bacteria g AFDM^{-1} (A), Bacteria g AFDM^{-1} (B), and Archaea g AFDM^{-1} (C) in the rhizosphere sediments of an active cattail in PCB-treated and control samples (Bars represent average + SEM (n=5); 60 d Bacteria PCB (n=4) and 90 d Archaea (n=2); ND = no data; *indicates P<0.05).

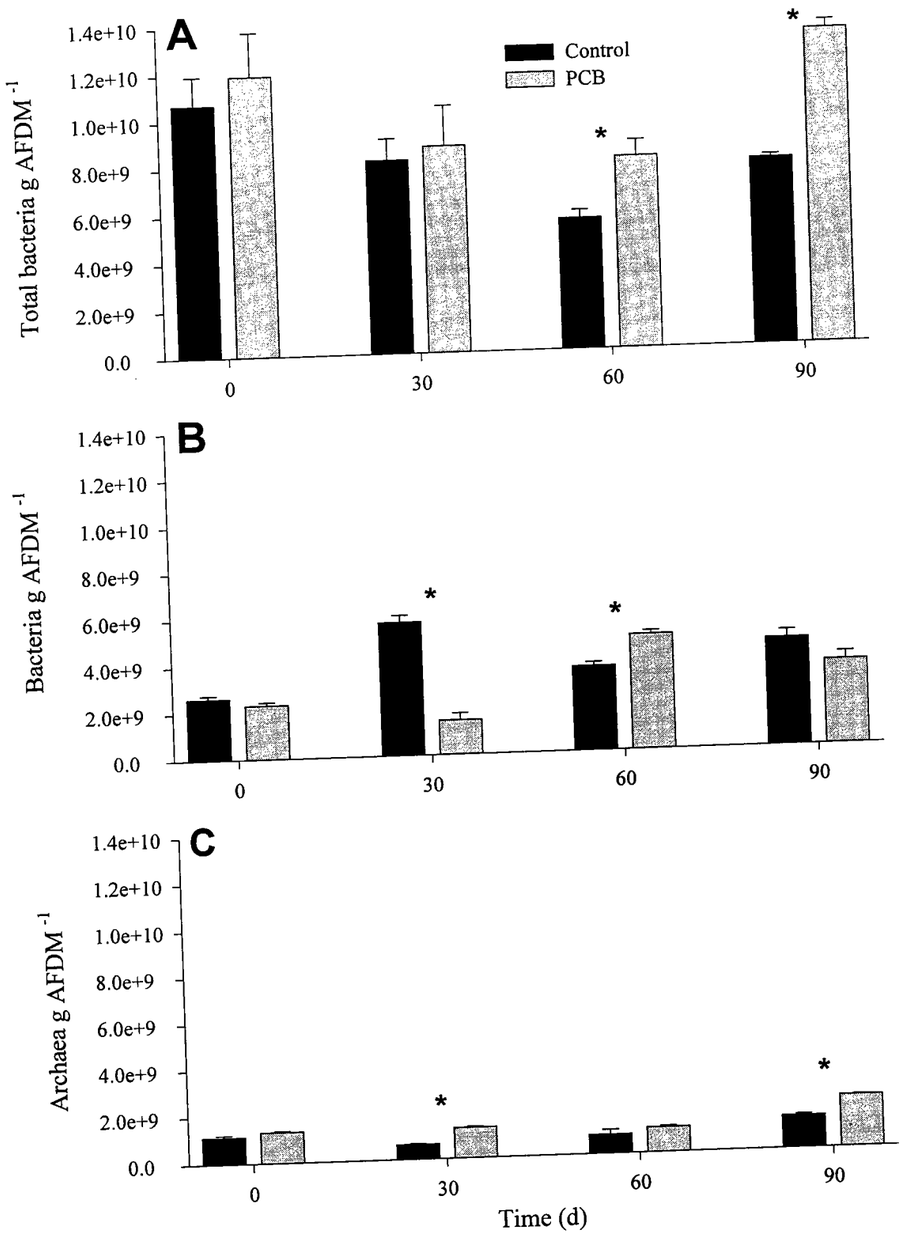

Figure 2. Comparison of the total number of bacteria g AFDM^{-1} (A), Bacteria g AFDM^{-1} (B), and Archaea g AFDM^{-1} (C) in the nonrhizosphere sediments of an active cattail in PCB-treated and control samples (Bars represent average + SEM; for all bars n=5 except 90 d PCB bacteria (n=4), 30 d PCB Bacteria (n=4), and 90 d PCB and control Archaea (n=2); * indicates P<0.05).

With respect to time, Bacteria numbers in the 60 d PCB-treated rhizosphere samples were higher than the 0 d and 30 d samples (P=0.003, 1-way ANOVA) (Figure 1B). In the rhizosphere control, 30 d Bacteria numbers were lower than 60 d and 90 d while 60 d numbers were higher than 0 d and 30 d (P<0.001). In contrast, nonrhizosphere PCB-treated samples had higher Bacteria g $AFDM^{-1}$ at 60 d than at 0 d, 30 d, and 90 d, and 90 d numbers were higher than 0 d (P=0.001). The nonrhizosphere control, 30 d Bacteria numbers were higher than numbers found on all other dates (90 d = 60 d, 0 d = 60 d, P<0.001) (Figure 2B).

There were significantly higher numbers of Archaea in the PCB-treated samples in both zones at 30 d and 90 d (P<0.001 for both, 2-way ANOVA) (Figure 1C and 2C). Average values are shown for 60 d Archaea measurements, however statistical analysis was not possible due to low sample number (n=2). Archaea numbers g $AFDM^{-1}$ ranged from 0.6×10^9 to 2.3×10^9 overall (Table 1). The 90 d rhizosphere PCB treatments were higher than on all other sampling dates for Archaea g AFDM-1 (P<0.001) (Figure 1C). Archaea numbers in the 30 d rhizosphere control samples were lower than the 0 d and 90 d numbers. Archaea numbers in the PCB-treated nonrhizosphere sediments again showed 90 d numbers greater than samples taken at other times and 60 d numbers less than 0 d and 30 d numbers (P<0.001) (Figure 2C). Similarly, 90 d Archaea numbers in the control samples were greater than the 60 d and 30 d samples with 30 d numbers being lower than 0 d (P<0.001).

Chemical Analysis. Six peaks in Aroclor 1242 were monitored for changes in concentration from 0 to 90 d that would indicate dechlorination (Table 2). A sample treated with the vehicle was tested to determine the amount of PCBs present in the soil prior to amendment. PCB concentration in the soil was determined to be 2.6 $\mu g\ g^{-1}$dry weight. Peaks were compared by determining the percent of individual peaks of the total. The peak percentages at 0 d in the rhizosphere and nonrhizosphere were similar. At 90 d the percentage of the peaks changed. Peaks 6 and 4, which have longer retention times

Table 2. Gas chromatography-electron capture detector (GC-ECD) analysis of Aroclor 1242 in control and PCB treated active root samples showing the relative peak percentages over the 90 d experiment time period.

Peak Number[a]	Control	Rhizosphere		Nonrhizosphere	
	0 d	0 d	90 d	0 d	90 d
1	6.5	12.8	18.5	13.9	22.3
2	4.7	14.5	13.7	15.1	13.3
3	6.9	17.3	19.4	17.3	21.3
6	4.5	17.3	14.1	16.9	12.1
4	21.8	19.4	15.7	17.8	13.9
5	56.6	18.7	18.6	19.0	17.1

[a]Peaks are listed in order of retention time from shortest to longest.

and therefore more chlorines decreased. Peaks 1 and 3, with shorter retention times increased. The magnitude of change in the peaks was greater in the nonrhizosphere than in the rhizosphere.

DISCUSSION

The root effect was tested by comparing the microbial assemblage in plants with an active root to the assemblage in samples with an inactive root. In the inactive root, the oxygen exchange capacity of the plants had been removed and therefore no difference should have been expected between samples taken from the rhizosphere and nonrhizosphere because both areas should have both been anaerobic. There were no differences in the abundance of total microbes, Bacteria, or Archaea when the rhizosphere and nonrhizosphere were compared in active and inactive root samples. The active root samples, where oxygen exchange should have still occurred, showed no difference between the rhizosphere and nonrhizosphere although a higher abundance of total bacteria in the rhizosphere was expected given the ability of the root to supply oxygen to aerobic bacteria, particularly methanotrophs (Gilbert and Frenzel 1995)

Although measurements of the microbial assemblage did not show an overall difference between the rhizosphere and nonrhizosphere, several similarities and differences are evident. Judging from the number of bacteria g $AFDM^{-1}$, the microbial assemblage in both the rhizosphere and nonrhizosphere took the first 30 d to establish, or possibly re-establish, itself. Numbers in each successive sampling were greater in both PCB-treated and control samples indicating that conditions for growth were similar at this level of analysis.

However, when comparing the change in the number of Bacteria and Archaea between the rhizosphere and nonrhizosphere, a time and root effect is evident. In the rhizosphere, Bacteria numbers in the PCB-treated samples are significantly higher at day 60 and subside by day 90 at which time the Archaea numbers increase significantly. On the other hand, Bacteria appeared to have established themselves earlier in the

nonrhizosphere sediment than in the rhizosphere (i.e., day 30), and sustained its abundance throughout the remainder of the experiment. This difference may be accounted for in that PCBs could have a toxic affect on Bacteria, thus allowing establishment of a different, Archaeal assemblage or that the rhizosphere inhibits establishment of an anaerobic assemblage.

Although PCB-treated nonrhizosphere samples never reach the numbers of Bacteria seen in the rhizosphere (i.e., 6.3×10^9 at 60 d), there is a significant increase in Bacteria at day 60 similar to what is seen in the rhizosphere. A similar trend is seen in the nonrhizosphere and rhizosphere in the Archaea numbers; 30 d and 90 d PCB-treated samples have greater numbers than the control, perhaps representing a shift or of substrate preference in the microbial assemblage.

Ideally the numbers of Bacteria and Archaea should equal the total numbers of bacteria. The sensitivity of wholecell hybridization is determined by metabolic activity. In general, cells that are metabolically inactive will contain less target rRNA, thereby generating a weaker signal (Hahn et al. 1992, Manz et al. 1993, Poulsen et al 1993) which may not be detected. DAPI, which is a nonspecific stain, targets the DNA and is not dependent on metabolic activity for detection and will therefore detect cells that hybridization will not. On average the Bacteria and Archaea in the active root samples accounted for approximately 52% of the total bacteria indicating that a portion of the total bacterial number are inactive or not prokaryotic.

The results of the PCB analysis indicate that dechlorination occurred in the treated samples in both the rhizosphere and nonrhizosphere. At 0 days the percentages of each peak in the rhizosphere and nonrhizosphere are similar; however, at 90 days the peak percentages have changed. Peaks 1 and 3 with shorter retention times and therefore fewer chlorines increased while peaks 4 and 6 with longer retention times and more chlorine decreased.

Results from the microbial analysis and from the chemical analysis show that both the Bacteria and the Archaea respond to the presence of PCBs and that one or more microorganisms in the sample is able to degrade PCBs. Kim and Rhee (1997) reported similar results and showed that PCB-dechlorinating microorganisms increased two orders of magnitude when exposed to 300 $\mu g\ g^{-1}$ PCBs.

This study shows that root activity does not significantly affect the bacterial assemblage in either the rhizosphere or nonrhizosphere at the domain level of measurement. The Bacteria and Archaea both responded to treatment with PCBs at 60 and 90 d respectively, and changes in the Aroclor 1242 were observed from 0 to 90 d demonstrating that some portion of the assemblage is able to degrade PCBs. A study by Lemke et al (1997a) showed that while no difference in total bacteria occurred in polluted versus unpolluted stream waters, differences at the species level showed significant changes in abundance. In order to specifically describe the microorganisms responsible for PCB degradation, further investigation should be done at the genus and species level in order to accurately describe the microbial ecology associated with the rhizosphere, how root activity effects the ecology, and what effects this interaction has on the degradation of PCBs.

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