ELSEVIER

Soil Biology & Biochemistry 39 (2007) 735–743

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Plant secondary metabolites, biphenyl, and hydroxypropyl-β-cyclodextrin effects on aerobic polychlorinated biphenyl removal and microbial community structure in soils

Wensui Luo*, Elisa M. D'Angelo, Mark S. Coyne

Soil and Water Biogeochemistry Laboratory, Plant and Soil Sciences Department, University of Kentucky, Lexington, KY 40546

Received 23 September 2005; received in revised form 12 September 2006; accepted 21 September 2006

Abstract

Biphenyl and several plant secondary metabolites (PSM) (carvone, isoprene, limonene, naringin, and coumarin) and surfactant (hydroxypropyl-β-cyclodextrin, (HP-β-CD)) have been shown to improve aerobic polychlorinated biphenyl (PCB) removal by several bacterial species. The objective of this study was to determine whether these treatments also affect PCB removal and microbial community structure in a high organic matter soil (Pahokee soil series with 67% organic matter) and low organic matter soil (Woolper soil series with 6% organic matter), as determined by monitoring changes in PCB levels and phospholipid fatty acids (PLFA) profiles in laboratory microcosms amended with these compounds. Biphenyl enhanced di-chlorinated and tri-chlorinated biphenyl removal in both soils, but PSM did not improve removal of these congeners. On the contrary, HP-β-CD decreased PCB removal when used in combination with biphenyl. Two-way analysis of variance indicated that HP-β-CD significantly increased tetra- and penta-chlorinated biphenyl removal from the high organic matter soil, but not from the low organic matter soil. Principal components analysis of PLFA data indicated that HP-β-CD increased proportions of 18:1ω7c associated with Gram-negative bacteria, but decreased 10me16 and 10me17 lipid associated with Gram-positive bacteria, while biphenyl and PSMs had no detectable effects on soil microbial communities. PCB removal was not correlated to any PLFA. In conclusion, PSM previously shown to enhance PCB removal in soil-free systems were not effective in two divergent soils evaluated in this study, and HP-β-CB had increase, decrease, or no effect on PCB removal depending on types of PCB congeners, soils, and co-amendments.

Keywords: Polychlorinated biphenyls; Plant secondary metabolites; Surfactant; Phospholipid fatty acid; Microbial community structures

1. Introduction

Polychlorinated biphenyls (PCB) are a group of compounds previously used in transformers, capacitors, paints, and other industrial appliances. PCB are widely disseminated in the US, and several major rivers have fish consumption advisories due to PCB contamination. Ingestion of PCB can lead to serious health problems such as impaired reproduction, immune deficiency, cancer, and skin and liver damage. Due to the widespread nature of PCB contamination and potential health risks, PCB removal from the environment has become a national priority (ATSDR, 2003). Bioremediation is considered a viable PCB removal strategy because many microorganisms can degrade PCB in diverse environments, including soils and sediments (Focht and Reineke, 2002).

Amending soils with biphenyl can enhance PCB degradation. For example, Focht and Brunner (1985) observed that biphenyl enhanced PCB removal from soils, and several researchers have successfully applied this strategy to bioremediate PCB contaminated environments (Harkness et al., 1993; Fava and Bertin, 1999; Singer et al., 2001). Biphenyl is believed to promote PCB degradation by inducing PCB-degrading enzyme synthesis and microbial growth (Donnelly et al., 1994). Unfortunately, biphenyl may not be an ideal compound for enhancing in situ PCB remediation, because of its own toxic effects and high cost (Park et al., 1999; Tandlich et al., 2001).

*Corresponding author. Current address: Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA.
E-mail address: lwens2@uky.edu (W. Luo).

0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.09.019

Many other compounds have been screened for their capacity to increase PCB degradation. For example, Donnelly et al. (1994) found that naringin and coumarin not only supported growth of PCB degraders *Corynebacterium* spp. MB1, *Burkholderia cepacia* LB400 (previously known as *Pseudomonas putida* LB400), and *Ralstonia eutropha* H850 (previously known as *Alcaligenes eutrophus* H850), but also fostered PCB metabolism by these microorganisms. Gilbert and Crowley (1997) discovered that an extract from spearmint (*Mentha spicata*) effectively induced *Arthrobacter* spp. B1B to degrade Aroclor 1242. The effective ingredients in the spearmint extract were identified as carvone and limonene. They suggested that carvone induced PCB cometabolism, which may function as a detoxification mechanism by this strain. Hernandez et al. (1997) achieved complete removal of Aroclor 1242 in 6 months by amending soils with orange peels, eucalyptus leaves, pine needles, and ivy leaves, but the effective ingredients in those plant residues were not identified. In these studies, it was postulated that plant secondary metabolites (PSM) may function as natural inducers of PCB degrading enzyme, and may be more environmentally friendly alternatives to biphenyl for this purpose.

Low PCB bioavailability is a major obstacle that must be overcome before biotransformation can be considered a viable PCB removal option. PCB bioavailability is influenced by its low water solubility ($<0.5\,mg\,kg^{-1}$) and strong adsorption to soil organic matter ($Log\,K_{ow}>5$) (Chou and Griffin, 1987). Bioavailability can be improved by employing surfactants such as Triton X-100; however, many surfactants could have limited field applicability because they are toxic and/or recalcitrant to degradation (Fava, 1996; Fava and Gioia, 1998; Billingsley et al., 1999; Singer et al., 2000). Fava and his colleagues found that cyclodextrins, a group of non-toxic, biodegradable annular glucose-oligosaccharides commonly used in food and pharmaceutical industries, significantly enhanced aerobic PCB biodegradation in bioreactors. They suggested that cyclodextrins form PCB water-soluble inclusions and liberate PCB molecules from soil particles, thus cyclodextrins could be used to improve PCB bioavailability and bioremediation in soils (Fava and Grassi, 1996; Fava et al., 1998, 2002). While cyclodextrins can increase PCB bioavailability, they may also be utilized as carbon and energy sources by microbial populations, which could enrich for PCB degraders and non-degraders and/or repress PCB degradation enzyme synthesis. The opposing nature of these processes needs to be investigated before cyclodextrins can be widely used in bioremediation treatment.

Little is known about soil microbial populations responsible for PCB degradation or how they are affected by bioremediation treatments. Several studies have described the composition of microbial communities at PCB contaminated sites using culture dependent and independent techniques (Lloyd-Jones and Lau, 1998; Nogales et al., 2001). However, there have been few if any reports on microbial population changes during PCB degradation in soils and this knowledge could be very useful to improve bioremediation strategies.

In this study, we examined the effects of biphenyl and several PSM (carvone, isoprene, limonene, naringin, coumarin) and surfactant (hydroxypropyl-β-cyclodextrin (HP-β-CD)) on PCB removal from two soils with different organic matter content. The soil microbial communities were monitored by PLFA methods. The hypothesis of the study was that biphenyl, PSMs and HP-β-CD affect soil microbial community and lead to changes in PCB removal. The results of this study will be useful for developing PCB remediation strategy with these tested amendments.

2. Materials and methods

2.1. Soils and chemicals

Woolper soil, a fine, mixed, mesic Typic Argiudoll (National Cooperative Soil Survey, 2005a), was collected from the top 0 to 15 cm soil at Spindletop Farm, University of Kentucky, Lexington, KY. Pahokee soil, a euic, hyperthermic Lithic Haplosaprist (National Cooperative Soil Survey, 2005b), was collected from the top 0 to 15 cm of a sugarcane plantation at the Belle Glade Research Station, Belle Glade, FL. These soils were chosen because they are typical soil of these geographic regions and have different organic matter content, which can significantly influence PCB biodegradation. Soils were sieved through a 4 mm mesh to remove large stones and debris and stored moist at 25 °C before use. Selected physical and chemical properties of soils were determined by the Soil Testing Lab of the Regulatory Services Unit at the University of Kentucky, Lexington, KY (Table 1). Soil texture and particle size analysis were determined by the pipette method. Soil pH was determined in a 1:1 soil/water ratio. Organic matter and total nitrogen of oven-dried soils (60 °C) were determined with a LECO combustion

Table 1
Selected physical and chemical properties of Pahokee and Woolper soils used in the study

Soil property	Units	Pahokee soil series	Woolper soil series
Soil texture		N.A.[a]	Silt loam
Sand	%	60.5	18
Silt	%	31.9	71
Clay	%	7	11
PH		4.8	6
Organic matter	%	67	6
Total nitrogen	%	2.8	0.4
Mehlich III P	$mg\,kg^{-1}$	8.5	125
Mehlich III K	$mg\,kg^{-1}$	126	113
Mehlich III Ca	$mg\,kg^{-1}$	5018	2654
Mehlich III Mg	$mg\,kg^{-1}$	312	141

[a]N.A. The textural assignment is not applicable to soils in the Histosol order.

instrument (LECO, St. Joseph, MI). Phosphorus, K, Ca, and Mg were extracted with Mehlich III reagent and determined with inductively coupled plasma spectroscopy (ICP) (Thermo Jarrell Ash Model 61, Franklin, MA).

Chlorinated biphenyl (CB) congeners, including di-CB (2-2 CB, 2-3 CB, 2,3- CB, 4-4 CB), tri-CB (2,4-4 CB), tetra-CB (2,5-2,5 CB, 2,4-2,4 CB, 2,3-2,5 CB, 2,3-2,3 CB, 3,4-3,4 CB, 2,5-3,4 CB, 2,4-3,4 CB), penta- CB (2,4,5-2,5 CB, 2,4,5-2,3 CB, 2,3,4-2,5 CB, 2,4,5-2,5 CB, 2,4,6-2,4 CB), and hex-CB (2,4,5-2,4,5 CB) were obtained from AccuStandard Inc. (St. Louis, MO). Carvone, limonene, naringin, coumarin, isoprene, biphenyl, and HP-β-CD were obtained from Aldrich Chemical Inc. (Milwaukee, WI). Hexane, acetone, dichloromethane and other organic reagents were of optimal grade and obtained from Fisher Scientific Inc. (Pittsburgh, PA).

2.2. PCB removal in soils

PCB removal was investigated in 96 microcosms with Woolper soil (50 g dry weight/microcosm) and Pahokee soil (30 g dry weight/microcosm) in 200 mL Mason glass jars. Different amounts of soil were used because they had significantly different bulk densities and similar volume of soil was used. Microcosms were spiked with 1 mL (Woolper) or 0.6 mL (Pahokee) stock PCB solution, which consisted of a mixture of 17 PCB congeners in acetone and hexane. The solvent was evaporated by placing the glass jars in a fume hood for 16 h. Because there were significant difference in evaporation pressure (EP) between solvent ($EP_{hexane} = 20.2$ kPa, $EP_{acetone} = 30.6$ kPa at 25 °C) and solute ($EP_{PCB} < 6.7 \times 10^{-5}$ kPa at 25 °C), the PCB stayed while hexane and acetone evaporated. The final PCB concentration in the soil microcosms was 10 mg kg^{-1} for each PCB congener. Congeners and concentrations were selected because they represent major chlorine substitution patterns and levels found in commercial PCB mixtures and contaminated sediments (Bedard et al., 1986). Six microcosms for each soil type were randomly selected and amended with either carvone, isoprene, limonene, naringin, coumarin, or biphenyl (inducer treatment) to give 2 mmol chemical kg^{-1} soil on days 1 and 40 after adding PCB to soils. Three microcosms for each inducer treatment were randomly selected and amended with HP-β-CD to achieve 30 mmol kg^{-1} soil on day 1. After amendment, soils were mixed with a stainless steel spatula to homogenously distribute the amendments. Amendment types and levels were selected based on results from previous studies (Donnelly et al., 1994; Fava et al., 1998).

Three additional microcosms for each soil type were prepared with either distilled water (no amendment treatment) or by autoclaving and adding Hg (121 °C, 0.10 MPa, 1 h on 2 separate days followed by $HgCl_2$ additions to achieve 100 mg Hg kg^{-1} soil) (Wolf and Skipper, 1994). These treatments were included to evaluate treatment effects on biotic and abiotic PCB removal and microbial populations. Soil moisture was adjusted to field capacity by adding distilled water. The gravitational water content of Woolper soil was 55% at the beginning of incubation and decreased to 46% by the end of incubation. The water content of Pahokee soil was kept around 110% during the incubation. Microcosms were sealed with the Mason jar lids to reduce PCB and inducer volatilization losses, and incubated in the dark at 25 °C. Samples were aerated at weekly intervals by opening the containers and mixing the soils with a sterile spatula. An aliquot of the beginning PCB-contaminated samples ($PCB_{initial}$) were preserved at −20 °C and extracted at the same time with the samples from the end of 80 days incubation (PCB_{final}). Percent PCB removal was determined by

$$\text{Percent removal} = [(PCB_{initial} - PCB_{final})/PCB_{initial}] \times 100.$$

2.3. PCB removal kinetics

In a separate experiment, PCB removal kinetics was evaluated in Woolper soil amended with biphenyl and naringin, which both supported PCB degrader growth in the previous study (Donnelly et al., 1994), but had different effects on PCB removal in the microcosm study described in the previous section. Microcosms were created with 20 g dry weight soil in Mason glass jars spiked with a mixture of 17 PCB congeners at 5 mg kg^{-1} soil each. Biphenyl and naringin were added to soil at 4 mmol kg^{-1} soil. Soil without biphenyl and naringin amendment was used for comparison. At 15, 40, 80, 120 and 166 days, three replicates were destructively sampled and stored at −20 °C before PCB extraction. A first-order degradation model [$C_t = C_{initial}\, e^{-kt}$, C_t (mg kg^{-1}): the PCB concentration at the time t, $C_{initial}$ (mg kg^{-1}): the initial PCB concentration, k (day^{-1}): the first order constant, and $e = 2.302$] was used to fit the PCB concentration in soil using the function of Microsoft Excel software (Microsoft Inc., Redmond, WA).

2.4. PCB extraction and analysis

Five g soil were extracted with 20 mL 1:5 acetone:hexane (volume:volume) solution in a 120 mL serum bottle sealed with Teflon-lined septum and aluminum crimp on an orbital shaker at 200 rpm for 1 h at 30 °C. After centrifugation at 1800g for 5 min, the hexane phase was splitless injected (250 °C) into a Shimadzu 14A gas chromatograph equipped with a 30 m HP-1 column (0.25 μm film thickness and 0.32 mm i.d.) (J & W Scientific, Folsom, CA) and an electron capture detector (300 °C), using helium as carrier gas, N_2 as makeup gas, and oven temperature program of 100 °C for 1 min, increasing at 2.5 °C min^{-1} to 240 °C and holding for 5 min. PCB extraction efficiencies using this protocol were >95% for Woolper soil and >90% for Pahokee soil as determined by comparison with authentic standards (AccuStandard Inc., St. Louis, MO).

2.5. PLFA analysis

Phospholipids were extracted from soil samples by the single-phase dichloromethane-methanol-buffer according to the procedure outlined by Findlay and Dobbs (1993). Briefly, soil lipids were obtained by extracting 5 g soil overnight with buffer containing 7.5 mL dichloromethane, 15 mL methanol, and 3 mL phosphate buffer (50 mM, pH 7.4). Organic and aqueous phases were separated by adding additional 7.5 mL dichloromethane and 7.5 mL water and allowing phases to separate overnight at 4 °C. Total lipids in the organic layer (dichloromethane) were separated into neutral lipid, glycolipid, and phospholipid by sequential elution through a silicic acid solid phase extraction column (J.T. Baker Inc., Phillipsburg, NJ) using chloroform, acetone, and methanol. Phospholipids were converted to fatty acid methyl esters (FAMEs) by mild alkaline esterification. The FAMEs were purified with octadecyl (C18) resin (J.T. Baker Inc., Phillipsburg, NJ). Purified FAMEs were analyzed with a Shimadzu 14A GC equipped with flame ionization detector (FID) and HP-1 column. Injector and detector temperatures were 250 and 260 °C, respectively. The carrier gas was helium (100 kPa) and the detector makeup gas was N_2 (150 kPa). Samples were splitless injected by a Shimadzu AOC20i automatic sampler. The temperature program was as follows: 80 °C for 1 min, increasing at 2.5 °C min^{-1} to 240 °C and holding for 15 min. Peak identification and quantification were determined by comparison with FAME standards from Supelco Inc. (Bellefonte, PA). The quantification and identification of FAME peaks were confirmed with gas chromatography-mass spectroscopy (GC-MS) analysis by an independent lab (Microbial Insights Inc., Rockford, TN).

The prevalence of microbial groups in soil samples was indicated from the mole percentage of signature PLFA as follows: Gram-positive bacteria (i15, i16, i17, a17, 10me16, 12me16, and 10me17), Gram-negative bacteria (16:1ω9, 16:1ω7, i17:1ω7, cy17, 18:1ω7t, 18:1ω7c, and cy19), actinomycetes (10me18), bacteria (16:1ω5, a15, b18:1, 18, and b19:1), fungi (18:1ω9 and 18:2ω6), and other eukaryotes (b20:1, 22:1, 22:6ω3) (DeForest et al., 2004).

2.6. Statistical analysis

Analysis of variance (ANOVA) was used to determine significance of inducer, surfactant, and their interaction effects on PCB degradation, microbial enumeration, and PLFA content. The percentage data were arcsine square root-transformed before evaluated by ANOVA. Inducer effects on PCB removal were compared with the least significant difference (LSD) multiple comparison method. Surfactant effects on PCB removal with different inducers were compared with Student's *t*-test. The difference in PCB removal rates were determined by Student's *t*-test. Treatment effects on microbial community structure were compared by principal component analysis (PCA) of PLFA data. Statistical analysis was performed with SAS package 6.0 (SAS Inc., Cary, NC).

3. Results

3.1. Biphenyl and PSM effects on PCB removal

Considerable amounts of PCB were removed in all treatments during the 80-days incubation. The extent of PCB removal, however, depended on types of soil, PCB congeners, and amendments (Table 2). In Woolper soil, no inducer treatment removed 18.4% of overall PCB, which was an average cross all congeners. On a congener basis,

Table 2
Effects of biphenyl and several plant secondary metabolites on polychlorinated biphenyl (PCB) removal in Woolper and Pahokee soil

PCB	Autoclaved	No inducer	Biphenyl	Naringin	Coumarin	Limonene	Isoprene	Carvone	*P*-values
	% PCB removal ± one standard deviation ($n = 3$)								
Woolper									
Di-PCB	37.8 ± 19.1	37.8 ± 2.9a	70.7 ± 4.9b	46.8 ± 9.7a	45.8 ± 4.7a	47.9 ± 11.0a	45.8 ± 3.4a	43.4 ± 3.6a	<0.01
Tri-PCB	27.2 ± 24.5	21.3 ± 2.2	38.5 ± 9.5	25.3 ± 12.3	26.5 ± 5.6	30.2 ± 13.9	27.9 ± 3.4	23.6 ± 8.1	0.38
Tetra-PCB	21.2 ± 21.8	11.0 ± 2.6	18.4 ± 12.8	12.5 ± 14.0	13.6 ± 5.1	17.8 ± 17.5	15.6 ± 4.2	15.6 ± 6.0	0.95
Penta-PCB	21.8 ± 21.5	10.1 ± 2.8	16.4 ± 11.7	10.3 ± 14.3	12.1 ± 5.0	16.1 ± 18.6	14.3 ± 3.6	14.1 ± 5.7	0.97
Hex-PCB	28.3 ± 22.3	18.4 ± 6.3	24.7 ± 8.4	17.4 ± 13.4	20.8 ± 6.5	22.5 ± 16.4	22.3 ± 1.2	22.2 ± 4.8	0.93
Average PCB	26.1 ± 21.5	18.4 ± 2.9	32.3 ± 10.1	21.4 ± 12.9	22.3 ± 5.1	25.7 ± 15.9	23.8 ± 3.7	22.9 ± 5.4	0.67
Pahokee									
Di-PCB	42.4 ± 1.0	44.9 ± 8.3	62.9 ± 4.0	50.0 ± 10.9	44.9 ± 8.6	47.6 ± 6.2	48.5 ± 10.7	40.6 ± 9.7	0.13
Tri-PCB	40.7 ± 1.5	41.3 ± 8.7a	67.2 ± 4.7b	47.4 ± 12.7a	38.7 ± 9.8a	41.8 ± 6.3a	44.1 ± 13.8a	32.6 ± 12.6a	0.03
Tetra-PCB	37.9 ± 3.2	39.9 ± 7.8	45.2 ± 2.9	44.4 ± 11.6	36.6 ± 9.8	39.5 ± 5.8	41.1 ± 11.9	29.6 ± 14.2	0.52
Penta-PCB	37.3 ± 3.8	38.5 ± 7.8	42.6 ± 3.1	42.8 ± 11.5	34.6 ± 10.3	37.9 ± 5.3	39.5 ± 12.5	27.1 ± 14.4	0.50
Hex-PCB	52.9 ± 17.6	52.1 ± 29.1	56.6 ± 23.6	53.7 ± 26.2	48.2 ± 31.5	51.9 ± 7.4	50.4 ± 29.2	49.6 ± 24.3	1.00
Average PCB	39.1 ± 2.7	41.5 ± 9.0	50.3 ± 3.3	46.0 ± 12.2	38.4 ± 9.6	41.3 ± 5.8	42.8 ± 11.8	32.9 ± 13.5	0.47

Each value represents the mean of three replications ± one standard deviation averaged for between one and seven congeners with the same degree of chlorination (see text for a description of congeners used in the study). Treatment effects were first evaluated by one-way analysis of variance after arcsine square root-transforming the data (excluding the autoclaved treatment). In cases where $P < 0.05$, treatment effects were further evaluated by the least significant difference test, with significant treatment differences indicated by different letters following mean values.

dichlorinated biphenyls were removed to a greater extent (37.8%) than higher chlorinated congeners (10.1–21.3%). Among six inducers tested, biphenyl caused the greatest overall PCB removal (32.3%). The degree of enhanced PCB removal by biphenyl depended on the type of PCB congener. Biphenyl significantly enhanced di-CB removal, but did not significantly improve removal of higher chlorinated congeners. PCB removal from soils amended with naringin, coumarin, isoprene, carvone, and limonene treatments were similar to each other and not significantly higher than control soils.

In Pahokee soil with no amendments, between 38% and 52% of PCB were removed (Table 2). Among the treatments, biphenyl significantly increased PCB removal compared to the control, which was primarily due to enhanced di-CB and tri- CB removal. PCB removal from soils amended with naringin, coumarin, isoprene, carvone, and limonene were not significantly different from control soils.

Considerable amounts of PCB were also removed from autoclaved and Hg-treated soils (21–53%). Extensive fungal growth was observed in these treatments, which could explain PCB removal in these microcosms.

3.2. Surfactant effects on PCB removal

HP-β-CD also had significant effects on PCB removal depending on the types of soil, PCB congeners, and other amendments (Table 3). In both soils in the absence of an inducer, HP-β-CD treatments did not significantly increase or decrease PCB removal. Compared with the biphenyl only treatment, HP-β-CD plus biphenyl treatment significantly reduced removal of di-CB in Woolper soils and reduced removal of di-CB and tri-CB in Pahokee soils. On the other hand, in carvone-amended Pahokee soil, HP-β-CD significantly increased removal of tetra- and penta-PCB. No significant effect of HP-β-CD on highly chlorinated PCB removal was found in Woolper soils.

3.3. PCB removal kinetics in Woolper soil

Biphenyl and naringin were selected to compare the inducer effects on PCB removal kinetics during 166-days incubation. Biphenyl was selected because it had the most significant effect in enhancing PCB removal in Woolper soil among the treatments. Naringin was tested because it had limited effect on PCB removal, but was similar to biphenyl supporting PCB degrader growth in a previous study (Donnelly et al., 1994). At the end of 166-days incubation, PCB removal on average was around 45.8% in soil without amendment, which was higher than the average removal of 18.4% in the 80-day incubation previously described. The PCB removal followed a first order degradation model. Correlation coefficients (r^2) ranged from 0.93 to 0.98 for lower chlorinated PCB (di-CB and tri-CB) and from 0.55 to 0.83 for higher chlorinated PCB (tetra- CB, penta- CB, and hex- CB) (Table 4). The PCB removal rates ranged between 0.016 and 0.022 $days^{-1}$ for di-CBs and <0.001 day^{-1} for hex-CB. On a congener basis, biphenyl increased di- CB, and tri-CB removal rates compared to naringin and the control, but had no significant effect on tetra-CB, penta-CB, and hex- CB.

3.4. Treatment effects on soil microbial communities

The total PLFA concentrations in Woolper soil ranged from 56 to 143 nmol g^{-1}. PCA of PLFA indicated that the microbial communities in Woolper soil were not changed by the inducer treatment, but were affected by HP-β-CD

Table 3
Hydroxylpropyl-β-cyclodextrin (HP-β-CD) effects on polychlorinated biphenyl (PCB) removal in Woolper and Pahokee soil relative to treatments without HP-β-CD, which were provided in Table 2

PCB	No inducer	Biphenyl	Naringin	Coumarin	Limonene	Isoprene	Carvone	*P*-values
	% PCB removal change related to treatments without HP-β-CD							
Woolper								
Di-PCB	−5.9	−28.4*	−20.8	−6.5	−2.9	−18.5	−7.2	0.02
Tri-PCB	17.0	−18.4	−0.1	23.1	23.9	1.2	29.4	0.56
Tetra-PCB	32.5	6.0	41.8	62.0	59.0	31.9	51.6	0.17
Penta-PCB	31.9	8.0	72.6	74.4	69.9	44.6	65.7	0.12
Hexa-PCB	15.7	3.8	51.2	25.7	46.4	29.5	48.2	0.09
Pahokee								
Di-PCB	2.6	−25.9*	−1.0	5.7	2.9	−1.8	31.0	1.00
Tri-PCB	7.3	−31.9*	−2.0	14.3	8.5	0.4	55.8	0.67
Tetra-PCB	6.5	−1.2	4.4	19.9	13.2	6.8	68.9*	0.04
Penta-PCB	10.8	3.7	8.6	29.0	20.1	11.0	85.3*	0.01
Hexa-PCB	10.6	2.9	14.5	23.8	15.5	18.1	32.1	0.29

The mathematic calculation was based on: percentage of PCB removal related to treatments without HP-β-CD (%) $= 100 \times$ (average PCB removal with HP-β-CD—average PCB removal without HP-β-CD)/average PCB removal without HP-β-CD. Significant HP-β-CD effects are indicated by "*" as determined by pairwise comparison of treatment with HP-β-CD and treatment without HP-β-CD for each inducer using Student's *t*-test ($t<0.05$). HP-β-CD effects on PCB removal across all treatments were evaluated by two-way ANOVA, and are indicated by *P*-values.

Table 4
Biphenyl and naringin effects on the first order PCB removal constants k (day^{-1}) in Woolper soils

PCB	No amendment		Biphenyl		Naringin	
	$k \pm SD(\times 10^{-3})$	r^2	$k \pm SD(\times 10^{-3})$	r^2	$k \pm SD(\times 10^{-3})$	r^2
Di-CB	16.12±0.71a	0.98	21.84±1.46b	0.97	17.49±1.42a	0.95
Tri-CB	4.26±0.32a	0.96	5.85±0.36b	0.97	4.82±0.46a	0.93
Tetra-CB	2.10±0.41a	0.79	2.04±0.37a	0.81	2.61±0.44a	0.83
Penta-CB	1.48±0.37a	0.70	1.23±0.35a	0.66	2.08±0.42a	0.78
Hex-CB	1.03±0.39a	0.55	0.75±0.37a	0.45	1.64±0.44a	0.68

SD stands for the standard deviation with three replicates. CB stands for chlorinated biphenyl. The *k*-values within a row followed by the same letter are not statistically different based on a Student's *t*-test ($t<0.05$).

amendment (Fig. 1A). The PCA plot showed that microcosms gathered into two groups based primarily on the presence and absence of HP-β-CD. One group was composed of 20 units, and 18 of them were treatments without HP-β-CD amendment. The other group was composed of 22 units, and 19 of them were treatments with HP-β-CD amendment. PLFA 10me16, 10me17, 16:1ω9, i17:1ω7, 18:1ω7, 16:1ω5, b18:1a, b19:1a, 21/22:6ω3 had most of their influences on the first principal component (PC1) with absolute loading scores ranging from 0.20 to 0.31 while i15, i16, i17, a17, 16:1ω7, 18:1ω5, a15, 10me18, 18:1ω9 had most of their influences on the second principal component (PC2) with absolute loading scores ranging from 0.21 to 0.40. Lipid by lipid comparison indicated that HP-β-CD significantly increased the proportion of 18:1ω7c 18:0, and b19:1a, and decreased the proportion of 10me16, 10me17, and 16:1ω5; other lipids were relatively unaffected by HP-β-CD (Fig. 2).

The total PLFA concentrations in Pahokee soil ranged from 104 to 179 nmol g^{-1}. Similar to what was found with Woolper soil, PCA of PLFA data showed that treatments clustered into two groups based on the presence and absence of HP-β-CD, but not on other amendments (Fig. 1B). PLFA i15, i16, 10me16, a17, 16:1ω7, 18:1ω5, cy19, 16:1ω5, a15, 18:0, and 10me18 had most of their influences on PC1 with absolute loading scores ranging from 0.20 to 0.30 while i17, 10me17, 16:1ω7, i17:1ω7, 18:1ω7, b18:1a, 18:1ω9, and b20:1 had most of their influences on PC2 with absolute loading scores ranging from 0.24 to 0.41. Treatments with HP-β-CD had significantly higher proportions of 18:1ω7c, lower proportions of i17, 10me17, and b20:1, and no effects on other PLFA (Fig. 2). No significant correlations were found between PLFA concentrations and PCB removal.

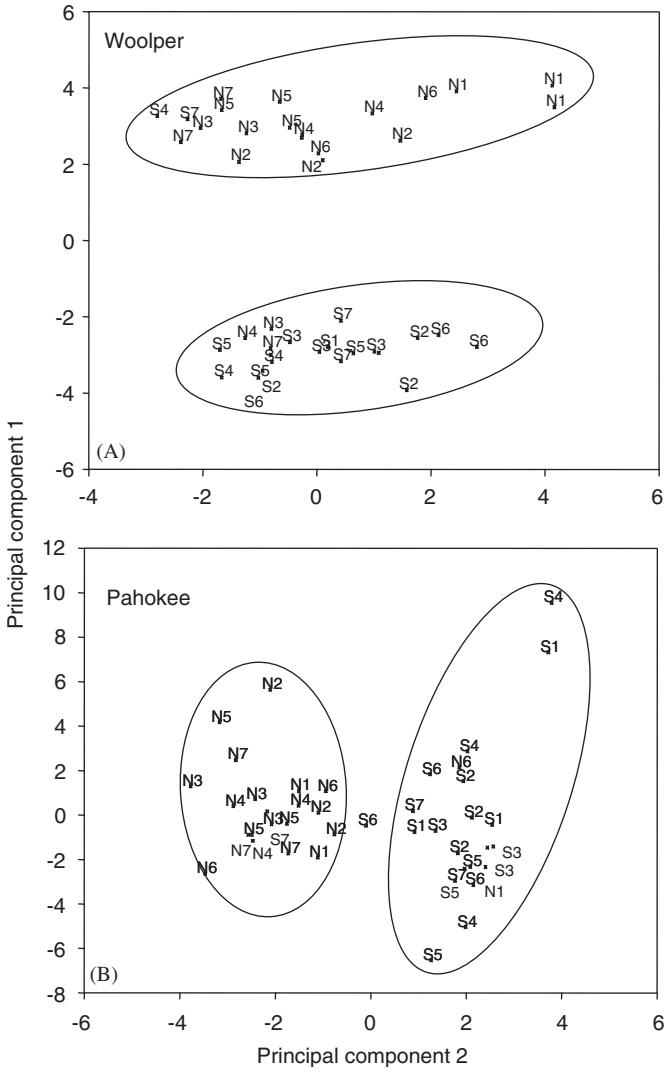

Fig. 1. Principal component analysis (PCA) plots of phospholipid fatty acids (PLFAs) in (A) Woolper soils and (B) Pahokee soils 80 days after being amended with biphenyl, plant secondary metabolies, and hydroxypropyl-β-cyclodextrin. Phospholipid fatty acids contributing to more than 1% of total lipids were used in the PCA. "S" and "N" signify treatments with and without hydroxypropyl-β-cyclodextrin, respectively. The numbers identify various treatments: 1—carvone; 2—naringin; 3—limonene; 4—coumarin; 5—isoprene; 6—biphenyl; 7—no amendment. For the Woolper soil, principal component axes 1 and 2 explains 34% and 20% of the variance, respectively. For Pahokee soil, principal component axes 1 and 2 explains 37% and 20% of the variance, respectively.

4. Discussion

4.1. Treatment effects on PCB removal

This study evaluated the effects of biphenyl, several PSMs, and surfactant HP-β-CD on indigenous soil microbes and PCB removal in two soils with divergent soil properties. Biphenyl improved removal of di- and

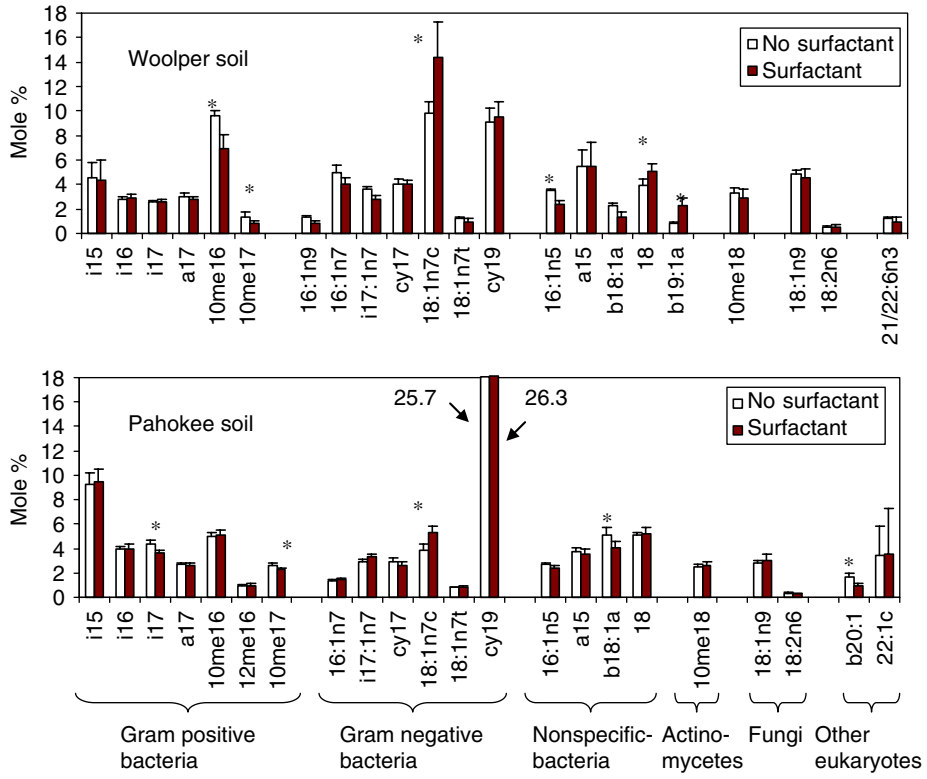

Fig. 2. Hydroxypropyl-β-cyclodextrin effects on phospholipid fatty acid (PLFA) composition at the end of the 80-days incubation in (A) Woolper soil and (B) Pahokee soil. Bars represent the mean of seven samples and error bars represent one standard deviation. The values of cy19 of Pahokee soil are out the range of *Y*-axis and are indicated on the figure. The symbol "*" were placed above PLFA that were significantly affected hydroxypropyl-β-cyclodextrin at significance level of $P<0.05$.

tri-PCB in both soils. However, it had little effect on removal of higher chlorinated PCB. A possible explanation for conger specificity of biphenyl stimulation is that lower chlorinated PCB congeners are cometabolically degraded by enriched biphenyl degraders due to structural similiarities to biphenyl. Restricted removal of higher chlorinated PCBs was likely attributed to steric hindrance of chlorine atoms on attack of the biphenyl ring by oxygenases and other PCB-degrading enzymes (Dorn and Knackmuss, 1978). Moreover, highly chlorinated PCB congeners typically have lower bioavailability than less chlorinated congeners, which could also explain differences in PCB removal rates.

In contrast to biphenyl, PSM had limited effect on PCB removal in either soil. These results contrasted with those obtained from pure-culture studies, in which PSM significantly improved PCB removal (Donnelly et al., 1994; Gilbert and Crowley, 1997). A possible explanation was that different microbial groups have variable responses to these plant compounds (Master and Mohn, 2001; Tandlich et al., 2001). Another explanation was that PCB and PSM bioavailability are more restricted in soils compared to liquid media (Jung et al., 2002). Static incubation conditions employed in this study could also limit PCB and PSM diffusion as compared to well-mixed liquid culture systems or soils with earthworms under field conditions (Lueprom-chai et al., 2002), even though this explanation seems somewhat unlikely as soils were mixed periodically during this study.

PSM were applied as pure chemicals in this study, and in association with plant residues in other experiments (Hernandez et al., 1997; Dzantor et al., 2002). In the latter studies, high PCB removal (80% or 100%) was observed with orange peels, eucalyptus leaves, pine needles or ivy leaf amendment. Considering that plant residues provide organic carbon and nutrients supporting microbial growth, it is not surprising that the pure chemicals were less effective than plant residues even though PSM were suspected to be the active ingredients affecting PCB removal.

There appeared to be significant interactions between biphenyl and HP-β-CD on PCB removal. In both Woolper and Pahokee, biphenyl in combination with HP-β-CD decreased removal of low chlorinated congeners compared to treatments with biphenyl alone. A possible explanation was that HP-β-CD, as a carbon source, could suppress biphenyl-induced PCB degrading enzymes and/or PCB degrading microbial populations. These results contrasted with those of Fava et al. (1998), who found that biphenyl in combination with HP-β-CD generally enhanced PCB removal compared with biphenyl alone. In that study, biphenyl was added to soil at the beginning of the incubation and HP-β-CD was added several weeks and months after biphenyl. Under those conditions, biphenyl

could have enriched for PCB degrading populations, then, the addition of HP-β-CD at a later time increased PCB bioavailability and transformation rates. In this study, HP-β-CD increased tetra- and penta-chlorinated PCB removal in Pahokee soil, which also suggests the function of HP-β-CD on increasing PCB bioavailability.

Interestingly, PCB removal was greater in the organic-rich Pahokee soil compared to Woolper soil, which may be attributed to higher humic substance levels in Pahokee soil that functioned as natural inducers of PCB-degrading enzyme synthesis. It was also possible that Pahokee soils supported greater microbial biomass and different microbial groups that were capable of degrading PCB, which was indicated by higher PLFA concentrations and different PLFA patterns in the two soils (e.g. cy19 and i15) (Fig. 2).

A lack of sterile control could complicate the interpretation of results because loss of PCB may due to non-biological mechanisms such as hydrophobic adsorption by soil organic matter, chemical destruction, and evaporation. Complete sterilization with autoclaving was also found to be difficult in experiments conducted by Fava and his colleagues (Fava and Bertin, 1999; Fava and Piccolo, 2002). In this study, the role of sorption on PCB removal was minimized because PCB removal was calculated by comparing with initial PCB levels in soils, which was extracted from the same soil with the same procedure. The extraction efficiencies, which was calibrated with PCB-aged sediments, were >90% for the Pahokee soil and >95% for the Woolper soil. Also, microcosms were tightly sealed and kept in the dark during the incubation, so it was unlikely that evaporation and photodegradation were major PCB loss processes. Significant enhanced PCB removal by biphenyl comparing to the no-amendment control also suggests that it was at least partially a microbially mediated process (Harkness et al., 1993).

4.2. Treatment effects on soil microbial communities

PSM are recognized for their influential role in plant–plant and plant–microbe interactions in the rhizosphere and phyllosphere (Dakshini et al., 1999; Dalton, 1999; Inderjit et al., 1999). For example, Oger et al. (2004) demonstrated that transgenic *Lotus* plants produced opine (a PSM) and enriched rhizosphere bacteria capable of utilizing this compound as sole carbon source. In the present study, however, PSM did not significantly affect microbial community structure as determined by PLFA analysis. On the other hand, HP-β-CD significantly influenced PLFA patterns in both soils. Most significant was enrichment of several lipids indicative of Gram-negative bacteria and depletion of lipids reflective of Gram-positive bacteria. In addition, many PLFA contributed to the PC1 and PC2 in PCA, implying that HP-β-CD, a readily available carbon and energy source, has affected growth of a wide variety of microorganisms in soils.

This study attempted to establish a link between soil microbial community information (PLFA) and microbial activity (PCB removal). However, no significant correlation between PCB removal and PLFA was detected in this study. Microbial community changes were influenced by surfactant, but no changes were detected in soils amended with biphenyl. These results were surprising considering biphenyl affected PCB removal. This could be because biphenyl functioned only as an inducer of PCB degrading enzymes, while surfactant was utilized as a labile carbon and energy source that significantly affected microbial community composition. Alternatively, PLFA methods may not be sensitive enough to detect small shifts in microbial community composition. Researchers could not discern the microbial community change caused by different tillage and residue management (Spedding et al., 2004), elevated atmospheric CO_2 treatment (Zak et al., 1996), and chronic NO_3^- amendment (DeForest et al., 2004). From these results, it is clear that more sensitive techniques are required to monitor microbial community shifts in soils. Incorporation of ^{13}C or ^{14}C into PLFA or nucleic acids from labelled PCB may provide a more sensitive and selective approach to identify PCB-degrading populations.

The present study demonstrated that PSM had no significant effects on PCB removal while biphenyl accelerated removal of lower chlorinated congener in high and low organic matter soils, except in the presence of HP-β-CD. HP-β-CD enhanced removal of higher chlorinated PCB in the high organic matter soil, but not in the low organic matter soil. These results demonstrated that there were significant interactions among biphenyl, HP-β-CD, and soil type. HP-β-CD changed microbial community structure based on PLFA pattern, while neither PSM nor biphenyl had detectable effects on PLFA composition. However, none of the PLFA biomarkers were correlated to PCB removal. More sensitive methods are hence required to identify PCB degrading populations in soil environments.

Acknowledgements

This research was funded by a National Science Foundation grant and the College of Agriculture, University of Kentucky.

References

ATSDR (Agency for Toxic Substance and Disease Registry), 2003. Toxicological Profile Information Sheet: PCB. Internet file: 〈http://www.atsdr.cdc.gov/toxprofiles/〉.

Bedard, D.L., Unterman, R., Bopp, L.H., Brennan, M.J., Haberl, M.L., Johnson, C., 1986. Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. Applied and Environmental Microbiology 51, 761–768.

Billingsley, K.A., Backus, S.M., Ward, O.P., 1999. Effect of surfactant solubilization on biodegradation of polychlorinated biphenyl

congeners by *Pseudomonas* LB400. Applied Microbiology Biotechnology 52, 255–260.

Chou, S.F.J., Griffin, R.A., 1987. Solubility and soil mobility of PCB. In: Waid, J.S. (Ed.), PCBs and the Environment. CRC Press Inc., Boca Raton, FL, pp. 101–113.

Dakshini, K.M.M., Foy, C.L., Inderjit, 1999. Allelopathy: one component in a multifaceted approach to ecology. In: Dakshini, K.M.M., et al. (Eds.), Principles and Practices in Plant Ecology: Allelochemical Interactions. CRC Press, Boca Raton, FL, pp. 3–14.

Dalton, T., 1999. The occurrence and behavior of plant phenolic acids in soil environments and their potential involvement in allelochemical interference interactions: methodological limitations in establishing conclusive proof of allelopathy. In: Dakshini, K.M.M., et al. (Eds.), Principles and Practices in Plant Ecology: Allelochemical Interactions. CRC Press, Boca Raton, FL, pp. 57–74.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004. Atmospheric nitrate deposition, microbial community composition, and enzyme activity in northern hardwood forests. Soil Science Society of America Journal 68, 132–138.

Donnelly, P.K., Hegde, R., Fletcher, J.S., 1994. Growth of PCB-degrading bacteria on compounds from photosynthetic plants. Chemosphere 28, 981–988.

Dorn, E., Knackmuss, H.J., 1978. Chemical structure and biodegradability of halogenated aromatic compounds: substituent effects on 1,2-dioxygenation of catechol. Biochemical Journal 174, 85–94.

Dzantor, K.E., Woolston, J.E., Momen, B., 2002. PCB dissipation and microbial community analysis in rhizosphere soil under substrate amendment conditions. International Journal of Phytoremediation 4, 283–295.

Fava, F., 1996. The presence of glass beads or triton X-100 in the medium enhances the aerobic dechlorination of aroclor 1221 in *Pseudomonas* spp. CPE1 culture. Chemosphere 32, 1469–1475.

Fava, F., Bertin, L., 1999. Use of exogenous specialized bacteria in the biological detoxification of a dump site-polychlorinated biphenyl-contaminated soil in slurry phase conditions. Biotechnology and Bioengineering 64, 240–249.

Fava, F., Gioia, D.D., 1998. Effects of triton X-100 and quillaya saponin on the *ex-situ* bioremediation of a chronically polychlorobiphenyl-contaminated soil. Applied Microbiology and Biotechnology 50, 623–630.

Fava, F., Gioia, D.D., Marchetti, L., 1998. Cyclodextrin effects on the *ex-situ* bioremediation of the chronically polychlorobiphenyl-contaminated soil. Biotechnology and Bioengineering 58, 345–355.

Fava, F., Gioia, D.D., Marchetti, L., Fenyvesi, E., Szejtli, J., 2002. Randomly methylated-cyclodextrins (RAMEB) enhance the aerobic biodegradation of polychlorinated biphenyl in aged-contaminated soils. Journal of Inclusion Phenomena and Macrocyclic Chemistry 44, 417–421.

Fava, F., Grassi, F., 1996. Cyclodextrins enhance the aerobic degradation and dechlorination of low-chlorinated biphenyls. Biotechnology Techniques 10, 291–296.

Fava, F., Piccolo, A., 2002. Effects of humic substances on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in a model soil. Biotechnology and Bioengineering 77, 204–211.

Findlay, R.H., Dobbs, F.C., 1993. Quantitative description of microbial communities using lipid analysis. In: Kemp, P.F., et al. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, pp. 271–284.

Focht, D.D., Brunner, W., 1985. Kinetics of biphenyl and polychlorinated biphenyl metabolism in soil. Applied and Environmental Microbiology 50, 1058–1063.

Focht, D.D., Reineke, W., 2002. Biotransformations of polychlorinated biphenyls. In: Hurst, C.J., et al. (Eds.), Manual of Environmental Microbiology, second ed. Blackwell Publishing, Malden, MA, pp. 1028–1037.

Gilbert, E.S., Crowley, D.E., 1997. Plant compounds that induce polychlorinated biphenyl biodegradation by *Arthrobacter* spp. strain B1B. Applied and Environmental Microbiology 63, 1933–1938.

Harkness, M., McDermott, J., Abramowicz, D., Salvo, J., et al., 1993. In situ stimulation of aerobic PCB biodegradation in Hudson River sediments. Science 259, 503–507.

Hernandez, B.S., Koh, S.C., Chial, M., Focht, D.D., 1997. Terpene-utilizing isolates and their relevance to enhanced biotransformation of polychlorinated biphenyl in soil. Biodegradation 8, 153–158.

Inderjit, Cheng, H., Nishimura, H., 1999. Plant phenolics and terpenoids: transformation, degradation, and potential for allelopathic interactions. In: Dakshini, K.M.M., et al. (Eds.), Principles and Practices in Plant Ecology: Allelochemical Interactions. CRC Press, Boca Raton, FL, pp. 255–266.

Jung, K.J., Kim, B.H., Kim, E., So, J.S., Koh, S.C., 2002. Monitoring expression of bphC gene from *Ralstonia eutropha* H850 induced by plant terpenes in soil. Journal of Microbiology 40, 340–343.

Lloyd-Jones, G., Lau, P.C.K., 1998. A molecular view of microbial diversity in a dynamic landfill in Quebec. FEMS Microbiology Letters 162, 219–226.

Luepromchai, E., Singer, A.C., Yang, C.H., Crowley, D.E., 2002. Interactions of earthworms with indigenous and bioaugmented PCB-degrading bacteria. FEMS Microbiology Ecology 41, 191–197.

Master, E.R., Mohn, W.W., 2001. Induction of *bph*A, encoding biphenyl dioxygenase, in two polychlorinated biphenyl-degrading bacteria, psychrotolerant *Pseudomonas* strain Cam-1 and mesophilic *Burkholderia* strain LB400. Applied and Environmental Microbiology 67, 2669–2676.

National Cooperative Soil Survey Staff, 2005a. Website address: 〈http://www.bae.uky.edu/UK-ARC/downloads/Files/soils/WOOLPER.htm〉.

National Cooperative Soil Survey Staff, 2005b. Website address: 〈http://soils.usda.gov/survey/online_surveys/florida/dade/classify.html#pahokee〉.

Nogales, B., Moore, E.R.B., Llobet-Brossa, E., Abraham, W.R., Timmis, K.N., 2001. Combined use of 16S ribosomal DNA and 16S rRNA to study the bacterial community of polychlorinated biphenyl-polluted soil. Applied and Environmental Microbiology 67, 1874–1884.

Oger, P.M., Mansouri, H., Nesme, X., Dessaux, Y., 2004. Engineering root exudation of *Lotus* toward the production of two novel carbon compounds leads to the selection of distinct microbial populations in the rhizosphere. Microbial Ecology 47, 96–103.

Park, Y.I., So, J.S., Koh, S.C., 1999. Induction by carvone of the polychlorinated biphenyl (PCB)-degradative pathway in *Alcaligenes eutrophus* H850 and its molecular monitoring. Journal of Microbiology and Biotechnology 9, 804–810.

Singer, A.C., Gilbert, E.S., Luepromchai, E., Crowley, D.E., 2000. Bioremediation of polychlorinated biphenyl-contaminated soil using carvone and surfactant-grown bacteria. Applied Microbiology and Biotechnology 56, 838–843.

Singer, A.C., Jury, W., Luepromchai, E., Yahng, C.S., Crowley, D.E., 2001. Contribution of earthworms to PCB bioremediation. Soil Biology and Biochemistry 33, 765–776.

Spedding, T.A., Hamel, C., Mehuys, G.R., Madramootoo, C.A., 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biology & Biochemistry 36, 499–512.

Tandlich, R., Brezna, B., Dercova, K., 2001. The effect of terpenes on the biodegradation of polychlorinated biphenyls by *Pseudomonas stutzer*i. Chemosphere 44, 1547–1555.

Wolf, C.D., Skipper, D.H., 1994. Soil sterilization. In: Weaver, R.W., et al. (Eds.), Methods of Soil Analysis, Part 2: Biochemical and Microbiological Properties. Soil Science Society of America Inc., Madison, WI, pp. 41–51.

Zak, D.R., Ringelberg, D.B., Pregitzer, K.S., Randlett, D.L., White, D.C., Curtis, P.S., 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. Ecological Applications 6, 257–262.