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Disappearance of Polychlorinated Biphenyls (PCBs) When Incubated with Tissue Cultures of Different Plant Species

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Polychlorinated Biphenyls (PCBs) were widely used in a number of industrial application in the United States for nearly 50 years (Bedard et al., 1980). PCB molecules have a biphenyl nucleus which contain 1 to 10 chlorine atoms; hence, there are 209 possible PCB molecules that differ by the location and/or number of the chlorine atoms on the rings (Fig. 1) (U.S. EPA, 1990). The manufacture of PCBs was halted 1976, when they were banned in the United States because it was determined that they can bioaccumulate in humans and are persistent in the natural environment (Lee and Fletcher, 1990; U.S. EPA, 1990). Up until 1976, approximately 100 PCB isomers had been routinely used as mixtures in industry under the commercial name Aroclor©. The wide-spread use of PCBs has resulted in their detection in 25% of the nations' superfund sites, as well as, their inclusion on the EPA priority pollutant list (Lee and Fletcher, 1990). Certain aerobic bacteria have been shown to readily degrade the lesser chlorinated PCBs (nonrecalcitrant), while they have difficulty degrading the greater chlorinated PCBs (recalcitrant) (Bedard et al., 1980).

It has been reported that the degradation of selected monophenolic xenobiotic compounds is qualitatively similar in both cell cultures and whole plants of the same species (Harms and Kottutz,1990). This suggests that callus tissue from a species that is capable of degrading a hazardous compound in the lab, can provide similar results when the whole plant is used out in the field. Harms and Kottutz (1990) have also suggested that callus tissue can provide results with less analytical expense, and in less time than using whole plant bioassays (Harms and Kottutz, 1990). In this study, callus tissues from three plant species were tested to determine their capacity to reduce the amount of extractable nonrecalcitrant and recalcitrant PCBs *in vitro*.

MATERIALS AND METHODS

Callus cells from the perennial grass *Vetiveria zizanioides* Nash (vetiver), the gymnosperm *Ginkgo biloba* L. (ginkgo), and the woody dicot *Ailanthus altissima*

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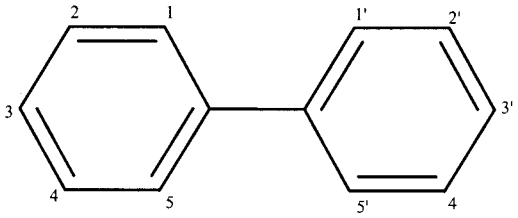

Figure 1. Biphenyl molecule indicating the numbering positions for possible chlorine substitutions

Swingle (tree-of-heaven) were grown in individual flasks containing their appropriate liquid culture media along with a defined six congener (PCB isomer) stock solution (Table 1). All of the semi-solid media used in this study contained Murashige and Skoog organic mixture, 3% sucrose, 0.8% agar, various Plant Growth Regulators (PGR), and adjusted to a pH of 5.6. The Murashige and Skoog organic mixture was purchased from Carolina Biological Supply Company (Burlington, NC). Analysis of the extracts from these experiments were done to determine if the plants selected had the capacity to cause a decrease in the amount of extractable PCBs recovered from the experimental flasks. In this study, no attempt was made to recover any of the bi-products formed during the conversion of the PCB molecule to the non-extractable form(s).

Table 1. Sources of callus tissue and corresponding media.

Plant	**Source of Explant**	**Media**
Vetiveria zizanioides Habit: Tropical to Subtropical	Node of stem to include the intercalary meristem	Murashige and Skoog Organic Mixture 4 mg/L 2,4-D 10% Coconut milk
Ginkgo biloba Habit: Temperate to Subtropical	Stem pieces from six-week-old seedlings	Murashige and Skoog Organic Mixture 1 mg/L 2,4-D
Ailanthus altissima Habit: Temperate to Subtropical	Leaf sections	Murashige and Skoog Organic Mixture 2 mg/L Kinetin 4 mg/L 2,4-D

Note: 2,4-D = 2,4,-dichlorophenoxy acetic acid

All three plant species selected are known to grow under harsh environmental conditions. For example, *V. zizanioides* is known to survive flooding, drought, and fire (National Research Council, 1993). *G. biloba* can survive air pollution and other environmental insults as does *A. altissima,* which is commonly found in urban settings (National Research Council, 1983; Campbell, 1987). The organs

from which explants were obtained varied among the taxa (Table 1). This was necessary because certain plant parts provided more vigorous germplasms than others. In order to establish and maintain callus cells, the procedure outlined in "Experiments In Plant Tissue Culture" (Doods and Roberts, 1982) was used. Upon the production of callus cells, tissue stocks were maintained on semi-sold media in 25 mL glass test tubes (125 x 25 mm) which were placed in a growth chamber set at 25 ± 1 ^{0}C with a 12 hr. photoperiod (0800-2000 hr. EST), and a light intensity of 250 μmol/m^2 sec^{-1} supplied by grow-light fluorescent lights. The callus cells from the explants were used as tissue stocks for this study. The stock cultures of *V. zizanioides* and *G. biloba* callus cells were subcultured every 3 weeks, while the *A. altissima* cells were subcultured every 4 weeks. Callus cell subcultures were made under a laminar flow hood to prevent microbial contamination.

A defined six congener PCB stock solution in hexane was used in this study. PCB congeners were purchased from Ultra Scientific (Kingston, RI). The bottles were labeled as 99% pure. The stock solution contained the following six congeners at the respective concentrations: 1) 2,2'-dichlorobiphenyl (0.45 μM); 2) 2,4',5-trichlorobiphenyl (0.39 μM); 3) 2,2',4,4'-tetrachlorobiphenyl (0.35 μM); 4) 2,2',4,4',5-pentachloro-biphenyl (0.31 μM); 5) 2,2',4,4',6 -pentachlorobiphenyl (0.31 μM); and 6) 2,2', 4,4', 6,6'-hexachlorobiphenyl (0.28 μM). These μM concentrations for each of the six congeners were equal to 100 ppm. The stock solution was stored in amber vials at 5 ± 1 ^{0}C to prevent degradation of the PCB congeners via photolysis (U.S. EPA, 1990).

Due to the hydrophobic nature of PCBs, they will adsorb onto cellular lipids whether the plants are living or dead (Fletcher et al., 1987). Once PCBs are attached to the cells, they are difficult to extract (Urey et al., 1976). Therefore, the following control of dead callus tissue was prepared: *V. zizanioides* and *G. biloba* was placed in liquid nitrogen for 3 minutes. This procedure froze the cells and provided dead cell controls. Control tissue of *A. altissima* was obtained by adding 3 mL of a 10% hypochloric acid solution to the flasks prior to adding the callus tissue (Lee and Fletcher, 1990).

Callus tissues used as test material were selected from stock cultures for their uniformity in texture, color, and lack of visible signs of embryogenesis or organogenesis. Healthy tissues from each germplasms (approximately 0.5 gm) were fragmented and placed into 125 mL culture flasks containing 10 mL of the appropriate liquid media (Table 1). The flasks containing tissues were sealed with an aluminum cap fitted with a cotton membrane (containing a 10 mm disc with a mesh of 0.02 μm) to assure aerobic conditions. Flask cultures were grown in the dark at 24-25 ^{0}C on a rotary shaker at 75 rpm. At the end of nine days, all of the flasks were removed from the shaker and placed under a sterile laminar flow hood.

A pin size hole was placed in the aluminum foil cap on the flasks using a sterile scalpel. At this point, a sterile micropipette tip was used to add 200 μL of the PCB-stock solution to all flasks, giving a final concentration of 2 ppm for each of the congeners. The hole in the aluminum caps was then sealed with tape before

Table 2. Mean of peak heights of GC chromatgraphs from extracts of living and dead plant callus cells after four days of incubation with a defined six congener PCB stock solution. [± S.D.]

	Vetiveria zizanioides		*Gingko biloba*		*Ailanthus altissima*	
PCB Congeners	Living	Dead	Living	Dead	Living	Dead
2,2'-Dichlorobiphenyl	0	80.7±26.5*	0	72.6 ±19*	95 ±2	31± 48
2,4',5-Trichlorobiphenyl	0	91.2 ±3.7*	0	62.9 ±16.8*	82.7 ±7.2	41.8±45.9
2,2',4,4'-Tetrachlorobiphenyl	0	88.5 ±5.6*	0	56.7 ±18.1*	90.3 ±1.5	74±10.5*
2,2',4,4',5-Pentachlorobiphenyl	0	87.7 ±3.1*	0	60.1 ±12.8*	93.3 ±4	78.2 ±11.6
2,2',4,4',6-Pentachlorobiphenyl	0	89 ± 2*	0	57.7 ±19*	93.7 ±4.7	76.7 ±10.5*
2,2',4,4',6,6'-Hexachlorobiphenyl	0	76.2 ±33.5*	0	71.4 ±17.8*	86.3 ±10.1	83.8 ±4.3

* Indicates a significant difference between the living and dead cells ($P \leq 0.05$).

returning the flasks to the shaker. After nine days in liquid culture, the cells reach their highest metabolism rate (Lee and Fletcher, 1990). Thus, incubating the cells with the PCB stock solution at this point may increase the amount of PCBs converted to a non-extractable form.

After four days of incubation, all of the flasks were extracted, using a hexane Triton-X-100® mixture (1% v/v) (Lee and Fletcher, 1992). Approximately 40 mL of the extraction mixture and 2.5 g of sodium sulfate were added to each flask prior to placing them on the shaker at 150 rpm at 24-25 ± 1 ^{0}C. After 24 hr, the organic layers were removed from the flasks and stored in amber vials at 5 ^{0}C. At the conclusion of each experiment, all of the flasks were examined to check for the presence of bacterial and fungal contamination, because these microorganisms have been reported to have the capacity of metabolizing certain PCB congeners (Yagi and Sudo, 1980; Thomas et al., 1992). Thus, the undetected presence of bacteria and/or fungi in the culture media may have given false positive results for the disappearance of PCBs caused by the plant cells. All of the experimental flasks were free of microbial contamination at the conclusion of the experiments.

The extracts were analyzed on a Gas Chromatograph (GC) 5890 Hewlett Packard (HP) series II equipped with an Electron Capture Detector (ECD). A computer station was attached to the GC, which was loaded with a 5890 Computer Program. An isothermal assay condition was used to analyze the extracts. The oven temperature for the ECD was 250 ^{0}C. The injection temperature was kept isothermal (250 ^{0}C), and the detector temperature was 260^{0}C. The carrier gas for the GC was ultrapure nitrogen and the makeup gas was 95% methane and 5% argon. The GC was calibrated with a standard (stock solution) everyday prior to analyzing the extracts, to ascertain the accuracy of the GC.

A decrease in peak height from the GC chromatographs was used to measure the conversion of the PCB molecule to a non-extractable form by the plant cells. The experiment was based on 6 samples for living and dead *V. zizanioides* cells, 7 samples for living and dead *G. biloba* cells, 3 samples for the living *A. altissima* cells, and 6 samples for dead *A. altissima* cells. SigmaStat® software package was used to perform all statistical analysis.

RESULTS AND DISCUSSION

Data from this study demonstrates that *V. zizanioides* and *G. biloba* caused a disappearance of all six congeners in the PCB-stock solution (Table 2).
On the other hand, a significant difference was not observed in the peak heights from the chromatographs of extracts from living *A. altissima* cells compared against the peak height from the chromatographs of extracts from the dead cells (Table 2). The recovery of PCBs from the dead cell controls was high for all three taxa, indicating that the zero percent recovery from the live cultures of *V. zizanioides* and *G. biloba* must be due to the conversion of the PCB molecules to non-extractable forms, which was not observed in the dead cells (Table 2).

It is unclear why a lower recovery of the tetra-, and pentachlorobiphenyls from the dead cells as compared to the living cells occurred in *A. altissima.* Since the control cells are not capable of any metabolic activity, the decrease in the chromatograph peak heights can be attributed to an abiotic factor. Also, the high recovery of PCBs from the living and dead *A. altissima* cells reinforces the fact that the extraction procedure used in this study was adequate to recover the PCBs from the flasks.

Plant cells remained viable when they were incubated with the PCBs. Once the incubation step was over, the cells did not show any discoloration or any other signs of death. This is in agreement with work conducted by Fletcher and Groeger (1987), who demonstrated that plant cells remained viable when they were incubated in a liquid culture medium with a defined 10 congener PCB cocktail, with each congener at 2 ppm.

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